Bioconversion of Cattle Manure by *Hermetia Illucens* Larvae: Mineral Content Changes in Manure and Larval Biomass.

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Abstract

Bioconversion by *Hermetia illucens* larvae is a novel technology for organic waste treatment and valorization. However, since the possible uses of products from this process are in agriculture and livestock, the bioconversion must guarantee the mineral quality of both the *Hermetia illucens* larvae frass and larval biomass. Therefore, this study aimed to assess the mineral content changes for both the larval biomass and larvae frass of *Hermetia illucens* after the manure bioconversion to determine their suitability as animal feed and organic fertilizer, respectively. *Hermetia illucens* larvae were put into a plastic box containing fresh cattle manure, and the control treatment with the same conditions without larvae was established. After the first pre-pupae were detected, frass and larvae were collected, and their mineral content was analyzed. At the end of the experiment, the larvae showed increases in some micro and macronutrients, especially calcium and manganese, increasing up to 2.6 and 22.6 times the initial concentration, respectively. The toxic elements concentration was increased in larval biomass, but these levels met the international legislation for animal feed. As a result, the mineral content in larval biomass revealed that *Hermetia illucens* could be potentially used as animal feed, which could be comparable with fish meal, and is probably better than soybean meal. However, the larvae frass could only be used as organic fertilizer in a Canadian context, with further treatment for decreasing the chromium content being necessary.

1. Introduction

Animal manure is a type of organic waste generated by livestock. With the increase of intensive production systems in response to the growing worldwide demand for animal protein, high amounts of manure must be managed (Sungur et al. 2016; Feng et al. 2018). Due to manure's nutrient and mineral content, the most common management method used for it is its direct application to soils with or without prior storage (IPCC 2006) to improve soil quality and crop yield (Fengsong et al. 2011). However, the presence of pathogens, antibiotics, veterinary drugs, and some toxic elements could limit its use on agricultural soils and carry a risk to the environment and human health (Sahito et al. 2016; Hu et al. 2017; Leclerc and Laurent 2017; Feng et al. 2018; Provolto et al. 2018; Zubair et al. 2020).

Macro and micronutrients and toxic elements are often found in a wide range of animal manures. They are linked with the animal's feed, supplementation, and manure's origin (Nicholson et al. 1999; Wang et al. 2014; Provolto et al. 2018; Qian et al. 2018; Li et al. 2019; Xu et al. 2019). Many of these are essential elements added to animal feed and play vital roles in animal health. They complement the animal's nutritional requirements and maintain proper animal growth (Dai et al. 2016; Hejna et al. 2018; Provolto et al. 2018). However, excessive supplementation and low absorption by animals of these elements can lead to high concentrations in manure (Feng et al. 2018; Guo et al. 2018; Li et al. 2019). For example, Cu, Zn, As, and Cr promote animal growth, disease mitigation, and feed use efficiency. Pig feed often contains higher concentrations of Cu and Zn, and the animal's gut absorbs only 10–20% of these metals in feed. Thus the remaining amount of metals is excreted, which is why pig manure often shows higher

levels of these metals than chicken and cattle manure (Nicholson et al. 1999; Fengsong et al. 2011; Ji et al. 2012; Wang et al. 2014; Ding et al. 2017; Feng et al. 2018).

The concern here is that toxic elements from manure entering the ecosystem may lead to accumulation, bioaccumulation, and biomagnification in the food chain (Zhao et al. 2014), through crop leaching and surface run-off to freshwater after their application to soils (Shi et al. 2018, 2019). Current studies reveal that land fertilizing with animal manure is the highest source of toxic elements in agricultural soils in countries with low industrial activities and the second source of toxic elements in soils, after atmospheric deposition, in industrialized countries (Shi et al. 2018, 2019). Annually, the land fertilizing with animal manure contributes, at a global level, approximately 2.9 kt of toxic elements to the environment (Leclerc and Laurent 2017).

