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# Black Soldier Fly-based bioconversion of biosolids creates high-value products with low heavy metal concentrations

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ABSTRACT

Annually, over 30 million dry tonnes of biosolids are produced worldwide, most of which are disposed into landfills or discharged into waterbodies, exacerbating eutrophication and water-borne diseases. Bioconversion of biosolids using Black Soldier Fly larvae (BSFL) can produce high-value biomass (protein, lipids, and chitin) while reducing the volume of waste that requires disposal. We aimed to determine the bioconversion performance of BSFL on different types of biosolids and biosolids blends and analysed the bioaccumulation of heavy metals in the larvae. BSFL converted all substrates into larvae biomass of similar protein content (31–37%). However, larvae growth and substrate reduction were significantly lower for unblended biosolids compared to wheat bran. Blending of biosolids with other organic waste such as food waste or wheat bran improved larvae performance (< 40% substrate reduction after 20 days; < 149 kg larvae/ tonne dry substrate) and fat content (< 31%). Despite initial high concentrations in biosolids (< 8700 mg/ kg), heavy metals were largely partitioned into the residues instead of the mature BSFL, resulting in low bioaccumulation of those elements in BSFL (< 180 mg/ kg). These concentrations were even below limits of international guidelines for animal feed. Therefore, this study demonstrated that BSF-based bioconversion can be an innovative and sustainable waste management and resource recovery technology to rapidly reduce the volumes of biosolids while transforming it into high-value biomass of low heavy metal concentrations.

## 1. Introduction

Globally, over 30 million dry tonnes of biosolids are produced annually (ANZBP, 2019; Mateo-Sagasta et al., 2015; Stantec, 2019; Wei et al., 2020). Management practices of biosolids such as stockpiling, landfilling, incineration or discharge into waterbodies can result in environmental degradation and present human health risks (Paramashivam et al., 2017). Biosolids are the product of dewatered and treated sewage sludge resulting from wastewater treatment processes (Mohajerani et al., 2019). Since biosolids are rich in carbon and plant nutrients they could be used as fertilizer or as a soil conditioner in agriculture, forestry, or ecological restoration (Simcock et al., 2019). Diverting biosolids from landfills by land application is a growing and cost-effective practice in some countries such as Australia or Czech Republic, where 83% and 91% of biosolids, respectively, are applied to land (ANZBP, 2019; LeBlanc et al., 2009). Other countries like Japan, USA and Germany have moved away from land application, towards incineration and energy-recovery (LeBlanc et al., 2009). However, this solution is expensive and represents a waste of potentially valuable organic carbon and plant nutrients (EPA, 2003; Mateo-Sagasta et al., 2015). It also releases greenhouse gases. On the other hand, land application of biosolids may negatively affect soil quality and human health while also being culturally unacceptable (James et al., 2016).

Biosolids contain pathogenic organisms, and depending on the type of wastewater treated (municipal or industrial), it may contain unacceptable levels of heavy metals and organic contaminants, such as pharmaceuticals, personal care products, halogenated organic compounds, and other xenobiotics (Brisolara and Bourgeois, 2019). Therefore, extensive treatment of sewage sludge is necessary to eliminate or reduce those contaminants, and to comply with current national guidelines for land application of biosolids (e.g. NZWWA, 2003). This is accompanied by particular sludge treatment costs covering up to 50% total costs for wastewater treatment (LeBlanc et al., 2009). In New Zealand, annual sludge treatment costs are around US$ 30 M per year

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(Tinholt, 2019). While 52% of biosolids are applied to land (largely for remediation purposes), over 40% are still discharged to landfills and oceans (Stantec, 2019). To overcome increasing costs for sludge treatment and landfilling as well as the environmental impacts from biosolids discharge to land and ocean, sustainable waste management solutions are needed.

In some countries like New Zealand vermicomposting of biosolids using earthworms is applied on a small scale (Tinholt, 2019). The resulting compost is traded as soil fertilizer and due to the transformation process, it is more socially accepted than direct application of biosolids to land (Quintern and Morley, 2017). However, vermicomposting is a relatively slow conversion process taking a minimum of eight weeks to reduce the volume by just 10% (Ndegwa et al., 2000). In contrast, insect-based bioconversion can reduce volumes of organic waste by up to 70% in two weeks (Gold et al., 2018; Myers et al., 2008; Rehman et al., 2017b) and produces high-value biomass from a waste product that would otherwise being disposed of in a linear economy system. This could create new revenue streams for the wastewater management from resulting insect-derived high-value products including fat or chitin (Gold et al., 2018).

*Hermetia illucens* L. (Diptera: Stratiomyidae), commonly known as the Black Soldier Fly (BSF) could be used for the bioconversion of biosolids. This insect is native to America but is now widespread in tropical and temperate regions (May, 1961; Sarpong et al., 2019). BSF larvae (BSFL) have a broad range of natural diets including animal manure and human faeces (Banks et al., 2014; Lalander et al., 2019; 2019; Rehman et al., 2017a). They have also been reported to consume fruit and vegetable waste and waste of milling or brewery side streams (Gold et al., 2018). Depending on the biowaste composition, they can consume up to four times their body weight of organic matter per day and convert it into high-value protein (35–50%) and fat biomass (17–36%) (Diener et al., 2009; Liu et al., 2018; Tomberlin et al., 2002). The resulting residues could be used as organic fertilizer for agricultural purposes (Sarpong et al., 2019).

While production of animal feed from BSF-based bioconversion of food waste is a growing market, there is limited knowledge on the use of high-value products (protein, fat, and chitin) from larvae grown on high-risk waste like biosolids containing high concentrations of heavy metals, salts, and other contaminants and how those contaminants could affect the survival and bioconversion efficiency of BSFL (Cho et al., 2020; Gold et al., 2018). Previous studies have demonstrated that BSFL can reduce microbial contaminants and pharmaceuticals during bioconversion processes (Awasthi et al., 2020; Cai et al., 2018b; Lalander et al., 2013; Lalander et al., 2015; 2016). BSFL are also able to tolerate microplastics and high concentrations of heavy metals in their feed (Cho et al., 2020; Diener et al., 2015; Gao et al., 2017; Purschke et al., 2017). Only a small set of studies indicated that biosolids or untreated sewage sludge could be a potential feed substrate for BSFL (Awasthi et al., 2020; Cai et al., 2018a; Lalander et al., 2019; Leong et al., 2015). In these studies, the risk of bioaccumulation of heavy metals in the larvae bodies was raised with exception of Cai et al. (2018a) but not further measured. In fact, studies have shown that heavy metals can accumulate during the bioconversion process of different substrates in BSFL, limiting their use as animal feed (Biancarosa et al., 2018; Cai et al., 2018a; Gao et al., 2017; Proc et al., 2020; Purschke et al., 2017; Schmitt et al., 2019; Tschirner and Simon, 2015; Van der Fels-Klerx et al., 2016; Wu et al., 2020). On the contrary, Diener et al. (2015) found that the bioaccumulation factor of Zn (BSFL/ substrate concentration quotient) decreased in BSFL with increasing Zn concentrations in their feed. Therefore, we hypothesise that the bioaccumulation factor of Zn and perhaps other metals in BSFL feeding on biosolids will be low. This may result in the production of valuable low-metal BSFL biomass.

