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Organic side streams (bioproducts) as substrate for black soldier fly (*Hermetia illucens*) intended as animal feed: chemical safety issues

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ABSTRACT

The growing world population accompanied by urbanisation and improved economies has resulted in an increased demand for diets rich in animal proteins. The rearing of livestock by existing practices is also becoming increasingly difficult due to changing environmental conditions. The use of black soldier fly larvae (BSFL) grown on organic side streams (bioproducts) as feed for animals presents a sustainable alternative to conventional feed ingredients. The nutritional and compositional profile of BSFL reared on organic side streams and their potential to be used as animal feed has been extensively explored. However, little information is available on the chemical safety of BSFL reared on organic side streams. This review addresses the chemical safety hazards of BSFL reared on substrates spiked with different chemical contaminants, including heavy metals, mycotoxins, pesticides, and pharmaceuticals. BSFL were observed to excrete mycotoxins and were able to reduce the concentration of pesticides and pharmaceutical compounds in the substrate. Certain heavy metals were found to be accumulated in BSFL when grown on spiked substrate. Initial studies have also indicated that the growth parameters and composition of BSFL are not altered by the presence of microplastics and organic pollutants in the substrate. Information about these feed-safety issues will assist in developing regulatory frameworks and appropriate processing methods to mitigate these hazards, thereby providing a framework of risks for the commercialisation of BSFL destined for animal feed.

Keywords: Australian legislation, black soldier fly larvae, heavy metals, mycotoxins, organic side streams, pesticides, pharmaceuticals, safety.

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Introduction

Rapid population growth combined with urbanisation and improving economies of developing nations has resulted in increased consumption and demand for animal-based proteins (Boland *et al.* 2013; Kim *et al.* 2019; Ismail *et al.* 2020). Livestock rearing by conventional agricultural practices in these economies is harder to achieve due to the reduced availability of land, feed and other resources, caused by climate change and socio-environmental factors (Kolasa-Więcek 2013; van Huis *et al.* 2013; Kim *et al.* 2019). Several studies have shown that insects as a source of feed for livestock farming can provide a sustainable solution to meet the demand for meat by the growing population (Dobermann *et al.* 2017; van Huis and Oonincx 2017; Guiné *et al.* 2021).

Black soldier fly (*Hermetia illucens*) larvae (BSFL) have been receiving great interest along with several other edible insects in both the food and animal feed sectors. BSFL can be reared on various organic side streams (bioproducts), including household waste, municipal sludge, spent grains, human and animal faeces (Nguyen *et al.* 2013; Banks *et al.* 2014; Oonincx *et al.* 2015*b*; Nyakeri *et al.* 2017; Barbi *et al.* 2020). The proximate composition of BSFL reared from these organic side streams is 32–46% crude protein, 15–38% crude lipid, and 20.4–30.55% fibre on a dry-matter basis (Zhou *et al.* 2013;

Mohd-Noor *et al.* 2017; Nyakeri *et al.* 2017; Spranghers *et al.* 2017; Gao *et al.* 2019; Shumo *et al.* 2019). BSFL has been observed to accumulate different amino acids from organic side streams. The composition and digestibility of these BSFL proteins and amino acids have been found to be better than those of conventional soymeal and fishmeal proteins, thereby suggesting their suitability as animal feed (Belghit *et al.* 2019; Lalander *et al.* 2019; Pieterse *et al.* 2019; Woods *et al.* 2019; Ravi *et al.* 2020). The lipid content of BSFL grown on organic side streams was mainly constituted of triglycerides containing predominantly saturated fatty acids (SFA; Ravi *et al.* 2020). SFA such as lauric acid and palmitic acid are distributed at higher concentrations in BSFL (Kroeckel *et al.* 2012; Oonincx *et al.* 2015*a*; Heuel *et al.* 2021). BSFL lipids, like proteins, have been observed to exhibit good production performance when used as feed for Atlantic salmon, Jian carp, and broilers (Li *et al.* 2016; Belghit *et al.* 2019; Kim *et al.* 2020).

Overall, BSFL fed with different organic side streams has demonstrated its ability to replace more conventional feed ingredients and offer a circular economy-based solution to the agri-food sector. Despite this, the commercialisation of BSFL as a feed ingredient is not widespread. The lack of specific regulations pertaining to the use of insects as feed is observed to be an important factor in governing their commercialisation (Domingues *et al.* 2020). Currently, countries including USA, Canada, and those belonging to the European Union (EU) have drafted certain regulations governing the use of BSFL as animal feed (Lähteenmäki-Uutela and Grmelová 2016; Lähteenmäki-Uutela *et al.* 2017). However, the usage of excreta and processed waste containing animal matter and several other organic side streams is prohibited as feed for rearing BSFL in these regions. The lack of information about safety issues associated with rearing BSFL with organic side streams is an important factor in influencing the development of regulatory framework (van der Fels-Klerx *et al.* 2018; Imathiu 2020).

This article reviews the current legislative standing of BSFL as animal feed in Australia. It will provide an overview of the prevalence of different chemical safety hazards such as heavy metals, mycotoxins, pesticides, and pharmaceutical compounds in organic side streams. The impacts imposed by these chemical contaminants to the safety and quality of BSFL intended to be used as animal feed are discussed. The review also briefly discusses the knowledge gaps regarding the chemical safety of BSFL and recommends directions for future research.