In addition to spreading manure on soils, the main methods of manure management used are anaerobic digestion and composting. However, toxic elements could be concentrated by these process (Hu et al. 2017; Zubair et al. 2020). Therefore, better methods of manure management must be developed which allow for the reduction and recycling of the toxic, macro and micronutrients of this waste.

A novel method for waste management that is gaining more attention due to the possibility of obtaining products of high value from this type of residue is bioconversion using fly larvae (Čičková et al. 2015; Huis 2019). *Hermetia illucens* is the most proposed species for the treatment and valorization of animal manure. Using this species in the process can produce larval biomass and larvae frass that could be used as alternative feed and biofertilizer, respectively (Liu et al. 2019). However, when the fly larvae are fed with manure from livestock activities, the generated larval biomass as feed is not recommended. Since 2017, the processed protein from seven edible insect species was approved by the European Union (EU) to use in aquaculture feeding. However, manure use is not allowed as an insect breeding substrate (European Commission 2017). Recently, the use of processed protein from these insects in poultry and swine feeds was authorized (European Commission 2021). Considering, that globally there are about 45.6 million tonnes per day of manure potentially available for processing (FAO; Chávez-Fuentes et al. 2017), and the potential for growing fly larvae on animal manure to recycle protein and manage organic waste from livestock production (Nordentoft et al. 2017), more research may be needed to confirm the suitability of the use of animal manure (Huis 2019).

Larval biomass can accumulate high levels of some toxic elements (up to 9 fold the cadmium concentration in the substrate) (Tschirner and Simon 2015; Purschke et al. 2017). Charlton et al. (2015) found that fly larvae fed with the swine manure of four different companies contained cadmium concentrations higher than the EU allowed limits for animal feed. Diverse studies agree with this finding and have highlighted the ability of *Hermetia illucens* to accumulate cadmium from spiked substrates (Diener et al. 2015a; Fels-Klerx et al. 2016; Gao et al. 2017; Purschke et al. 2017; Bulak et al. 2018) and substrates without cadmium, too (Tschirner and Simon 2015; Biancarosa et al. 2017; Schmitt et al. 2019). Despite this concern, *Hermetia illucens* can also accumulate essential minerals for animal nutrition, such as Cu, Zn, and Ca (Diener et al. 2015b; Tschirner and Simon 2015; Bulak et al. 2018). Given this, various

studies reveal that larvae meal from *Hermetia illucens* as feed for fish, pig, and poultry is a promising substitute for fish and soy meal (Stadtlander et al. 2017; Xiao et al. 2018; Biasato et al. 2019; Yu et al. 2019).

Similarly, after co-products bioconversion, larvae frass have shown to be an interesting alternative for chemical fertilizer, increasing the development of crops such as tomato and leaf lettuce (Setti et al. 2019). However, when the substrates are contaminated with toxic elements, some elements can become concentrated, and others reduced. For example, *Hermetia illucens* larvae can concentrate Pb in spiked substrates (Diener et al. 2015b; Fels-Klerx et al. 2016). In contrast, Cai et al. (2018) detected decreasing concentrations of Cd, Cr, Cu, Hg, Zn, and Pb from municipal sludge sewage. Miranda et al. (2021) demonstrated that *Hermetia illucens* could reduce animal manure's mineral content depending on the system scale. Accordingly, *Hermetia illucens* larvae could be a potential agent for treating toxic elements in contaminated wastes.

Thus, in the context of the need for manure treatment and valorization, besides the potential of *Hermetia illucens* for feed and biofertilizer production from decayed materials, the aims of this study were: 1) to assess the suitability of *Hermetia illucens* larvae and frass from animal manure bioconversion as animal feed and biofertilizer by taking into consideration its mineral content and 2) to analyze changes in mineral concentrations, from larvae to pre-pupae, on larvae biomass and cattle manure after the bioconversion.