In this study, we aimed to: (1) determine the survival and bioconversion efficiency of BSFL in a range of biosolids and blends of biosolids with other types of organic waste, (2) analyse and determine the partitioning and bioaccumulation factors of heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn), non-essential elements (Al, As) and macronutrients (N, Ca, Mg, Na, K, S) in BSFL fed on the range of substrates, (3) infer the potential factors affecting the bioconversion efficiency of BSFL, and (4) determine if the BSFL could be used as animal feed based on their heavy metal composition.

## 2. Material and methods

### 2.1. Source of larvae and feed substrates

Ten-day-old larvae of *Hermetia illucens* L.(BSFL), fed on moist wheat bran substrate, were obtained from Prescient Nutrition Ltd. (Palmerston North, New Zealand). Wheat bran substrate was freshly prepared before every feeding by mixing the dry bran flakes (Champion Professional Flour Food Bran, Champion Flour Milling Ltd., Auckland, New Zealand) with autoclaved deionized water in a ratio 1:4 (w/w). Household food waste consisted of cuttings and peelings of fruit and vegetables (feijoas, banana, mandarin, pineapple, mango, kiwi fruit, cabbage, onion, zucchini, sweet corn, carrot, potato, tomato, cucumber, lettuce), grounded tea, rice, noodles, and chicken eggshells. It was blended and kept at 4 °C until feeding to BSFL. Two types of biosolids, (1) dewatered sludge treated with Solid Grade Cationic Polyelectrolyte (Zetag® 8185, BASF) for thickening (referred to as Biosolids 1) and (2) digester sludge containing 3% solids (referred to as Biosolids 2), were retrieved from New Zealand municipal wastewater treatment plants in Wellington and Palmerston North, respectively. The sludge was kept at 4 °C until feeding to BSFL. Table A.1 (Supplementary Material, Part A) shows the nutritional composition of the different feed substrates. The water content of the substrates ranged between 78%, for Biosolids 1, to 82% for wheat bran, Biosolids 2 + Wheat and food waste (Figure A.2 B, Supplementary Material, Part A). Heavy metal and macronutrient composition of ten-day-old larvae and substrates are listed in Table A.3.

### 2.2. Experimental setup and rearing conditions

About 680 ten-day old larvae were fed with different blends of biowaste every three or four days with a feeding rate of 100 mg/ larvae/ day (Figure A.1, Supplementary Material Part A). Feed substrates (total amount of 1300 g per treatment) were wheat bran (Wheat), food waste (Food), dewatered sludge (Biosolids 1), dewatered sludge blended with food waste in ratio 50:50 (w/w based on fresh weight; Biosolids 1 + Food), dewatered sludge blended with wheat bran in ratio 50:50 (w/w based on fresh weight; Biosolids 1 + Wheat), or digester sludge blended with wheat bran in ratio 75:25 (w/w based on fresh weight; Biosolids 2 + Wheat). All treatments were set up in triplicates. BSFL were reared at 25 °C and 60–70% RH for 20 days in squared plastic boxes (12 cm height, 16 cm length), closed by a lid with a 10 × 10 cm hole that was covered with a polyester cloth. Samples of substrate and residues were taken at the beginning and end of the experiment to monitor chemical composition and electrical conductivity (EC). In addition, samples of the transformed substrates were collected after 4, 7, 11, and 14 days of rearing to monitor pH, moisture content, and dehydrogenase activity (Figure A.1). At the end of 20 days of rearing, boxes with larvae and residues were weighed to determine the mass of residues. The mature larvae (5th and 6th instar, based on coloration) were separated from the residues, disinfected with 70% ethanol, and rinsed several times with tap water. To empty their digestive tracts before further analysis, they were kept overnight at 13 °C in a clean box until another rinsing step with demi-water and gentle drying with clean tissue paper. After this overnight starvation period, the total weight of all mature larvae was determined and the average weight per larvae was calculated from repeated measurements of small subsets of larvae. BSFL were kept at −20 °C until further processing for chemical analysis.

### 2.3. Analysis of larvae growth rate and survival, substrate reduction and bioconversion efficiency

The final weight of the residues was calculated by subtracting the total weight of the rearing container, containing the mature larvae and residues, with the empty weight of the container and the total weight of mature larvae. The final number of surviving larvae was estimated by dividing the final weight of all larvae by the average weight per larvae. Growth Rate Index of larvae (GRI), substrate reduction (SR) based on total or volatile solids and Bioconversion rate (BCR) were calculated according to Diener et al. (2009) and Banks et al. (2014), Eqs. (1)–(4).

$$GRI(mg/days) = (L_{end} - L_0)t \quad (1)$$

*t, days of rearing;* $L_0$ *initial weight of ten-days old larvae;* $L_{end}$*, total weight of mature larvae*

$$Survivalrate(\%) = 100 * nL_{end}/nL_0 \quad (2)$$

$nL_0$*, number of larvae at beginning;* $nL_{end}$*, number of larvae at the end*

$$SR(\%) = ((S - R)/S) * 100 \quad (3)$$

*S, weight of total or volatile solids of total substrate; R, weight of total or volatile solid of residues*

$$BCR(\%) = (L_{DM} / S_{DM}) * 100 \quad (4)$$

$L_{DM}$*, total dry weight of mature larvae;* $S_{DM}$*, dry weight*

### 2.4. Chemical and biological analysis of substrate, residues, and larvae

#### 2.4.1. Dry weight, ash, organic matter, pH, and EC

The weight of total solids (TS; referred to as dry matter) was determined by drying 5–10 g material at 105 °C for 24 h. Ash was determined by ignition in a muffle furnace at 550 °C for 6 h according to APHA 2540 G (Baird et al., 2017). Mass of volatile solids (VS; referred to as organic matter) resulted from subtraction of 100 by mass of ash.

Changes in pH and EC were analysed with a pH metre (pH700, EUTECH Instruments) and EC electrode (CON700, EUTECH Instruments). 4 g of substrate or residues were mixed with 10 ml autoclaved deionized water and incubated at room temperature for 18 h before each measurement.

#### 2.4.3. Total carbon, nitrogen, recoverable phosphorous, larvae crude protein and fat content

Total carbon (C [%]) and nitrogen (N [%]) were determined by combustion analysis using a LECO CN828 according to AOAC method 972.43 and method 968.06 (Dumas Method), respectively (AOAC, 2005). Total recoverable phosphorus was measured by Hills laboratory using ICP-MS according to EPA-OST 200.2 (Martin et al., 1994); samples were dried, sieved and digested using nitric/hydrochloric prior measurements.

Crude fat content of larvae was extracted by Soxhlet extraction according to Liu et al. (2018). Crude protein content (%) of larvae was determined by multiplying N (%) with 4.67 (Janssen et al., 2017) and crude protein content of the substrate was calculated by multiplying N (%) with 6.25. A protein conversion rate (PrCR) was calculated according to Lalander et al. (2019), Eq. (5).

$$PrCR(\%) = (LDM * PrL)/(SDM * PrS) * 100 \quad (5)$$

*PrL, crude protein content of mature larvae; PrS, crude protein content of substrate*

#### 2.4.4. Heavy metals, non-essential elements, macronutrients, and bioaccumulation factor

The total extractable concentration of heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn), non-essential elements (Al, As) and macronutrients (Ca, K, Mg, Na, S) were determined by acid digestion with $HNO_3$. In brief, 0.2 g of dried material was mixed with 5 ml 67% $HNO_3$ and incubated overnight at room temperature, followed by digestion at 220 °C using a UltraWAVE Single Reaction Chamber Microwave Digestion System (Milestone Inc.). The digest was diluted with milli-Q water to reduce final $HNO_3$ concentration to below 10%. Digests were analysed with an Agilent 8900 Triple Quadrupole ICP-MS. Mean recovery of As, Co, Cu, Mo, and Cd in reference plant materials (Rice Flour 1568a, Tobacco Leaves c2015/76,210, and Tomato Leaves 1573a; International Plant analytical Exchange [IPE] 100), obtained from NIST (USA) and Ichtj (Poland), were analysed for quality assurance; mean recovery ranged between 80 and 102%.