BSFL as animal feed: Australian legislation

Insects are considered as farmed animals and their usage as animal feed is subject to different legislation across different states and territories in Australia (Insect Protein Association of Australia 2020). A summary of this legislation in different states is described in this section. Animal matter derived from vertebrate animals, such as blood, bone meal and feathers, is defined as restricted animal material (RAM; Alagappan *et al.* 2021). The feeding of RAM to ruminants is prohibited throughout Australia. Food waste containing meat, tissues and other materials extracted from mammals is declared prohibited pig feed (PPF). These RAM and PPF classifications restrict the use of insects as feed and feed ingredients for ruminants and swine across Australia (Department of Primary Industries and Regions-South Australia 2013; Western Australia Department of Primary Industries and Regional development 2013). Insects and insect proteins obtained using these substrates are allowed to be used as feed in aquaculture and poultry feed in most states across Australia (Alagappan *et al.* 2021). However, in Queensland the use of insects and their protein as feed is permitted only for aquaculture (Queensland Goverment 2019). It is noteworthy that rendered BSFL reared with PPF can be used as feeds for swine and poultry in different jurisdictions across Australia. Also, whole insects including BSFL when reared on non-RAM and PPF are approved to be used as feed for all livestock (Nolet 2020). Table 1 includes a list of chemical compounds and their maximum tolerable levels (MTL) in different animal feeds across Australia. BSFL intended to be used as feed in different States in Australia should comply with these regulations. It can be observed from the table that some states, namely, Northern Territory, Tasmania, Victoria, and Australian Capital Territory, have not established MTL for contaminants in animal feed. Queensland is the only state within Australia to establish MTL for organic pollutants such as dioxins and other dioxin-like poly-chlorinated biphenyls. It can also be observed that MTL for a same compound vary among different feeds, suggesting that MTL specific to BSFL has to be established so as to include BSFL under Australian animal feed legislation.

Heavy metals

Occurrence of heavy metals in organic side streams

Heavy metals are known to be associated with carcinogenicity, mutagenicity and several other detrimental effects to humans and animals on ingestion or inhalation (Verma *et al.* 2018). Organic side streams contain a diverse set of heavy metals, which can be an issue when they are used as a substrate for rearing insects. For example, raw and processed food organic side streams composed of banana peels, tapioca peel, coconuts, and rice husks that are potential dietary sources for rearing BSFL are known to contain heavy metals such as copper, cadmium, arsenic, nickel, and lead, depending on the regional source of the waste stream

Table 1. Maximum tolerable limits for contaminants in Australia.

Compound	Compound type	Type of livestock	State	Permitted concentration
Aflatoxin B1	Mycotoxin	Feed for dairy animals	Queensland	0.2 mg/kg[A]
		Feed for pigs	Western Australia and New south Wales	0.05 mg/kg[B,C]
		Feed for poultry	Western Australia and New south Wales	0.02 mg/kg[B,C]
		Feed for cattle, sheep, and goats	Western Australia and New south Wales	0.05 mg/kg[B,C]
Aldrin and dieldrin	Insecticide	All feeds	Queensland and South Australia	0.01 mg/kg[A,D]
Dichloro-diphenyl-trichloroethane	Insecticide	All feeds	Queensland and South Australia	0.05 mg/kg[A,D]
Polychlorinated bi-phenyls (PCB)	Insecticide	All feeds	Queensland and South Australia	0.05 mg/kg[A,D]
Dioxins and dioxin-like PCB's	Insecticide	Feed for fish	Queensland	7 ng/kg[A]
		Feeds other than for fish	Queensland	1.5 ng/kg[A]
Cadmium	Heavy metal	Feed for cattle, sheep, and goats	Queensland and New South Wales	1 mg/kg[A,C]
		Feed ingredients of animal origin	Queensland	2 mg/kg[A]
		Pig feed	Western Australia	0.5 mg/kg[B]
Lead	Heavy metal	All feeds	Queensland	0.5 mg/kg[A]
			Western Australia	1 mg/kg[B]
			New South Wales	0.2 mg/kg[C]
Mercury	Heavy metal	Fish feed	Queensland	0.2 mg/kg[A]
			Western Australia	0.4 mg/kg[B]
			New South Wales	0.4 mg/kg[C]
		Feed ingredients for aquaculture	Queensland	0.4 mg/kg[A]
		All other feed	Queensland	0.1 mg/kg[A]
			Western Australia	0.02 mg/kg[B]

[A]Queensland Goverment (2017).
[B]Western Australia Department of Primary Industries and Regional development (2013).
[C]New South Wales Department of Primary Industries (2017).
[D]Department of Primary Industries and Regions-South Australia (2013).

(Bożym *et al.* 2015; Kadir *et al.* 2017). Other heavy metals such as zinc and chromium (refer Table 2) are also found distributed in typical municipal sewage sludge, along with the above listed metals (Ščančar *et al.* 2000; Weng *et al.* 2014). However, the distribution of these heavy metals in these waste sources is highly variable, depending on the availability of the specific constituents in the wastes. Table 2 summarises the distribution of various heavy metals in different organic side streams, suggesting that BSFL when reared on such substrates might be susceptible to bioaccumulation of heavy metals and exhibit impaired growth attributes. The forthcoming section will discuss the impacts caused when BSFL is reared on substrates spiked with different heavy metals.