2. Materials And Methods

2.1. Materials

Hermetia illucens larvae and cattle manure collecting

Larvae of *Hermetia illucens* were obtained from eggs of wild flies found on the composting project of the State University of Santa Cruz (Ilhéus-Bahia-Brazil). The cattle manure was collected from an experimental farm from the university mentioned above. The *Hermetia illucens* larvae rearing process during the first six days and cattle manure collecting are described in the recently published paper of Sanchez Matos and colleagues (2021). Table 1 shows the mineral content of chicken feed and cattle manure used for the rearing of *Hermetia illucens* larvae and the experiments, respectively.

Table 1. Mineral content of chicken feed and cattle manure used in experiments for the growth of young larvae (mean ± 95% confidence interval)

Elements	Chicken feed	Cattle manure
Micronutrients		
Cu (g kg ⁻¹)	0.02±0.09	0.04±0.08
Fe (g kg ⁻¹)	1.87±0.41	1.81±0.81
Mn (g kg ⁻¹)	0.04±0.01	0.53±0.04
Zn (g kg ⁻¹)	0.32±0.11	0.32±0.74
Macronutrients		
Ca (g kg ⁻¹)	18.72±8.76	6.06±5.54
K (g kg ⁻¹)	11.45±0.61	2.06±1.39
Mg (g kg ⁻¹)	2.63±0.10	2.98±1.06
Na (g kg ⁻¹)	3.98±0.30	1.25±1.39
P (g kg ⁻¹)	10.79±2.97	3.06±2.35
S (g kg ⁻¹)	2.36±0.11	1.89±0.29
Toxic elements		
Cd (mg kg ⁻¹)	<0.001	0.29±0.48
Cr (mg kg ⁻¹)	2.91±2.61	3.05±1.78
Pb (mg kg ⁻¹)	<0.001	<0.001

2.2. Experimental setup

The cattle manure was homogenized, and one kg of this manure was introduced into a plastic box with four replicates. Subsequently, 1000 6 day-old larvae were spread over manure. For each box containing manure and larvae, a control with the same characteristics without larvae was established. The experiments were carried out until the first prepupae appeared. The experimental conditions and more details of the experimental setup can be found in the previous study of Sanchez Matos et al. (2021).

2.3. Analytical methods

Mineralization and elemental analysis

The larvae and manure were dried at 60°C for 48 hours and ground in a ceramic mortar. Dry samples of 0.3 g of manure and larvae were weighed and digested with 4 mL HNO₃, 0.5 mL HCl and 2.5 mL H₂O₂ into perfluoroalkoxy (PFA) vessels using a multimode microwave apparatus (CEM Mars Xpress). The samples were digested according to the following program: ramp in 2 min to 120 °C, 8 min at 120 °C, 5 min from 120 °C to 180 °C, and 15 min at 180 °C. After digestion, the contents in the PFA vessels were transferred to 50 mL conical centrifuge tubes, then filled to 15 mL with deionized water. For digested samples of larvae, aliquots of these were diluted 20-fold with ultrapure water. These solutions were analyzed by an inductively coupled plasma optical emission spectrometry (ICP OES), model 710-ES Varian (Mulgrave, Australia), with axial configuration. This instrument was equipped with a MEINHARD® concentric nebulizer (Santa Clara, USA), coupled to a cyclonic nebulization chamber - single pass Varian (Mulgrave, Australia), Varian quartz torch (Mulgrave, Australia), and solid-state detector with an arrangement of CCD diodes. Argon 99.998% White Martins / Praxair (Bahia, Brazil) was used to generate the plasma and the nebulization system. Specsol® primary monoelemental standards of 1000 mg L⁻¹ for Fe, Cu, Mn, Cd, Cr, Pb and, Zn; 10000 mg L⁻¹ for Ca, Mg, K, P, S and, Na in 5% v v⁻¹ were employed for secondary standards dilutions used on calibration curve generating. The spectral lines were, in turn, as follows: (nm) for Cu, 327.395; Fe, 238.204; Mn, 257.610; Zn, 213.857; Ca, 373.690; K, 766.491; P, 213.618; S, 181.972; Mg 279.800; Cd, 214.439; Cr, 267.716; Pb, 220.353 and Na 589.592. All glassware was decontaminated in 10% (v v⁻¹) nitric acid for 24 h and rinsed with ultrapure water.