Mass balances were calculated by multiplying the concentration with the dry weight of the whole sample. A bioaccumulation factor (BAF) was determined according to Diener et al. (2015), Eq. (6).

$$BAF = C_L/C \quad (6)$$

$C_L$*, concentration of elements in larvae;* $C_S$*, initial concentration of elements in substrate; based on dry matter.*

#### 2.4.5. Dehydrogenase activity (DHA)

DHA (related to total microbial activity) in the substrates and residues was determined by a colorimetric enzyme assay as previously published (Barrena et al., 2008; Wong and Fang, 2000). DHA led to reduction of 2,3,5-triphenyltetrazolium chloride to triphenylformazan (TPF). The production of TPF was measured after 20–24 h of incubation at 25 °C along with a calibration curve of different TPF standard concentrations using a BMG FLUOstar Optima spectrophotometer at 485 nm. Measurements were performed in triplicates per sample.

### 2.5. Statistical analysis

Data for larvae survival rate, SR of total solids and volatile solids, crude fat and crude protein content, BCR, PrCR, C/N ratio, total carbon and nitrogen content in substrates were arcsine transformed and analysed by One-way ANOVA followed by Tukey HSD tests (*agricolae* package, de Mendiburu and Yaseen, 2020). The same test was also performed to compare differences in GRI, organic matter, total P, pH, DHA, EC, macronutrient, heavy metal, and non-essential element content of the substrates. Type-III two-way ANOVA of log-transformed data was performed to compare concentrations of heavy metals, non-essential elements and macronutrients between treatments and sample types. Tukey HSD post-hoc tests were performed by using the R-Shiny application designed by Assaad et al. (2015). Changes in elemental composition between substrate, residues and larvae were analysed by principal component analysis (PCA, *stats 3.6.2* package [R-Core, 2021]) and visualized with fviz_pca_biplot function (*factoextra* package, Kassambara and Mundt, 2020). Differences in pH, moisture or DHA between treatments were analysed by ANCOVA (*agricolae* package). Spearman's rank correlation analysis was performed to test for potential relationships between initial elemental concentrations in the substrate and resulting elemental concentrations and BAF in mature larvae. The same analysis was also performed to test for correlations of larvae growth, BCR, crude fat or crude protein content to substrate nutritional factors (*corx* package, Conigrave, 2020). All statistical tests and graphical interpretations were performed with RStudio 1.4.1717 based on R 4.1.0.

## 3. Results

### 3.1. Survival and growth of BSFL on biosolids blends

BSFL survived and grew in all substrates including those containing biosolids. While the survival rate of BSFL for the different substrates did not differ, the growth rate of BSFL was significantly affected by the type of substrate (Table 1). The larvae growth rate for Biosolids 1 (6.3 mg/ day) was significantly lower than for the control substrate wheat bran (7.9 mg/ day). Blending biosolids with other organic waste led to similar (Biosolids 1 + Wheat and Biosolids 2 + Wheat: around 7.4–8 mg/ day) or higher larvae growth rates (Biosolids 1 + Food: 8.7 mg/ day) compared to wheat bran. The growth rate of BSFL was significantly lower when BSFL were reared on food waste (4.7 mg/ day) compared to wheat bran and significantly lower compared to Biosolids 1 (Table 1). There were no significant correlations between larvae growth rate and substrate nutritional factors (Table A.2, Supplementary Material Part A).

After 20 days of rearing, the substrate mass was reduced between 29.5% and 49.8% (Table 1) and was lowest for food waste (29.5% TS, 33.3% VS) or Biosolids 1 (34.9% TS, 34.9% VS). Blending biosolids with food waste (Biosolids 1 + Food) or wheat bran (Biosolids 2 + Wheat) increased the substrate reduction to similar ranges for wheat bran (46.3% TS, 49.8% VS). Overall, the bioconversion rate (BCR) was highest for wheat bran and biosolids blends compared to food waste or Biosolids 1 (Table 1). Furthermore, the BCR was positively correlated with the volatile solids content in the substrates (Table A.2). The protein conversion rate (PrCR) ranged between 9.3 and 35.8% and was highest for wheat bran, food waste (30.1%) and Biosolids 2 + Wheat (28.2%) and lowest for Biosolids 1 (Table 1).

### 3.2. Effect of biosolids on the fat and protein concentrations in the BSFL

The crude fat content of mature larvae ranged between 16.6% and 31.3% (Table 1). Larvae reared on food waste had the lowest concentration of fat, while larvae reared on biosolid blends Biosolids 1 + Wheat and Biosolids 2 + Wheat had the highest concentration of fat (31.3% and 30.2%, respectively). The crude fat content of the larvae had a significant positive correlation to volatile solids (i.e. organic matter), Mg as well as Mn and had a significant negative correlation to the concentration of Ca and overall sum of macronutrients in the substrates (Table A.2).

Mature larvae had a crude protein content of 28.3% to 37.1%. With exception of larvae reared on food waste or Biosolids 1 + Food, the larvae crude protein content was not significantly different from the control substrate wheat bran (Table 1). Crude protein content was significantly positive correlated with phosphorus, volatile solids, initial pH, Mg and Mn in the substrate. A significant negative correlation for crude protein content and substrate nutritional factors was obtained for Ca, and the sum of all macronutrients (Table A.2). Although there were significant differences in moisture content and DHA between the different substrates (Figure A.2 B and C), these factors had no significant effect on larvae crude fat or crude protein content (Table A.2).

### 3.3. Changes in the composition of substrate and larvae

Processing of the different substrates by BSFL led to significant increases in pH, from an initial pH of the substrate of 4.5–6 to a final pH in the residues of 7.5–8.0 (Figure A.2 A, Supplementary Material Part A). Except for Biosolids 1, moisture content of the substrates did not significantly change over time (Figure A.2 B). When BSFL were feeding on the different substrates, DHA increased significantly within the first four days of rearing (Figure A.2 C). The highest DHA at day 4 was measured for Biosolids 1 (80 μg/ g/ h) while lowest DHA was observed for food waste (32 μg/ g/ h).

Although initial ten-day-old larvae contained significantly higher concentrations of Ca (20,354 mg/ kg), K (15,694 mg/ kg), Mg (5966 mg/ kg) and S (4747 mg/ kg) as well as Mn (165 mg/ kg) (Fig. 2, Table A.3), overall total masses of these elements contributed by the ten-day-old larvae were significantly lower compared to the substrates (Figure A.3). Therefore, the total mass of macronutrients, heavy metals, and non-essential elements at the beginning of the feeding trial were largely introduced by the substrates.