Effect of heavy metals on BSFL growth parameters

When the rearing of BSFL on a plant-based substrate varying in concentrations (10–100%) of seaweed (*Ascophyllum nodosum*) was evaluated for 8 days, it was observed that the survival rate of larvae was more than 95% until the concentration of seaweed in the feed reached 70%. Similarly, the larval weight gain and the feed conversion ratio were found to be negatively affected when the concentration of seaweed in the substrate was increased above 60%. The concentrations of lead, mercury, cadmium and arsenic in substrate with 70% seaweed were found to be at 0.17, 0.015, 0.28, and 24 mg/kg respectively, which were higher than those in the control substrate, suggesting the impact of heavy metals on influencing the mortality rate of BSFL (Biancarosa *et al.* 2018). BSFL reared on wheat bran diet spiked (refer Table 3) with cadmium at concentrations ranging from 10–80 mg/kg and copper at concentrations ranging from 100 to 800 mg/kg did not bring about drastic changes to larval weight when compared with the control without the contaminants. It is also noteworthy that BSFL fed with diets containing copper and cadmium at concentrations of 400 and 20 mg/kg had greater bodyweights than those fed the control diets (Wu *et al.* 2020). The pre-pupation and

Table 2. Distribution of heavy metals in organic side streams.

Heavy metal	Waste type	Concentration (mg/kg of dry matter)
Lead	Spent grains[A]	<1.0[A]
	Vegetable and fruit peels[A]	21.7[A]
	Processed food waste[B]	145.8[B]
	Municipal sewage sludge[B]	32.1–141.1[B]
Cadmium	Spent grains[A]	<0.5[A]
	Vegetable and fruit peels[A]	0.9[A]
	Processed food waste[C]	3.2[C]
	Municipal sewage sludge[B,D]	1.4–2.7[B,D]
Copper	Spent grains[A]	8.1[A]
	Vegetable and fruit peels[A]	17.5[A]
	Processed food waste[C]	193[C]
	Sewage sludge[B]	164.6–517.4[B]
Arsenic	Processed food waste[C]	141.8[C]
Iron	Sewage sludge[D]	23 700[D]
Zinc	Spent grains[A]	96.6[A]
	Vegetable and fruit peels[A]	23.5[A]
	Sewage sludge[B,D]	846.5–2032[B,D]
Nickel	Spent grains[A]	<1.0[A]
	Vegetable and fruit peels[A]	<1.0[A]
	Processed food waste[C]	140[C]
	Sewage sludge[B,D]	35.6–621[B,D]
Chromium	Spent grains[A]	2.5[A]
	Vegetable and fruit peels[A]	3.5[A]
	Sewage sludge[B,D]	129.9–856[B,D]

[A]Kadir *et al.* (2017).
[B]Weng *et al.* (2014).
[C]Bożym *et al.* (2015).
[D]Ščančar *et al.* (2000).

eclosion rates were found to be unaffected by heavy metals cadmium and chromium when distributed at concentrations ranging from 20 to 80 mg/kg and from 100 to 300 mg/kg respectively, in wheat bran substrates (Gao *et al.* 2017). The growth and development of BSFL in feed spiked with arsenic, lead, and cadmium at three concentrations ranging between 4 and 16 mg/kg, 2.5 and 10 mg/kg, and 1 and 4 mg/kg respectively, was measured. The development time, survival rate, and total liveweight were not affected, except when the feed contained cadmium at a concentration of 1 mg/kg of feed (van der Fels-Klerx *et al.* 2016). BSFL reared on corn–semolina feed spiked with a mixture of heavy metals (arsenic, 2 mg/kg; cadmium, 1 mg/kg; mercury, 0.1 mg/kg; lead, chromium, and nickel, 10 mg/kg each) was found to negatively influence the larval weight gain. However, no significant differences were observed in the survival rates when compared with those fed the control diets. Cai *et al.* (2018) postulated that copper, cadmium, chromium, zinc, and mercury are the heavy metals that could possibly affect the survival rate of BSFL, whereas lead, boron, nickel, and mercury are expected to affect the liveweight gain. It was suggested that heavy metals when fed together exhibit complex interactions and do not lead to significant changes in the development and growth of BSFL (Cai *et al.* 2018).

Bioaccumulation of heavy metals by BSFL reared on spiked feeds

BSFL have the potential to accumulate several heavy metals from the feed. The accumulation of mercury, lead, and arsenic from seaweed by BSFL varied linearly with an increasing concentration of seaweed from 10% to 100% in the feed (Biancarosa *et al.* 2018). However, the accumulation of cadmium attained a plateau after the concentration of seaweed in the feed reached 60%. The maximum concentrations of mercury, lead, arsenic, and cadmium in the larvae reared on feed containing 100% seaweed in this study were found to be 0.02, 0.29, 23, and 2.3 mg/kg respectively (Biancarosa *et al.* 2018). The accumulation of copper and cadmium by BSFL increased by 25.2- and 15.3-fold with diets containing copper and cadmium at concentrations of 800 and 80 mg/kg respectively, when compared with the control diets free of these heavy metals (Wu *et al.* 2020). The term bioaccumulation factor (BAF) is defined as the ratio of the concentration of a contaminant in the target sample (BSFL) to the concentration of the contaminant in the substrate. A BAF value higher than 1 would suggest the accumulation of the contaminant from the feed to the host (Gao *et al.* 2017). BSFL reared on chicken feed spiked with arsenic, lead, and cadmium at concentrations ranging between 4 and 16 mg/kg, 2.5 and 10 mg/kg, and 1 and 4 mg/kg respectively resulted in larvae with BAF values ranging between 0.49 and 0.58 for arsenic, 1.4 and 2.2 for lead, and 6.1 and 9.5 for cadmium. Purschke *et al.* (2017) observed similar BAF values for cadmium accumulation when BSFL were fed with spiked semolina–corn feed. Animal feed containing the maximum concentration of heavy metals resulted in reduced BAF values in all three cases of heavy metals in the above-stated experiments (van der Fels-Klerx *et al.* 2016). The extent of accumulation of these heavy metals is also subject to change, based on the life stage of BSFL. BAF values for cadmium accumulation by BSFL (cadmium concentration in feed of 80 mg/kg) were observed to be 4.6, 4.1 and 0.5 for larvae, prepupae and pupae respectively. The BAF value for chromium (Cr concentration in feed of 300 mg/kg) ranged between 0.002 and 0.64, with the highest value being observed for larvae and the lowest for pupae (Gao *et al.* 2017). Overall, it can be observed from these studies that the BAF value in BSFL was highest for cadmium, followed by arsenic and lead. BAF values for nickel and chromium in BSFL were found to be <1 in the above-mentioned studies.