The Bioaccumulation factor (BAF) was calculated on a dried matter basis (Walker 1990; Diener et al. 2015b):

$$BAF = \frac{\text{Concentration in organism } (C_i)}{\text{Concentration in feed } (C_o)} \quad (1)$$

Therefore, a BAF greater than 1 implies bioaccumulation of the element from the substrate into the larvae (Fels-Klerx et al. 2016).

2.4. Data analysis

The data were analyzed using R Studio statistical software (version 3.4.2 R-Studio 2017). The homogeneity of variance and normality of distribution were tested using Levene's test and Shapiro Wilk's test. The statistical significance was determined by Student's t-test (p<0.05). The statistical significance was assessed only for Ca, Mg, Mn, Cd, Cr, and Pb content in larvae, using a non-parametric Wilcoxon test (p<0.05). These statistical tests to assess the significant difference were applied to CM+HI (treatment with *Hermetia illucens* larvae) vs. CM (control treatment) and prepupae (first prepupae) vs. young larvae.

3. Results And Discussion

3.1. Mineral content changes in *Hermetia illucens* larvae frass after manure bioconversion

The bioconversion of organic materials was able to cause changes in the mineral content of the substrates. In this study, as shown in Table 2, Cu, Mn, and Na content were significantly increased, and Ca and K content showed a significant reduction in *Hermetia illucens* frass after cattle manure bioconversion. However, Fe, Mg, P, S, Zn, and the content of the toxic metals showed no marked changes. This finding is similar to the effect of *Musca domestica* larvae only in Cu, Fe, Na, P, and S content of cattle manure reported by Hussein et al. (2017). These differences could be linked to two factors: 1) the difference between the bioaccumulation factors of *Musca domestica* and *Hermetia illucens*, and 2) the higher water and dry matter reduction by *Musca domestica* in the cited study (37%) than for *Hermetia illucens* in this study (16.8%). Considering the potential use of larvae frass as biofertilizers, great attention should be given to high rates of reduction of organic matter of waste and low levels of element uptake, which could lead to an accumulation of undesired elements. For example, Zhu et al. (2015) reported a slight increase of 11% and 9% of initial Cd and Cr concentrations, respectively, and a 2.5 fold increase in the Pb concentration after pig manure bioconversion by *Musca domestica* larvae. The authors also registered a reduction of 74% in water content. On the other hand, Proc et al. (2020a) found a significant increase in the Cd content and a considerable reduction in dry matter (64.7%) of fish feed not spiked by the action of *Hermetia illucens* larvae. Furthermore, in substrates spiked with cadmium and lead solutions, the concentrations of these elements increased by up to 10%; a 3.7 fold increase on the initial Cd and Pb concentration, respectively, after bioconversion by *Hermetia illucens* larvae (Fels-Klerx et al. 2016). This effect could be detrimental if the concentrations of the toxic metals in the substrate are very close to the limit values for a particular use. The *Hermetia illucens* larvae could increase the concentration of these elements, thereby causing these to exceed allowed values. Furthermore, monitoring of toxic elements levels in substrates used as feed for *Hermetia illucens* larvae is recommended.

Table 2

Mineral content in *Hermetia illucens* larvae and residues after cattle manure bioconversion (mean $\pm$ 95% confidence interval)