There were significant differences in the chemical composition of the feed substrates (Table A.3). Biosolids containing substrates had significantly higher ($p < 0.0001$) concentrations of heavy metals and non-essential elements but similar concentrations of macronutrients compared to the control substrate wheat bran (Table B.8 and B.9, Supplementary Materials Part B). Overall, concentration of heavy metals and non-essential elements were 7-times (for Co) to 150-times (for Pb) higher in Biosolids 1 as compared to wheat bran or food waste. Blending biosolids with other organic waste decreased concentration of these elements (Table A.3, Fig. 2). After 20 days of rearing, heavy metals, non-essential elements and macronutrients except for Ca and Mn were partitioned into the residues but not mature larvae (Fig. 1). Concentrations of most of these elements were significantly higher in the residues compared to the mature larvae as well as the substrates (Fig. 2, Table A.3). Biosolids 1, contained the highest concentrations of heavy metals and non-essentials elements (Fig. 2). The concentrations of As, Al, Cd, Co, Cr, Cu, Fe, Mo, and Ni significantly increased in the mature larvae fed on Biosolids 1 compared to the ten-day-old larvae. Concentrations of these elements were significantly higher (up to 60 times) in mature larvae feeding on Biosolids 1 compared to wheat bran or food waste (Table A.3). Spearman rank correlation analysis showed significant but moderate positive correlations ($p < 0.0004$, $R > 0.28$) between concentration of macronutrients (except for K, Na and S) and heavy metals in substrates and mature larvae (Figure A.4).

The bioaccumulation factor (BAF) indicates whether an element is portioned into (BAF > 1) or out of (BAF < 1) BSFL (Diener et al., 2015).

**Table 1**
Summary of performance data of BSFL that were reared on various feed substrates. Average and standard deviation ($n = 3$) are displayed. Different letters indicate significant differences amongst different treatments ($p < 0.05$, see Table B.7): wheat bran (Wheat), food waste (Food), dewatered sludge (Biosolids 1), dewatered sludge blended with food waste in ratio 50:50 (Biosolids 1 + Food), dewatered sludge blended with wheat bran in ratio 50:50 (Biosolids 1 + Wheat), or digester sludge blended with wheat bran in ratio 75:25 (Biosolids 2 + Wheat).

| Treatment | Survival rate (%) | Growth rate index (mg/ days) | Substrate reduction rate (%) | | Bioconversion rate (%)* | Protein conversion ratio (%)* | Larvae composition* | |
|---|---|---|---|---|---|---|---|---|
| | | | Total Solids | Volatile Solids | | | Crude protein (%) | Crude fat (%) |
| Wheat | 87 ± 10 | 7.87 ± 0.23 ab | 46.30 ± 4.17 a | 49.85 ± 2.72 a | 15.68 ± 0.14 a | 35.78 ± 1.08 a | 37.07 ± 0.75 a | 28.58 ± 2.95 ab |
| Food | 92 ± 9 | 4.73 ± 0.55 d | 29.53 ± 1.48 c | 33.32 ± 1.04 c | 9.98 ± 0.54 b | 30.06 ± 0.55 b | 28.26 ± 1.29 c | 16.61 ± 0.78 c |
| Biosolids 1 | 83 ± 13 | 6.27 ± 0.55 c | 34.93 ± 1.11 bc | 34.92 ± 0.26 c | 7.08 ± 0.54 c | 9.34 ± 0.22 d | 35.96 ± 1.42 a | 23.29 ± 1.60 b |
| Biosolids 1 + Food | 82 ± 9 | 8.67 ± 0.57 a | 40.75 ± 6.03 ab | 45.48 ± 5.69 ab | 16.1 ± 0.55 a | 16.49 ± 1.73 c | 31.59 ± 1.02 b | 26.05 ± 3.41 ab |
| Biosolids 1 + Wheat | 95 ± 8 | 8.07 ± 0.38 ab | 32.16 ± 4.1 bc | 37.61 ± 5.37 bc | 13.84 ± 0.31 a | 14.38 ± 1.86 c | 34.96 ± 1.21 a | 31.29 ± 1.12 a |
| Biosolids 2 + Wheat | 92 ± 4 | 7.37 ± 0.06 bc | 40.48 ± 2.64 ab | 44.86 ± 2.72 ab | 14.6 ± 0.07 a | 28.15 ± 2.35 b | 36.72 ± 1.50 a | 30.24 ± 1.72 a |

* based on dry weight.