Table 3. Bioaccumulation of heavy metals by black soldier fly larvae (BSFL) and its effects on BSFL growth and survival rate.

Heavy metal	Substrate	Initial concentration in feed (mg/kg)	Concentration in BSFL (mg/kg)	Bioaccumulation factor value	Effect on BSFL
Arsenic	Seaweed	36[A]	23[A]	1.56[A]	Survival rate, feed conversion ratio, and weight gain affected[A]
	Spiked chicken feed	4[B]	1.96[B]	0.49[B]	Development time, survival rate, and weight gain unaffected[B]
	Spiked semolina–corn feed	3[C]	2.8[C]	0.93[C]	Survival rate unaffected; weight gain and development time affected[C]
Chromium	Spiked semolina–corn feed	15.2[C]	3.4[C]	0.22[C]	Survival rate unaffected; weight gain and development time affected[C]
Lead	Seaweed	0.25[A]	0.29[A]	1.16[A]	Survival rate, feed conversion ratio and weight gain affected[A]
	Spike chicken feed	10[B]	12[B]	1.2[B]	Development time, survival rate, and weight gain unaffected[B]
	Spiked semolina–corn feed	15.2[C]	35.6[C]	2.3[C]	Survival rate unaffected; weight gain and development time affected[C]
Nickel	Spiked semolina–corn feed	15.2[C]	4.2[C]	0.27[C]	Survival rate unaffected; weight gain and development time affected[C]
Cadmium	Seaweed	0.34[A]	2.3[A]	6.76[A]	Survival rate, feed conversion ratio and weight gain affected[A]
	Spiked wheat bran	80[D]	25.6[D]	0.32[D]	Weight gain unaffected[D]
	Spiked chicken feed	1[B]	6.9[B]	6.9[B]	Development time, survival rate, and weight gain affected[B]
	Spiked semolina corn feed	1.5[C]	13.7[C]	9.1[C]	Survival rate unaffected; weight gain and development time affected[C]
Mercury	Seaweed	0.021[A]	0.023[A]	1.09[A]	Survival rate, feed conversion ratio and weight gain affected[A]
	Spiked semolina–corn feed	0.2[C]	0.1[C]	0.5[C]	Survival rate unaffected; weight gain and development timeaffected[C]
Copper	Spiked wheat bran	800[D]	549[D]	1.65[D]	Weight gain naffected[D]

[A]Biancarosa *et al.* (2018).
[B]van der Fels-Klerx *et al.* (2016).
[C]Purschke *et al.* (2017).
[D]Wu *et al.* (2020).

Bioaccumulation of heavy metals in BSFL reared on organic side streams

BSFL raised on organic side streams such as aquaculture sludge that were not spiked with contaminants, resulted in reduced accumulation of some heavy metals (Biancarosa *et al.* 2019; Schmitt *et al.* 2019; Shumo *et al.* 2019). The compositional analyses of the aquaculture sludge showed the presence of several heavy metals, including magnesium, iron, zinc, manganese, chromium, cobalt, copper, arsenic, molybdenum, silver, cadmium, mercury, and lead. The BAF values for most of these heavy metals were less than 1, except for cadmium (2.7) and mercury (1.9). Despite the higher BAF values, the concentrations of mercury (0.2 mg/kg) and cadmium (1.4 mg/kg) in BSFL were within the maximum tolerance limits allowed by Australian legislative bodies for animal feed. This is probably due to lower concentration of cadmium (0.5 mg/kg) and mercury (0.1 mg/kg) in the aquaculture sludge used for rearing BSFL (Schmitt *et al.* 2019). Table 3 displays the extent of accumulation of different heavy metals by BSFL. Atlantic salmon when fed with insect meal did not accumulate arsenic, probably due to the reduced bioavailability of arsenic from BSFL in the fish muscle (Biancarosa *et al.* 2019). Similarly, BSFL fed with organic waste such as chicken manure, kitchen waste and spent grains did not accumulate heavy metals such as cobalt, iron, copper, and zinc that were found to be naturally present in these organic wastes (Shumo *et al.* 2019).

Mycotoxins

Occurrence of mycotoxins in organic side streams

Organic side streams obtained from households and sewage sludge from municipal corporations potentially used as feed

Table 4. Distribution of mycotoxins in different waste stream.