Elements	Young larvae	Larvae	<i>p</i> -value*	CM (Residue)	CM + HI (frass)	<i>p</i> -value*	Fish meal**	Soybean meal***
Micro and macronutrients								
Ca (g kg ⁻¹)	24.62 $\pm$ 1.93	66.35 $\pm$ 1.62	0.00	7.98 $\pm$ 1.74	4.95 $\pm$ 0.34	0.01	30.3	3
Cu (g kg ⁻¹)	< 0.006	0.02 $\pm$ 0	-	0.04 $\pm$ 0.01	0.05 $\pm$ 0.01	0.04	0.03	0.01
Fe (g kg ⁻¹)	0.75 $\pm$ 0.03	1.06 $\pm$ 0.12	0.00	2.06 $\pm$ 0.98	2.00 $\pm$ 0.38	0.83	0.91	0.15
K (g kg ⁻¹)	17.84 $\pm$ 1.11	18.69 $\pm$ 0.6	0.06	4.78 $\pm$ 0.65	8.05 $\pm$ 1.89	0.01	8.5	21.5
Mg (g kg ⁻¹)	4.57 $\pm$ 0.29	6.65 $\pm$ 0.23	0.00	4.83 $\pm$ 0.82	5.14 $\pm$ 0.83	0.31	3	3.1
Mn (g kg ⁻¹)	0.10 $\pm$ 0.01	2.26 $\pm$ 0.05	0.00	0.69 $\pm$ 0.16	0.34 $\pm$ 0.06	0.01	0.01	0.03
Na (g kg ⁻¹)	2.53 $\pm$ 0.11	2.63 $\pm$ 0.13	0.08	1.88 $\pm$ 0.03	2.3 $\pm$ 0.16	0.01	11.1	0.01
P (g kg ⁻¹)	12.68 $\pm$ 1.03	12.15 $\pm$ 0.39	0.15	3.31 $\pm$ 1.44	3.67 $\pm$ 0.61	0.40	21.6	6.2
S (g kg ⁻¹)	4.35 $\pm$ 0.44	4.88 $\pm$ 0.15	0.02	2.58 $\pm$ 0.61	2.78 $\pm$ 0.23	0.29	4.00 ^a	4.2
Zn (g kg ⁻¹)	0.43 $\pm$ 0.03	0.54 $\pm$ 0.05	0.00	0.41 $\pm$ 0.15	0.50 $\pm$ 0.17	0.17	0.05	0.05
Toxic elements								
Cd (mg kg ⁻¹)	0.02 $\pm$ 0.04	0.34 $\pm$ 0.03	0.00	0.45 $\pm$ 0.04	0.42 $\pm$ 0.05	0.08	-	-
Cr (mg kg ⁻¹)	1.09 $\pm$ 0.13	2.46 $\pm$ 0.11	0.00	2.89 $\pm$ 2.03	5.76 $\pm$ 5.51	0.14	-	-
Pb (mg kg ⁻¹)	< 0.002	0.19 $\pm$ 0.36	-	< 0.002	< 0.002	-	-	-

The mineral content of *Hermetia illucens* larvae frass obtained in this study was compared with European, Canadian, American, and Brazilian maximum limits of contaminants in organic fertilizers to assess its suitability for agricultural use. As shown in Tables 2 and 3, Cu, Zn, Cd, and Pb content met the maximum limits established by cited legislations; however, Cr content only met the Canadian maximum limits. A reason for this finding could be linked to Cr supplementation in cattle feeds and subsequent transfer to excreta. Usually, diverse forms of Cr are used in feed to treat mental, physical, or metabolic stress in cattle. They sometimes can exceed the maximum limits for animal feed by six fold, as reported by Li et al.(2019). Therefore, in this case, it would be recommendable that a subsequent treatment process can be used to improve the suitability of larvae frass, for example, for co-composting with other residual materials that have low Cr content.