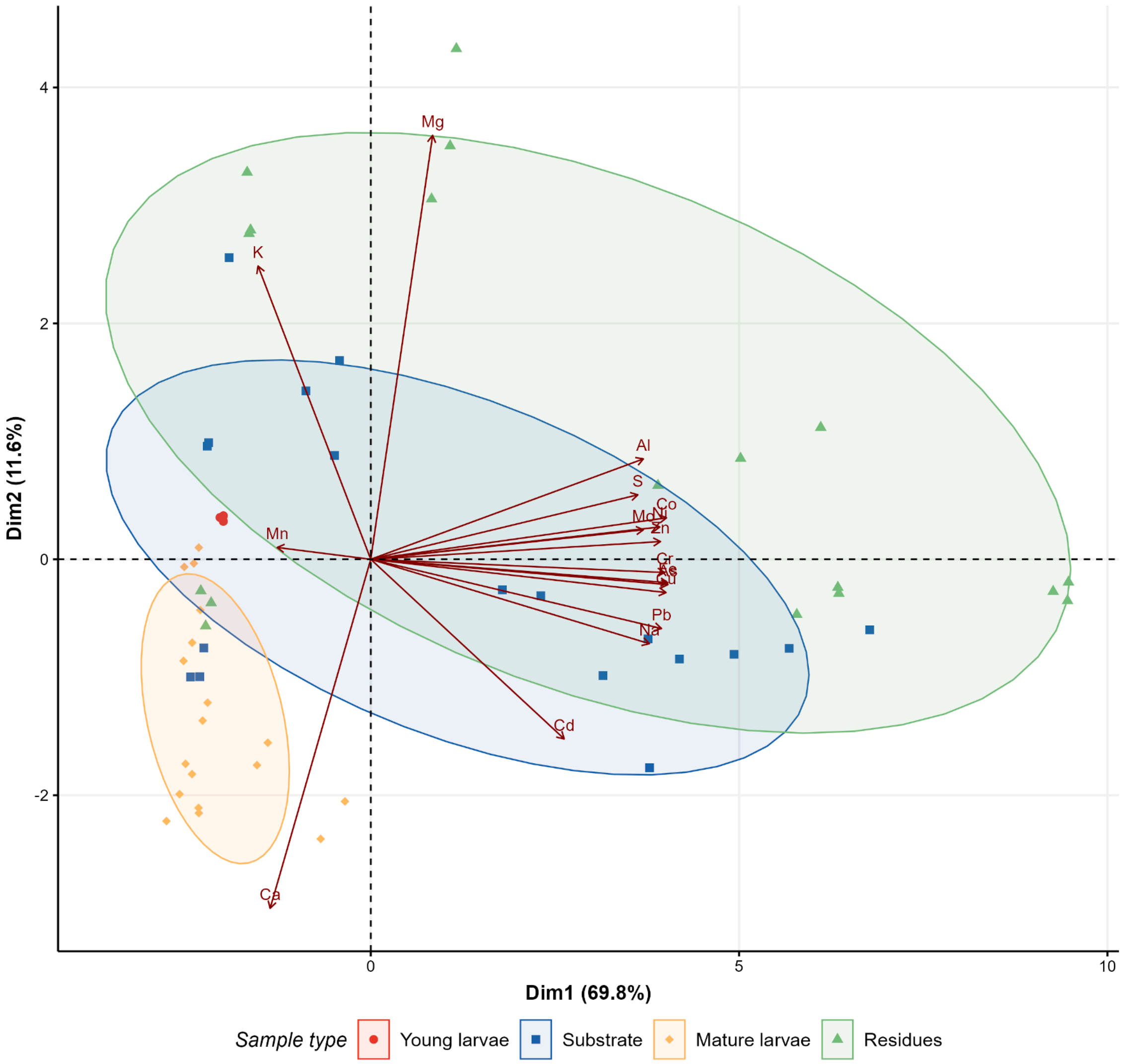


**Fig. 1.** Principal Component Analysis over macronutrients, heavy metals, and non-essential elements concentrations in different sample types across all treatments. Dim: principal component. Young larvae:10-day old larvae at the start of the experiment. Mature larvae: larvae after 20 days of experiment.

For wheat bran and food waste, a BAF > 1 occurred for the macronutrients Ca, Mg, Na, S and the heavy metals Cd, Cu, Mn, Pb and Zn (Fig. 3). In comparison, a BAF > 1 was only obtained for Ca, K, Mn as well as Cd (only for Biosolids 1) for resulting mature larvae when feeding on biosolid containing substrates (Fig. 3). There was a significant and for some elements very strong negative correlation ($p < 0.0001$, $R < -0.52$) between initial substrate concentration and BAF in mature larvae for all macronutrients, heavy metals, and non-essential elements except for Mn (Fig. 4).

## 4. Discussion

In this study, we aimed to determine the tolerance of BSFL to different types of biosolids and blends of biosolids. We showed for the first time that high-value insect biomass of low heavy metal concentration can be produced from biosolid substrates containing high background concentrations of those elements. In particular, concentrations of As, Zn and Cu were increased in the biosolids that limits their use as soil conditioner (NZWWA, 2003). BSFL grew successfully on different types of biosolids substrates with similar levels of crude protein (35.9–36.7%) and crude fat content (23.3–30.2%) compared to the control wheat bran. However, the larvae growth and bioconversion efficiency was strongly affected by the different feed substrates. To upscale BSF-based bioconversion of biosolids it is important to understand which nutritional factors of the feed substrate may be attributed to a better performance of the larvae.

### 4.1. *Factors affecting bioconversion efficiency of biosolids substrates by BSFL*

While some studies have shown that elevated concentrations of Zn and Cu can affect the survival of BSFL (Cai et al., 2018a), the larvae survival was not significantly different amongst the treatments in this study. However, BSFL mass was significantly lower when biosolids were supplied as the only diet substrate (i.e. Biosolids 1) with highest concentrations of Zn, Cu, and other heavy metals. This is consistent with other BSF-studies (Purschke et al., 2017; Wu et al., 2020). Blending biosolids with other organic waste improved larvae growth rate, substrate reduction rate and bioconversion efficiency to similar or even higher levels compared to the control substrate wheat bran. Although not identified in this study, out-diluting of heavy metals and non-essential elements Cu, Pb, Zn and As, by blending biosolids with other organic waste, might have led to improved larvae growth rate, similarly to what was reported by Cai et al. (2018a). In addition, high concentration of total macronutrients and especially Ca were identified to negatively affect the formation of larvae fat and protein biomass. This

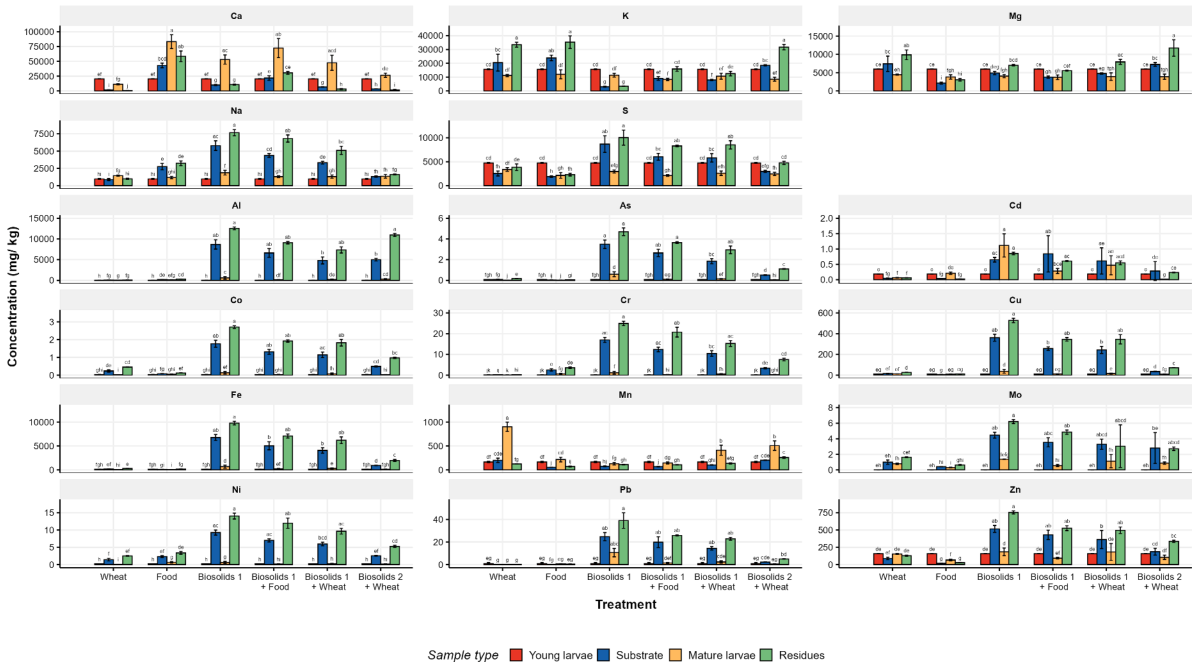


**Fig. 2.** Concentration of macronutrients (Ca, K, Mg, Na, S), non-essential elements (Al, As), and heavy metals in young and mature larvae well as substrate and residues for different treatments: wheat bran (Wheat), food waste (Food), dewatered sludge (Biosolids 1), dewatered sludge blended with food waste in ratio 50:50 (Biosolids 1 + Food), dewatered sludge blended with wheat bran in ratio 50:50 (Biosolids 1 + Wheat), or digester sludge blended with wheat bran in ratio 75:25 (Biosolids 2 + Wheat). Mean values ($n$ = 3) with standard deviation are displayed. Different letters indicate significant difference between sample type and treatments (see Table A.3. and Table B.10, Supplementary Material Part A and B). Young larvae:10-day old larvae at the start of the experiment. Mature larvae: larvae after 20 days of experiment.

is consistent with studies showing delayed development of *Drosophila melanogaster* under high concentrations of Ca (Clark, 1958). Furthermore, high salt concentrations in unblended biosolids substrates may decrease larvae growth (Cho et al., 2020).