Mycotoxin	Waste type	Concentration (µg/kg)
Viomellin	Municipal sludge[A]	3400[A]
T-2 Toxin	Agricultural waste landfills[B]	222.4[B]
Aflatoxin B1	Municipal sludge[A]	69[A]
	Pig slurry	12.2
	Organic fraction of municipal solid waste[C]	13.04[C]
Ochratoxin	Residential food waste landfills[B]	19.36[B]
Zeralenone	Agricultural and residential landfills[B]	3.43[B]
Mycophenolic Acid	Summer household waste[D]	75–19 000[D]
	Winter household waste[D]	4800[D]
Penitrem A	Household waste[D]	35–7500[D]
Thomitrem A	Household waste[D]	20–2100[D]
Thomitrem E	Household waste[D]	20–3300[D]
Roquefortine C	Summer household waste[D]	70–90[D]
	Winter household waste[D]	190[D]

[A]Youssef et al. (2020).
[B]Murray et al. (2016).
[C]Tacconi et al. (2019).
[D]Rundberget et al. (2004).

for BSFL can contain mycotoxins (refer Table 4) produced by different species of toxic and pathogenic fungi. The commonly observed fungal species include *Pencillium* sp., *Aspergillus versicolor*, *A. niger*, and *Saccharomyces cerevisiae* (Bień and Nowak 2014). The filamentous fungi, especially those belonging to the genus *Aspergillus*, *Penicillin*, *Alternaria* and *Fusarium*, produce low molecular-weight secondary metabolites called mycotoxins (Santos Pereira *et al.* 2019). Approximately 300–400 mycotoxins have been identified and classified into different groups on the basis of their structure and functional properties (Bennett and Klich 2003). The mycotoxins belonging to the groups of aflatoxins, fumonisins, ochratoxins, trichothecenes, and zearalenone are known to cause serious concerns to the health and wellbeing of livestock (Santos Pereira *et al.* 2019). These mycotoxins are found to be highly stable under natural conditions and are resistant to several processing methods including thermal processing (Bień and Nowak 2014; Santos Pereira *et al.* 2019).

Raw municipal sludge collected from a sludge treatment plant in Egypt had mycotoxins aflatoxin B1 and viomelline at concentrations of 69 and 3400 µg/kg respectively (Youssef *et al.* 2020). The concentration of aflatoxin B1 in municipal solid waste and pig slurry was determined to be 13.04 and 12.2 µg/kg respectively. However, it was observed that the concentration of mycotoxins was reduced by the action of microbes present in the wastes on composting (Tacconi *et al.* 2019). Mycotoxin analysis in 26 different landfill sites in Canada meant for food composting showed the presence of three mycotoxins, namely zearalenone, ochratoxin A and T-2 toxin. It was observed that 24 landfill sites contained at least one of these toxins and six piles had two of these toxins. Zearalenone was found to be prevalent (79%) in most of these landfill sites at a concentration of 3.43 µg/kg, followed by ochratoxin A and T-2 toxin at concentrations 19.36 and 222.4 µg/kg respectively (Murray *et al.* 2016). Barley, wheat, and several other spent brewer's grains that can potentially be used as substrate to rear insects are also prone to contamination by the fungal species *Fusarium* (Piacentini *et al.* 2019). *Fusarium* species are known to produce a diverse set of trichothecene mycotoxins such as deoxynivalenol (DON), acetyldeoxynivalenol, and diacetoxyscirpenol (Bianco *et al.* 2020). Ninety-seven samples of food waste were collected from households in Hønefoss, Norway, during summer (48 samples) and winter (49 samples) and were analysed for *Pencillium* and its mycotoxins. Mycotoxins including mycophenolic acid ($n = 36$), roquefortine C ($n = 13$), penitrem A ($n = 11$), thomitrem A ($n = 11$), and thomitrem E ($n = 11$) were prevalent in samples collected during summer, but household waste collected during winter had only mycophenolic acid and roquefortine C found in two samples (Rundberget *et al.* 2004). Hence, from a health perspective, it is important to investigate whether BSFL will accumulate these mycotoxins when reared on such contaminated organic side streams.

Effects of mycotoxins on growth and bioaccumulation in BSFL

BSFL were able to grow normally on the cereal grains spiked with DON (0.63 mg/kg of feed), a Type B trichothecene mycotoxin produced by several species of *Fusarium*, but accumulated DON during their larval stages. However, on reaching pupation they started excreting the toxin back into the residual feed (Gulsunoglu *et al.* 2019). When BSFL standard wheat-based feed was spiked with four different mycotoxins, namely, aflatoxin B1 (0.5 mg/kg of feed), zearalenone (12.5 mg/kg of feed), DON (125 mg/kg of feed), and ochratoxin A (2.5 mg/kg of feed), the growth rate was not affected. BSFL were able to either excrete or reduce these toxins either individually or as a mixture to significantly lower concentrations than those present in the feed (Camenzuli *et al.* 2018). Similar results were obtained when cornflour–semolina-based feed was spiked with zearalenone (860 µg/kg of feed), DON (697 µg/kg of feed), ochratoxin A (260 µg/kg of feed) and three different types of aflatoxin, namely aflatoxins B1 (88 µg/kg of feed), B2 (17 µg/kg of feed) and G2 (41 µg/kg of feed). It was also observed that these mycotoxins did not cause any negative effect to larval weight. The concentration of the mycotoxins in the residual substrate was comparable with the initial concentrations in spiked feed for all mycotoxins except DON (Purschke *et al.* 2017). DON was distributed at higher concentrations in residual substrate than in the spiked feed, suggesting that it might have a negative impact for the use of residual substrate as a fertiliser. Camenzuli *et al.* (2018) observed that the concentrations of DON, zearalenone, and ochratoxin A were found to be higher in the residual substrate than the initial spiked concentration. It is speculated that BSF convert the DON that was bound to the protein and carbohydrate matrix in the feed to freely available DON, thereby increasing their concentration in the residual feed material (Schrögel and Wätjen 2019). When poultry feed spiked with aflatoxin B1 at six different concentrations varying from 0.01 to 0.5 mg/kg was used to rear BSFL, the survival rate and live larval weight were found to be unaffected, and the accumulation of the toxin was found to be lower than the detectable level of 0.01 μg/kg. Also, BSFL did not accumulate the metabolite aflatoxin M1 obtained through the bioconversion of the parent toxin, thereby suggesting the ability of the larvae to excrete aflatoxin B1 (Bosch *et al.* 2017). Overall, the growth parameters of BSFL when reared on substrates spiked with different mycotoxins in these studies remained unaltered and did not accumulate the mycotoxins (Table 5).