Table 3
Maximum limits of mineral content in animal feed and organic fertilizer

Elements	Maximum Limits for feed			Maximum Limits for organic fertilizer			
	EU ¹	USA ²	Canada ³	EU ⁴	USA ⁵	Canada ⁶	Brazil ⁷
Ca (g kg ⁻¹)	-	-	-	-	-	-	-
Cu (g kg ⁻¹)	-	0.25 ^{p, s} ; 0.1 ^f	-	0.3	1.5	0.4	-
Fe (g kg ⁻¹)	-	0.5 ^p ; 3.0 ^s	-	-	-	-	-
K (g kg ⁻¹)	-	10.0 ^a	-	-	-	-	-
Mg (g kg ⁻¹)	-	5.0 ^p ; 2.4 ^s ; 3 ^f	-	-	-	-	-
Mn (g kg ⁻¹)	-	2.0 ^p ; 1.0 ^s	-	-	-	-	-
Na (g kg ⁻¹)	-	-	-	-	-	-	-
P (g kg ⁻¹)	-	10.0 ^a	-	-	-	-	-
S (g kg ⁻¹)	-	4.0 ^a	-	-	-	-	-
Zn (g kg ⁻¹)	-	0.5 ^p ; 1 ^s ; 0.25 ^f	-	0.8	2.8	0.7	-
Cd (mg kg ⁻¹)	2.0	10 ^a	0.4	1.5	39	3	3.0
Cr (mg kg ⁻¹)	-	100 ^s ; 500 ^p	-	2.0	-	210	2.0
Pb (mg kg ⁻¹)	10.0	10.0 ^a	8.0	120.0	300	150	150.0

¹Source: Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. ²Source: National Research Council (2005). Mineral tolerance of animals. ³Source: Canadian Food Inspection Agency (2015). RG-8 Regulatory Guidance: Contaminants in Feed. ⁴Source: Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products. ⁵Source: Code of Federal Regulations Part 503-Standards for the Use or Disposal of Sewage Sludge. ⁶Source: Canadian Council of the Ministers of the Environment (2005). Guidelines for compost quality. ⁷Source: Instrução Normativa SDA N°27 do 05 de Junho de 2006, Anexo V: Limites máximos de contaminantes admitidos em fertilizantes orgânicos e condicionadores de solo. ^a: concentration value for poultry, swine and fish feed. ^f: concentration value for fish feed. ^p: concentration value for poultry feed. ^s: concentration value for swine feed.

3.2. Mineral content changes in *Hermetia illucens* larval biomass

Fly larvae can degrade different types of substrates, assimilating the minerals therein for growth and development. Table 2 shows the mineral concentration of initial larvae (young larvae) and larvae (first prepupae appeared) of *Hermetia illucens*. Some macronutrients (K, Na, and P) did not have marked changes at the end of the bioconversion process. On the other hand, other micro and macronutrients, such as Ca, Cu, Fe, Mg, Mn, S, and Zn, presented significant increases. Proc et al.(2020b) also found increases in Ca, Cu, Fe, Mg, and Mn concentrations; however, K, Na, P, S, and Zn content decreased in larvae at the end of fish feed bioconversion. The reason for the differences between these studies could be linked to the nutritional composition of substrates. In this regard, Tschirner and Simon (2015) revealed that mineral content changes in biomass of *Hermetia illucens* larvae depend on the type of substrate. For example, the authors found that K, Na, P, and Mg content decreased in larvae fed with a mixture of middlings from a feed mill. When larvae were fed with dried distillers' grains with solubles made from barley, corn, wheat, and sugar syrups (protein group), the Cu, Fe, K, Mg, Mn, Na, P, and Zn content decreased. However, in larvae fed with dried sugar beet pulp, only Cu, Fe, P and Cu decreased, and the remaining elements increased.

Among all the evaluated elements, calcium was the most increased in larval biomass at the end of the experiment, with a 2.6 fold increase in the initial concentration. This result is consistent with the increase in the calcium content in *Hermetia illucens* larvae fed with fish feed, up to 2.3 times the initial concentration of young larvae, reported by Proc et al.(2020b), and it is also similar to the results obtained in larvae fed with co-products (dried sugar beet pulp), up to 2.7 times the initial calcium concentration, highlighted by Tschirner and Simon (2015). Previous studies with other fly larvae (*Musca autumnalis*) have revealed that large amounts of Ca are ingested and stored in the Malpighian tubules during the larval stage, to subsequently be used during the pupariation process (Darlington et al. 1983; Grodowitz and Broce 1983). This calcium uptake by fly larvae depends on the concentration of this element in the diet (Dube et al. 2000).