Values for crude protein and fat content as well as protein conversion ratios of larvae fed on biosolids were comparable to other BSF-studies using human faeces as feed substrate (Gold et al., 2020; Leong et al., 2015; Nyakeri et al., 2019). However, these values contrast with findings of Lalander et al. (2019) who measured a protein conversion ratio for undigested sludge of around 7.8%, while in this study the protein conversion ratio for Biosolids 1 was around 9.3%. The authors also reported a decreased performance of the larvae when reared on digested sludge compared to undigested sludge. However, no comment on heavy metal content for the different types of substrates was made. In this study, Biosolids 2 was obtained from a digester but mixing with wheat bran as bulking substance led to a similar bioconversion and protein conversion rate compared to the control substrate.

Aside from high heavy metal concentrations, BSFL growth and survival may be affected by C/N ratio, protein, and volatile solids content of the provided substrate (Gold et al., 2020; Lalander et al., 2019; Rehman et al., 2017a). Here, similarly to Lalander et al. (2019), the volatile solids content was attributed to positively affect larvae protein and fat biomass formation as well as the bioconversion efficiency. Volatile solids constitute the organic fraction in biowaste that can be easily decomposed by BSFL for energy consumption (Gold et al., 2018). This could partially explain the improvement of the bioconversion efficiency for the substrate Biosolids 1 + Wheat (13.8%) compared to Biosolids 1 (7.1%) as blending increased the volatile solids content from 88% to 91.1%. However, volatile solids content seemed to only explain a better bioconversion efficiency and growth rate partially. This was the case for the feed substrate Biosolids 1 + Food compared to Biosolids 1 + Wheat. Rehman et al. (2017a) postulated a good balance of volatile solids and nitrogen content in the substrate is essential for a good bioconversion performance which could explain the performance differences of the larvae for the discussed biosolids blends. The C/N ratio for Biosolids 1 + Food was higher and similar to the control substrate compared to Biosolids 1 + Wheat.

In contrast to former studies (Gold et al., 2018; Gold et al., 2020; Lalander et al., 2019; Li et al., 2011; Nyakeri et al., 2019), in our study the larvae growth and bioconversion efficiency including substrate reduction rate was lowest for food waste compared to all other substrates including biosolids blended with food waste. Besides the low nitrogen and volatile solids content in food waste a higher fraction of fibres, originating from fruit and vegetable as prominent part of the blend, would have contributed to the low performance of the larvae. High concentrations of fibres in BSFL feed have been shown to negatively affect the larvae growth and the bioconversion efficiency accordingly (Barragán-Fonseca et al., 2018; Li et al., 2011; Liu et al., 2018; Palma et al., 2019). Generally, the low protein and fat content of the mature larvae fed on food waste were comparable to former studies (Gold et al., 2018; Gold et al., 2020; Liu et al., 2018; Nyakeri et al., 2019).

Correlation analysis indicated higher concentrations of Mn or Mg as well as an initial high pH of the feed substrate as important factors that enhance larvae fat and protein accumulation. Manganese is an essential element for the development and growth of insects (Ben-Shahar, 2018). Harrison (1928) showed that melanism in moth larvae can be increased when exposed to food enriched with Mn. Similarly, higher levels of Mn may improve anabolism of BSFL to produce more protein and fat. Magnesium was reported to be essential for the development of *D. melanogaster* (Clark, 1958) which might be also the case for BSF. Moreover, low pH (< 4) of the substrate was shown to decrease larvae growth and development (Ma et al., 2018). This may explain why the larvae fed on food waste of initially pH 4.5 did not gain equally weight and accordingly had a significantly lower protein and fat content compared to the larvae fed on the other substrates with initial higher pH of above 5.5.

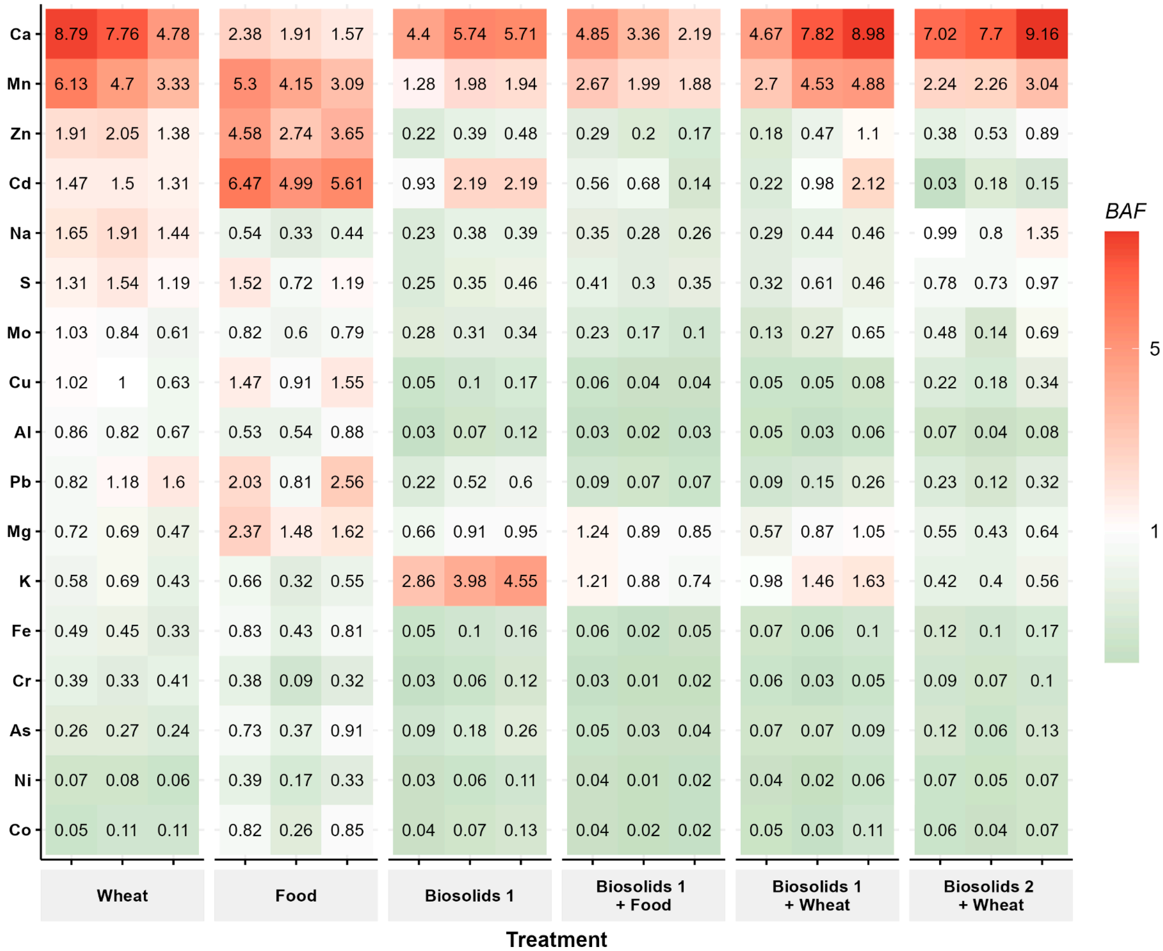


**Fig. 3.** Bioaccumulation factor (BAF) for macronutrients (Ca, K, Mg, Na, S), heavy metals, and non-essential elements (Al, As) in mature larvae for different treatments: wheat bran (Wheat), food waste (Food), dewatered sludge (Biosolids 1), dewatered sludge blended with food waste in ratio 50:50 (Biosolids 1 + Food), dewatered sludge blended with wheat bran in ratio 50:50 (Biosolids 1 + Wheat), or digester sludge blended with wheat bran in ratio 75:25 (Biosolids 2 + Wheat). If BAF ≥ 1 (displayed in white to red) than a bioaccumulation of the respective element occurred in the larvae body.