Pesticides

Occurrence of pesticides in organic side streams

The occurrence of pesticides and pharmaceutical compounds in organic wastes obtained from restaurants and households should be very low, considering that these products have been manufactured and packed according to government-specified guidelines. Organic side streams such as fruit and vegetable peels contain a diverse set of pesticides, with endosulfans and thiabendazole distributed at prominent concentrations (Taube *et al.* 2002). A non-profit organisation, Environmental Working Group (EWG) published a report in 2019 with a list of prevalent fruits and vegetables with high concentrations of pesticide residues (Nguyen *et al.* 2020). The list comprised fruits and vegetables, including strawberries, kale, apples, pears, cherries, spinach and potatoes (Nguyen *et al.* 2020). Insecticides such as tetrahydrophthalimide, permethrin, and formetantae hydrochloride were observed to be predominantly distributed in these fruits and vegetables (Nguyen *et al.* 2020). The exposure of these pesticides at specific concentrations can cause genotoxicity and neurological disorders in humans and animals (Hołyńska-Iwan and Szewczyk-Golec 2020; Jepson *et al.* 2020). Hence, it is important to monitor the impact caused by these pesticides to the safety and quality of BSFL.

Effect of pesticides on BSFL growth parameters

When a BSFL diet composed of wheat, potatoes and yeast was spiked with six different insecticides including chlorpyrifos, propoxur, imidacloprid, spinosad, tebufenozide and cypermethrin at concentrations of 0.05, 0.05, 0.1, 2.0, 0.05, and 0.3 mg/kg respectively (Meijer *et al.* 2021), the survival rate of the larvae was found to be unaffected, and no statistical difference was observed between the control and the treatments, except for cypermethrin (survival rate = 87%; Meijer *et al.* 2021). The larval weight gain was unaffected by most of the pesticides, except for substrate spiked with cypermethrin and tebufenozide. Interestingly, BSFL reared on feed spiked with the same

Table 5. Effects of mycotoxins on BSFL reared with spiked feeds and its effects on BSFL growth and survival rate.

Mycotoxin	Substrate	Concentration in substrate	Concentration in BSFL	Effects on BSFL
Deoxynivalenol	*Fusarium*-infected cereals[A]	0.63 mg/kg[A]	NM*[A]	Excreted by pupal stage; growth rate unaffected[A]
	Wheat-based diet[B]	125 mg/kg[B]	Excreted by BSFL[B]	Growth rate unaffected[B]
	Semolina-based feed[C]	697 μg/kg	Excreted by BSFL[C]	Growth rate unaffected[C]
Aflatoxin G1	Semolina-based feed[C]	46 μg/kg[C]	Excreted by BSFL[C]	Growth rate and weight gain unaffected[C]
Aflatoxin B2	Semolina-based feed[C]	17 μg/kg[C]	Excreted by BSFL[C]	Growth rate and weight gain unaffected[C]
Aflatoxin B1	Semolina-based feed[C]	88 μg/kg[C]	Excreted by BSFL[C]	Growth rate and weight gain unaffected[C]
	Wheat-based feed[B]	0.5 mg/kg[B]	Excreted by BSFL[B]	Growth rate unaffected[B]
	Poultry feed[D]	0.5 mg/kg[D]	Excreted by BSFL[D]	Survival rate and weight gain unaffected[D]
Zearalenone	Semolina-based feed[C]	860 μg/kg[C]	Excreted by BSFL[C]	Growth rate and weight gain unaffected[C]
	Wheat-based feed[B]	12.5 mg/kg[B]	Excreted by BSFL[B]	Growth rate unaffected[B]
Ochratoxin A	Semolina-based feed[C]	260 μg/kg[C]	Excreted by BSFL[C]	Growth rate and weight gain unaffected[C]
	Wheat-based feed[B]	2.5 mg/kg[B]	Excreted by BSFL[B]	Growth rate unaffected[B]

[A]Gulsunoglu *et al.* (2019).
[B]Camenzuli *et al.* (2018).
[C]Purschke *et al.* (2017).
[D]Bosch *et al.* (2017).
NM*, not mentioned.

insecticides (cypermethrin excluded) at concentrations 10 times higher than that of the initial concentration exhibited survival rate and weight gain similar to those of the control trials (Meijer *et al.* 2021). Purschke *et al.* (2017) observed similar trends (no change in survival rate and larval weight gain) in their trials when the substrate was spiked with an insecticide mixture comprising chlorpyrifos, chlorpyrifos methyl and pirimiphos methyl at a total concentration of 2.5 mg/kg of feed.

In their experiments, Meijer *et al.* (2021) observed that imidacloprid when spiked at 0.1 mg/kg positively stimulated larval weight gains. Similarly, spiking the BSFL feed with azoxystrobin and propiconazole resulted in larvae with a greater fat content than in the controls (Lalander *et al.* 2016). This increase in larval weight and fat content in BSFL grown on spiked feeds is possibly due to insecticide-induced hormesis at lower concentrations, although these compounds may be toxic at higher concentrations (Meijer *et al.* 2021). Pyriproxifen when spiked at 58 mg/kg in BSFL diets resulted in an increase in larval weight; however, higher concentrations negatively influenced the gain in larval biomass (Tomberlin *et al.* 2002).