Regarding the toxic elements, the Cd, Cr, and Pb concentrations in larvae were significantly increased (Table 2). The Cd content increase could be linked with the high Ca content in *Hermetia illucens* larvae, inherent to insects. Cd uptake is through Ca^{2+} channels in the intestinal cells (Braeckman et al. 1999; Craig and Hare 1999; Buchwalter and Luoma 2005) and this element is mainly accumulated in the larvae body (47%-93%) and less amount is excreted in the feces (Wu et al. 2020). However, the Pb content increase would be linked with the Pb content in the substrate as related by Tschirner and Simon (2015) and Diener et al.(2015b), both for spiked and not spiked substrates with Pb solutions, respectively.

On the other hand, a comparison of the mineral content in larval biomass was carried out in other studies to identify if the concentrations of minerals depend on the type of substrate (not spiked with mineral solutions) when it is feed co-product or waste. Table S1 (in supplementary material) shows a wide variation in the mineral content of larval biomass with apparently no pattern according to the type of substrate used for rearing. For instance, calcium content obtained in larvae was higher than the values obtained in larvae fed with feeds and co-products, and only similar with larvae reared in dried sugar beet pulp. However, this does not mean that all larvae reared in residues will have more calcium than those

obtained from co-products or animal feed. For example, in the case of larvae fed with restaurant waste (Sprangers et al. 2017), kitchen waste and chicken manure (Shumo et al. 2019) had lower amounts of calcium than those from the other substrates. In this study, the Cu content in larvae was higher than that fed with feeds and in line with larvae fed with chicken manure and kitchen waste (Shumo et al. 2019) but lower than the Cu content in commercial larvae (Irungu et al. 2018). Furthermore, regarding K, Mg, and P content, these were similar to the content of larvae fed with chicken feed (Dierenfeld and King 2008) and Na content was in line with the content in larvae grown in poultry and pig manure (Newton et al. 2005). Zn and Fe content in the present study were higher in larvae fed with feeds or co-products and only lower than larvae reared in chicken manure (Shumo et al. 2019), and the Mn content was higher than all the Mn concentrations in larvae from the cited studies.

Regarding the content of the toxic element, the Cd concentration in larvae fed with cattle manure of this study was lower than all the Cd content in larvae fed with other types of waste and co-products, and only in line with larvae fed with chicken feed (Fels-Klerx et al. 2016). However, Cr content was only lower than the content of this metal in larvae fed with municipal sewage sludge and Pb concentrations were higher than for all the Pb contents in larvae grown in feeds, some co-products, and especially municipal sewage sludge.

The high variations among the mineral contents in larvae fed both feeds, co-products or waste, could be linked to different factors, such as different experimental setups (Bosch et al. 2019a, b), different harvest times (Liu et al. 2017), different fly strains (Zhou et al. 2013) and the different nutritional contents of substrates (Tschirner and Simon 2015).

The micro and macronutrients in larvae were compared with the mineral content of fish and soybean meals, as shown in Table 2. The larvae's Ca, Fe, Mg, Mn, and S contents were higher than in fish and soybean meal. The Cu, Na, and P contents were higher in the fish meal than in the others; however, they were higher in larvae than in the soybean meal. The K content in larvae was lower than in soybean meal but higher than in fish meal. The Zn concentration was similar to that in larvae, fish meal, and soybean meal. Therefore, the mineral content of *Hermetia illucens* larvae is comparable with fish meal and possibly better than soybean meal.

The mineral content in larvae was also compared with the maximum limits for feeds established by different countries. As shown in Tables 2 and 3, the toxic elements in larvae met the EU, USA (United States of America), and Canada concentration limits for Cd, Cr, and Pb. For micro and macronutrients, EU and Canadian legislation has not established maximum limits; therefore, the values of these elements in larvae were compared with the limits established by the USA. The Cu level in larval biomass met the maximum limit for pig, poultry, and fish feed. Furthermore, Fe and Zn content only met the maximum limit for pig feed. However, K, Mg, Mn, P, and S concentrations were slightly higher than the maximum limits for poultry, pig, and fish feed. These results are promising for two reasons: 1) the safe levels of toxic elements and 2) despite the slightly higher mineral levels compared with maximum levels for feed, the

larval biomass could be used partially in feeds by adjusting the mineral content according to the animal's nutritional requirements.