### *4.2. Partitioning of heavy metals and macronutrients in larvae and residues*

High protein and fat levels as well high Ca levels in BSFL reared on biosolid substrates in this study, similarly to what was observed for other BSF studies (Proc et al., 2020; Schmitt et al., 2019; Tschirner and Simon, 2015), makes them advantageous to use as feed for livestock (Kim et al., 2021). However, concentrations of toxic elements in BSFL that could accumulate from the substrate may represent a health risk when used as animal feed. In this study, we showed that most heavy metals and non-essential elements from the substrates were partitioned into the residues, not the mature larvae. Concentrations of heavy metals in mature larvae that fed on biosolid substrates were up to 50-times lower as compared to the substrates and the BAF was < 1 for almost all elements except for Mn. This stands in contrast with a former BSF-study on sewage sludge (Cai et al., 2018a). However, other studies that reared BSF with corn or wheat-based substrates spiked with high concentrations of particular heavy metals such as Zn, Cu or Cr (Diener et al., 2015; Gao et al., 2017; Wu et al., 2020) demonstrated that BAF in mature larvae is decreasing with increasing substrate levels. This is also supported by the correlation analysis (Fig. 4) showing a significant negative correlation between initial concentration of most heavy metals (except Mn) and non-essential elements in the substrate and BAF in mature larvae. Hence, BSFL demonstrate a mechanism of exclusion of toxic concentrations of heavy metals by actively excreting those into the residues. Similar phenomena were reported for other insects such as mealworms or grasshoppers (Crawford et al., 1996; Lindqvist and Block, 1995). At low concentrations some heavy metals such as Cu or Zn are essential for cellular functions. Therefore, BSFL might actively uptake those elements to maintain metabolism. This would support the increased BAF (> 1) when wheat bran or food waste was provided as feed substrate. Besides Cu and Zn, Mn is an essential element for maintaining metabolism. The concentration of Mn was already elevated initially in the ten-day-old larvae. During the bioconversion process the concentration of this element increased in the mature larvae. This is consistent with Proc et al. (2020). In BSF, Mn is linked to the synthesis of melanin, especially in the late stage of BSFL development, as a protection mechanism against solar radiation when migrating from the substrate (Ushakova et al., 2017).

When unblended biosolids (Biosolids 1) were supplied as a feed substrate, Cd accumulated in the larvae (BAF >1). However, this was not the case for BSFL reared on Biosolids 1 + Wheat although the initial substrate concentrations were similarly high (around 0.6 mg/ kg). In contrast, the BAF for larvae fed on Biosolids 1 + Food was lower than Biosolids 1 although the initial substrate concentration of Cd was higher (0.8 mg/ kg). Interestingly, Purschke et al. (2017) observed an accumulation of Cd in BSFL (BAF = 1.5) fed on corn semolina spiked with heavy metals with a concentration of 1.5 mg/ kg while no bioaccumulation (BAF = 0.68) was observed for BSFL fed on sewage sludge mixed with chicken manure and wheat bran that had an alike concentration of 1.8 mg/ kg (Cai et al., 2018a). These contrasts show that differences in the accumulation of Cd in BSFL may not only be attributed

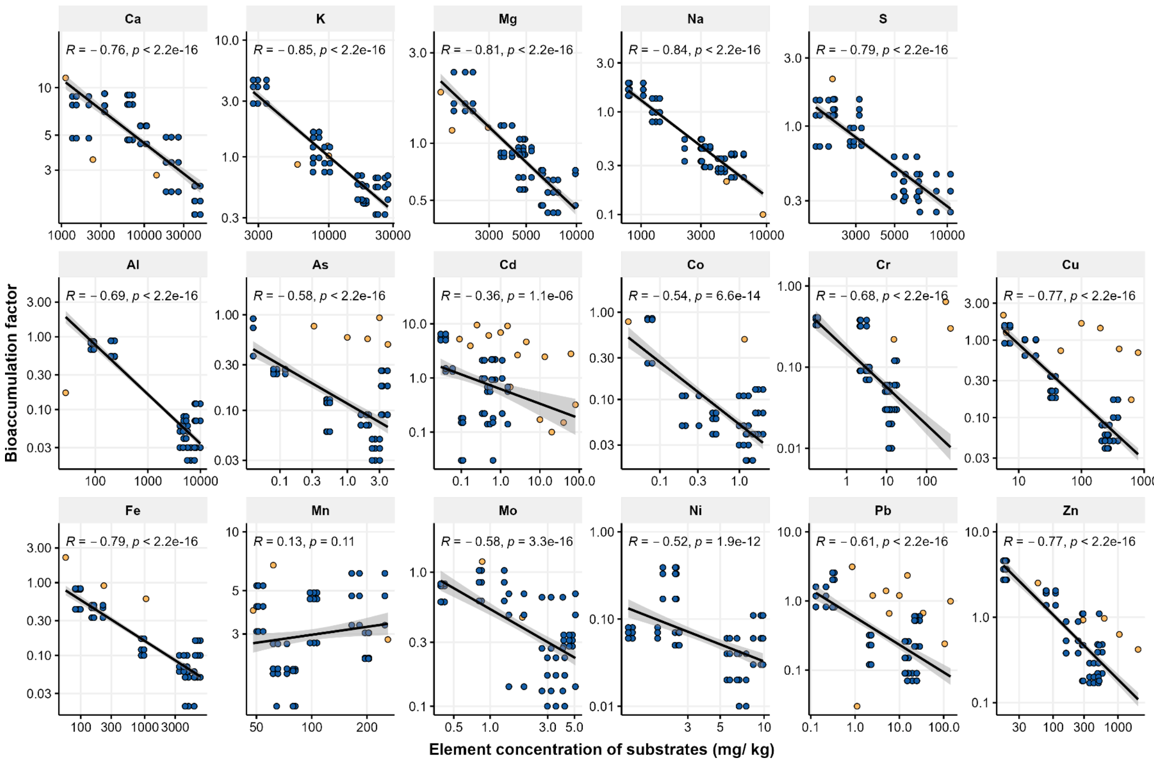


**Fig. 4.** Spearman rank correlation plots for concentrations of macronutrients (first row) or heavy metals and non-essential elements in the substrate and BAF of those elements in mature larvae. Blue dots represent data collected in this study and yellow dots represent data from previous BSFL feeding studies: Biancarosa et al., 2018; Cai et al., 2018a; Diener et al., 2015; Gao et al., 2017; Proc et al., 2020; Purschke et al., 2017; Schmitt et al., 2019; Tschirner and Simon, 2015; van der Fels-Klerx et al., 2016; Wu et al., 2020 (Table A.4 and A.5, Supplementary Material Part A).