Bioaccumulation of pesticides by BSFL

BAF values for different pesticides in BSFL and their effect on larval growth parameters are shown in Table 6. BSFL did not bioaccumulate most of the pesticides reported in the table and the BAF value was less than 1 for all of the insecticides included in these trials (Purschke *et al.* 2017; Meijer *et al.* 2021). Mass balance analysis on the residual substrate/frass from these trials showed that the concentrations of these pesticides in the frass were lower than the starting concentrations (Lalander *et al.* 2016; Purschke *et al.* 2017; Meijer *et al.* 2021). It is speculated that the degradation of these pesticides is brought about by the enzymatic reactions mediated by the microbial flora present in the substrate and the microbiome of the BSF larvae. However, the mechanism for this degradative action is yet to be identified.

Pharmaceutical compounds: occurrence and the effects on BSFL growth and bioaccumulation

Use of veterinary pharmaceuticals is widespread to promote the health of animals reared on livestock enterprises and the shedding of these compounds is commonly observed in animal manure (Song and Guo 2014). Pharmaceutical compounds belonging to a diverse set of families including tetracyclines, sufonamides, and fluorochinolones are frequently found in different animal manures (Wohde *et al.* 2016). The vast majority of veterinary pharmaceuticals undergo degradation except for certain persistent compounds, including roxithromycin, virginiamycin, and sarafloxacin (Song and Guo 2014). It is important to understand the effects imposed by these pharmaceuticals on BSFL, especially if they are being considered as a prospective feed source.

Pharmaceutical compounds, like pesticides, did not influence the growth parameters. The growth rate and

Table 6. Bioaccumulation factor values for pesticides in BSFL and their effects on BSFL growth and survival rate.

Pesticide	Feed	Initial concentration in feed (mg/kg)		Bioaccumulation factor		Effect on BSFL
Chlorpyrifos	Potatoes, wheat, and yeast-based diet[A]	0.05[A]	0.5[A]	NA[A]	0.024[A]	Survival rate and weight gain unaffected[A]
	Semolina-based feed	0.4[B]		0.015[B]		Survival rate and weight gain unaffected[B]
Propoxur	Potatoes, wheat, and yeast-based diet	0.05[A]	0.5[A]	NA[A]	0.007[A]	Survival rate and weight gain unaffected[A]
Imidacloprid	Potatoes, wheat, and yeast-based diet	0.1[A]	1[A]	NA[A]	0.007[A]	Survival rate and weight gain unaffected[A]
Spinosad	Potatoes, wheat, and yeast-based diet	2[A]	20[A]	0.08[A]	0.002[A]	Survival rate and weight gain unaffected[A]
Tebufenozide	Potatoes, wheat, and yeast-based diet	0.05[A]	0.5[A]	NA[A]	0.01[A]	Survival rate unaffected; weight gain affected[A]
Cypermethrin	Potatoes, wheat, and yeast-based diet	0.3[A]		0.59[A]		Survival rate and weight gain affected[A]
Chlorpyrifos-methyl	Semolina-based feed	0.4[B]		NA[B]		Survival rate and weight gain unaffected[B]
Pirimiphos-methyl	Semolina-based feed	0.4[B]		0.0025[B]		Survival rate and weight gain unaffected[B]

[A]Meijer *et al.* (2021).
[B]Purschke *et al.* (2017).
NA, not applicable because the concentration was found to be below detection limits in the larvae.

larval biomass gain were unaltered when BSFL were reared on dog food spiked with carbamazepine at 1.9 mg/g of dry matter, trimethoprim at 9.9 mg/g of dry matter, and roxithromycin at 5.9 mg/g of dry matter (Lalander *et al.* 2016). Trimethoprim was found to be bioaccumulated in BSFL during the early stages of larval lifecycle; however, the larvae harvested at the end of the feeding trial were free of trimethoprim (Lalander *et al.* 2016). It was also observed that the BSFL were degrading these pharmaceutical compounds rather than excreting them in the frass. The total concentration of compounds was found to be reduced by 1 log unit at the end of the rearing trials, but the mechanism of degradation was unknown (Lalander *et al.* 2016). Mei *et al.* (2022) investigated the bioaccumulation and degradation of tylosin and enrofloxacin in unsterile and sterile BSFL. Tylosin and enrofloxacin spiked at 100 mg/kg of feed were not bioaccumulated in either sterile or unsterile BSFL. Unsterile BSFL had better degradation rates (82.9%) for the pharmaceuticals, owing to the gut microbiota associated with them, than did sterile BSFL (43.6%) and the substrate free of BSFL (30.6%; Mei *et al.* 2022). The induced expression of genes conferring resistance to tylosin and enrofloxacin along with the change in microbial flora of BSFL further indicated the role played by the gut microbiota in influencing the degradation of these two antibiotics (Mei *et al.* 2022).

Knowledge gaps

The impact of different groups of heavy metals, mycotoxins, and pesticides on BSFL growth and bioaccumulation has been reported in previous studies. However, little information is available about the effects caused by other chemical contaminants that are found prevailing in organic side streams such as plastics and organic pollutants. This section will briefly describe the knowledge gaps regarding the safety aspects of BSFL in relation to the above-mentioned contaminants.