3.3. Bioaccumulation of toxic elements, micro and macronutrients in *Hermetia illucens* larvae

The bioaccumulation factors (BAF) of micro and macronutrients and toxic elements in larval biomass increased from young to adult larvae (Fig. 1). The BAF of elements in larvae that were higher than 1 showed the following trend in this study: Ca > K > Mn > P > S > Mg > Na > Zn > Cd.

These results were compared with the BAF of different elements in *Hermetia illucens* larvae fed with artificially uncontaminated substrates of previous studies (Tschirner and Simon 2015; Biancarosa et al. 2017; Schmitt et al. 2019; Proc et al. 2020a). As was previously observed in the larvae concentrations, there is a wide variation range in the BAF of elements of different studies; however, the BAFs of Ca, Mg, Mn, and Cd in all studies were > 1, thereby revealing bioaccumulation. Ca, Mg, and Mn are essential elements for insects' metabolic processing and development (Clark 1958; Ben-Shahar 2018); however, Cd is considered a toxic element and, as mentioned, is linked with the calcium content in fly larvae. For instance, in *Musca domestica* larvae that generally contain tenfold less calcium than *Hermetia illucens* larvae (Gold et al. 2018), the BAF of cadmium is < 1 (Wang et al. 2017; Negi et al. 2020), on the other hand, in *Hermetia illucens* larvae, this factor often exceeds the value of 1. This Cd and Ca relationship is also in line with the BAF obtained when *Hermetia illucens* larvae were fed with substrates spiked with cadmium solutions at different concentrations (Diener et al. 2015b; Fels-Klerx et al. 2016; Purschke et al. 2017; Bulak et al. 2018; Wang et al. 2021). Consequently, great care should be taken regarding the cadmium content in substrates used as feed for *Hermetia illucens* larvae since this can accumulate up to almost tenfold the cadmium concentration of substrate in larval biomass (Tschirner and Simon 2015).

4. Conclusion

In this study, the cattle manure bioconversion by *Hermetia illucens* larvae was studied by obtaining larval biomass and frass. The Ca, Cu, Fe, Mg, Mn, S, and Zn content in larvae increased through bioconversion. The biomass larval showed promise for partial use as animal feed in countries where waste-fed insects would be allowed due to the high micro and macronutrients content and safe toxic metal levels. However, more studies are needed to assess the microbiological and chemical (antibiotics, veterinary medicines, and allergens) hazards of using animal manure as growing media for *Hermetia illucens* larvae. Furthermore, considering the mineral content, *Hermetia illucens* larvae frass was only suitable as organic fertilizer in a Canadian context due to its Cr concentration. So further treatment, such as co-composting with low chromium residual materials, would be necessary and more controls in chromium supplementation for livestock to improve the suitability of larvae frass obtained from the bioconversion of manure.

Declarations

Availability of data and materials

The datasets supporting the conclusions of this manuscript are included within the article and the supplementary information.

Code Availability

Not applicable.

Authors' Contributions

Joan Sanchez-Matos, Jose Adolfo de Almeida Neto and Ivon Pinheiro Lôbo contributed to the study conception and design. Material preparation, data collection and analysis were performed by Joan Sanchez-Matos, Lara Pinto de Araujo, Vinnícius Henrique Cerqueira da Silva and Raildo Mota de Jesus. All authors contributed equally to the interpretation of the results and the writing of the manuscript.

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Declarations Ethics Approval

Not applicable.

Consent to Participate

Not applicable.

Consent for Publication (Include Appropriate Statements)

Not applicable.

Conflicts of Interest/Competing Interests

The authors declare no conflict of interest.

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Figures

Figure 1

Bioaccumulation Factors (BAF) of different elements for *Hermetia illucens* larvae fed with cattle manure

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