**Table 2**
International regulation guidelines for metal concentrations in animal feed in EU, USA, Canada, and China.

| Element | EU maximum limits[a] (mg/ kg) | | USA: NRC Minimal Tolerance[b] (mg/ kg) | Canadian Regulatory guidance[c] (mg/ kg) | Chinese feed standard[d] (mg/kg) | | Concentration in larvae fed on biosolids substrates[e] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Feed materials | Complete feed | | | Feed ingredient | Feed products | Biosolids1 | Biosolids 1 + Food | Biosolids 1 + Wheat |
| Al | n.a. | n.a. | n.a. | 200 (non-ruminant) | n.a. | n.a. | 613 ± 325.2 | 169.4 ± 25.7 | 229.8 ± 93 |
| As | 2 | 2 | 30 | 8 | 2 (stone powder)<br>40 (algae and processed products) | 2 (other compound feed)<br>10 (additive premixed feeds) | 0.59 ± 0.24 | 0.11 ± 0.03 | 0.14 ± 0.03 |
| Cd | 2 | 0.5 | 10 | 0.3 (other livestock)<br>0.4 (horses) | 0.75 (stone powder)<br>2 (feed ingredients derived from animals) | 0.5 (compound feed)<br>1.25 (Concentrated feed) | 1.11 ± 0.38 | 0.28 ± 0.08 | 0.47 ± 0.31 |
| Cr | n.a. | n.a. | 100 (swine, horse cattle sheep)<br>500 (poultry) | n.a. | 5 | 5 (compound feeds)<br>20 (additive for pigs) | 1.17 ± 0.66 | 0.24 ± 0.1 | 0.49 ± 0.21 |
| Cu | n.a. | n.a. | 40 (goat) | n.a. | n.a. | n.a. | 37 ± 18.5 | 11.1 ± 2.04 | 14.9 ± 4.58 |
| Hg | 0.1 | 0.1 | 0.2 | n.a. | 0.1 (other feed ingredients)<br>0.5 (fish and by-product feed ingredients) | 0.1 (other compound feed)<br>0.5 (aquatic compound feed) | n.a. | n.a. | n.a. |
| Pb | 10 | 5 | 10 (swine, horse, poultry) | 8 | 5 (single-cell protein feed)<br>30 (feed grass) | 5 (compound feed)<br>40 (additive premixed feed) | 10.61 ± 68 | 1.48 ± 0.41f | 2.35 ± 1.02 |
| Se | n.a. | n.a. | 0.5 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |

[a] EU Directive 2002/32/EC.
[b] NRC, 2005 (Guide used by FDA).
[c] CFIA, 2017.
[d] AQSIQ, 2017.
[e] This study.

to the initial concentration in the substrate and by feed exclusion mechanisms of the larvae but also other factors. For example, extraordinary high Ca content in BSF compared to other dipterans (Finke and Oonincx, 2014) have been related to Cd uptake in BSFL because of similar ionic sizes that fit through the same uptake channel (Braeckman et al., 1999). However, in this study accumulation of Cd in the larvae was not related to increases in larvae Ca content.

Because of increased levels of Cd, Cu, Pb and Al, BSFL fed on Biosolids 1 (Table 2) would not comply to international animal feed guidelines (Table 2). In contrast, the heavy metal content of larvae that were reared on blended biosolids substrates like Biosolids 1 + Wheat or Biosolids 1 + Food were below maximum levels of these guidelines and could be potentially used as animal feed if concentration of Hg for these larvae (not measured in this study) would also be below acceptable limits. Alternately to animal feed, larvae fed on biosolid substrates including Biosolids 1 could be used as fertilizer as the low metal content compared to resulting residues are below the limits of organic material for land application (NZWWA, 2003). Previous studies also indicated that heavy metals like Cd and Cr do not accumulate in the skin (Gao et al., 2017) and that BSFL reared on substrate with high levels of heavy metals such as Zn, Cu, Pb and Ni bioaccumulated these elements in their bodies but not in extracted larval fats (Cai et al., 2018a). Hence, chitin and fat of BSFL fed on biosolids substrates could be a valuable source for a range of other industrial use (Gold et al., 2018).

### 4.3. Economic potential of BSF-based bioconversion of biosolids

The reduction of heavy metals in biosolids continues to be a challenge and new (expensive) technologies have been developed to treat this problem (Brisolara and Bourgeois, 2019). Here, we have shown that BSF-based bioconversion of biosolids blends can create high-value biomass with low heavy metal concentrations and can reduce waste volume of up to 40% in 20 days which is up to 195-times faster than decay in landfills (Cruz and Barlaz, 2010). This could strongly reduce costs for landfilling as well as greenhouse gas emission. For example, in New Zealand a 40% reduction of normally landfilled biosolids (45,000 tonnes [Stantec, 2019]) to 27,000 tonnes/ year) by BSF-based bioconversion could lead to cost reduction of over US$12 M/ year (landfill disposal fee: US$660/ dry tonne [Beecroft and Prosser, 2020]) and reduction of carbon emissions of over 27,000 Mg $CO_2$e/ year (based on emission rate of 1.50 Mg $CO_2$e/ Mg substrate by decay on landfills [Brown et al., 2010]).

On the other hand, bioconversion of biosolids blends such as Biosolids 1 + Food and Biosolids 1 + Wheat could create 149 and 130 kg larvae per tonne dry substrate, respectively. This relates to 45–47 kg insect protein and 39–41 kg insect fat as well as 17–19 kg chitin per tonne dry substrate (based on 13% chitin content of the larvae [Wong et al., 2019]). Given the biosolids production in the EU, China, or NZ of 8.9 Mio., 7.8 Mio. and 66,000 dry tonnes/ year, respectively (Mateo-Sagasta et al., 2015; Stantec, 2019; Wei et al., 2020), BSF-based bioconversion could recover valuable resources from this waste product and could create up to 420,000, 370,000, and 3100 tonnes of protein/ year, 360,000, 320,000, and 2680 tonnes of fat/ year as well as 170,000, 150,000, and 1280 tonnes of chitin/ year, respectively. The market value of larvae protein, fat and chitin are estimated to be US$ 6000/t, US$ 2350/t, and US$ 34,500/t, respectively (Bloomberg, 2020; Byrne, 2021; Priyadarshi and Rhim, 2020). Therefore, under optimal conditions, revenues of about US$ 9 bn/ year, US$ 8 bn/ year, or US$ 70 M/ year could be created by BSF-based bioconversion of all biosolids produced in EU, China, and NZ, respectively.

### 4.4. Conclusion

We have shown that BSF-based bioconversion of biosolids can (1) produce valuable insect biomass rich in protein and fat and (2) reduce waste volume by up to 40% in less than 20 days. Furthermore, we demonstrated that (3) heavy metals and non-essential elements did not accumulate in the mature BSFL but rather in the residues. The heavy metal concentration in BSFL fed on biosolids was low enough to allow their use as animal feed. For a potential upscaling of the bioconversion technology of biosolids we identified nutritional factors such as volatile solids content, Mg, Mn, pH, and Ca to be relevant to control to improve the bioconversion efficiency of BSFL. Further research is needed to evaluate potential transmission of other contaminants than heavy metals during the BSF-based bioconversion process of biosolids. Overall, this study provided evidence that BSF-based bioconversion can be a promising waste minimization and resource recovery solution towards the creation of a circular economy around biosolids management.

## CRediT authorship contribution statement

**Kristin Bohm:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Project administration, Funding acquisition. **Gregory A. Hatley:** Investigation, Writing – review & editing. **Brett H. Robinson:** Validation, Supervision, Project administration, Writing – review & editing. **María J. Gutiérrez-Ginés:** Resources, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.resconrec.2021.106149.

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