Plastics and microplastics in organic side streams

Plastics and microplastics are said to be prevalent in organic-waste streams, especially in household waste and solid municipal waste. However, there have been limited studies to document the effects caused to BSFL by these plastics. Romano and Fischer (2021) exposed BSFL to polypropylene microplastics and observed no significant changes in length, biomass gain and survival rate of the larvae. The pupation percentage was found to be negatively affected by the presence of polypropylene (Romano and Fischer 2021). The composition of short-chain fatty acids in BSFL was found to be variable and it is speculated that this change in fatty acid composition might also bring a shift to the gut microbiota, which could influence several other metabolic activities in BSFL (Romano and Fischer 2021). When BSFL was reared on homogenised household food waste spiked with 20% of polypropylene and 20% of polystyrene separately, and the pupation rate, larval survivability, biomass gain, and substrate reduction was monitored, survivability and biomass gain of BSFL were not found to be affected when reared on substrate spiked with these plastics. However, the rate of pupation and the feed conversion ratio increased and decreased respectively, with both 20% polypropylene and 20% polystyrene (Cho *et al.* 2020).

Organic pollutants in organic side streams

The distribution of persistent organic pollutants such as dioxins, polychlorinated biphenyls (PCB) and polyaromatic hydrocarbons in animal feeds is regulated by legislative authorities in Australia. However, very little information is available regarding the effect of these compounds on BSFL. When BSFL larvae reared on spent brewer's grain waste were subjected to analysis for ortho- and non-ortho-PCBs, polychlorinated dibenzodioxins/polychlorinated dibenzofurans and polybrominated diphenyl ethers, the presence of these compounds was detected but their concentrations in larvae were below 10 μg/kg (Charlton *et al.* 2015). Similarly, the concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in BSFL meal prepared from seaweed as feed was lower than the maximum permissible levels for dioxins and dioxin-like PCBs in Australia (Biancarosa *et al.* 2019). It is noteworthy that these two studies focussed only on the bioaccumulation of the compounds in the BSFL and did not account for the effects caused by these organic pollutants in terms of larval growth rate or survivability.

Recommendations for future research

It can be noted from the previous sections in this article that BSFL were able to excrete different groups of mycotoxins, pesticides, and certain heavy metals without accumulating and having adverse effects on larval growth attributes. BSFL were also observed to reduce or degrade certain mycotoxins, pesticides, and pharmaceutical compounds but the mechanism by which the larva initiates this response is yet to be clarified. Mei *et al.* (2022) in their studies determined that the gut microbiota of BSFL plays a significant role in mediating the degradation of pharmaceuticals. Further research in relation to gene expression and proteomic studies should be conducted on BSFL to understand other functional pathways or mechanisms that might be responsible for metabolising these chemical contaminants. Cytotoxic and genotoxic effects caused by the exposure of BSFL to chemical contaminants should be investigated as they might influence the growth and reproduction of BSFL. Understanding of these degradation mechanisms and cytotoxic profiles would be beneficial to help maximise the productivity of BSFL in commercial rearing practices and to develop practices to reduce undesirable effects such as stunted growth, reduced weight gain, etc.

The integration of BSFL into Australian animal-feed legislation when reared on organic-waste streams including municipal waste, restaurant waste, abattoir waste, supermarket waste, etc. would require knowledge about their chemical safety. Information regarding the distribution of different chemical contaminants in different organic-waste streams and their effect on bioaccumulation, growth parameters, and compositional properties of the BSF larvae is limited. Also, further researched is needed to evaluate the cumulative impact caused by different contaminants (combined effect caused by mixture of mycotoxins, heavy metals, pesticides, etc.) to the safety and compositional properties of BSFL. This information will identify contaminants that are deleterious to the larvae and guide in the development of maximum tolerable limits for these contaminants, thereby promoting the development of legislation pertaining to the use of BSFL as animal feed.

Conclusions

The use of BSFL reared on organic side streams as a sustainable animal-feed ingredient is gaining prominence across the globe. However, commercialisation has not been widespread due to lack of specific regulations, particularly in regard to the safety of the larvae when reared on different organic side streams. This review article presents an overview of the prevalence of different chemical contaminants in organic side streams and the impact caused by them to the chemical safety of BSFL. The survival rate, growth rate and biomass gain of BSFL in the presence of these contaminants, including mycotoxins, heavy metals, pesticides, and pharmaceutical compounds, was found to be largely unaffected except in the case of the pesticides cypermethrin and tebufenozide. BSFL are prone to accumulate some heavy metals, including cadmium, arsenic and lead, which could potentially cause deleterious effects to animals fed BSFL or lead to residues in secondary animal products. The extent of risk from these safety hazards in BSFL appears to be influenced by the nature of the organic stream used for rearing. Rather than accumulating pesticides, pharmaceuticals, and mycotoxins, BSFL either excreted them or reduced the concentrations of these contaminants in the residual frass. The mechanism by which the larvae excrete or degrade these contaminants is yet to be explored and needs further investigation. Information regarding the chemical safety of the larvae when exposed to microplastics, dioxins and other organic pollutants that are commonly found in the environment is limited and requires further research. Current studies have demonstrated the chemical safety of larvae when reared on conventional stockfeed and other homogenous substrates. Further research is also needed to investigate the cumulative effect caused by various contaminants that might occur together in organic side streams. The findings from future research will aid us to better understand the chemical risk from BSFL fed on different side streams, which in turn will aid in the determination of management approaches to mitigate specific risks and the development of a regulatory framework to promote the commercialisation of BSFL in the animal-feed sector.

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