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Research article

Upcycling of recycled minerals from sewage sludge through black soldier fly larvae (*Hermetia illucens*): Impact on growth and mineral accumulation

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ABSTRACT

Phosphorous (P) resources are finite. Sewage sludge recyclates (SSR) are not only of interest as plant fertilizer but also as potential source of minerals in animal nutrition. However, besides P and calcium (Ca), SSR contain heavy metals. Under EU legislation, the use of SSR derivatives in animal feed is not permitted, but given the need to improve nutrient recycling, it could be an environmentally sound future mineral source. Black soldier fly larvae (BSFL) convert low-grade biomass into valuable proteins and lipids, and accumulate minerals in their body. It was hypothesized that BSFL modify and increase their mineral content in response to feeding on SSR containing substrates. The objective was to evaluate the upcycling of minerals from SSR into agri-food nutrient cycles through BSFL. Growth, nutrient and mineral composition were compared in BSFL reared either on a modified Gainesville fly diet (FD) or on FD supplemented with either 4% of biochar (FD + BCH) or 3.6% of single-superphosphate (FD + SSP) recyclate (n = 6 BSFL rearing units/group). Larval mass, mineral and nutrient concentrations and yields were determined, and the bioaccumulation factor (BAF) was calculated. The FD + SSP substrate decreased specific growth rate and crude fat of BSFL ($P < 0.05$) compared to FD. The FD + SSP larvae had higher Ca and P contents and yields but the BAF for Ca was lowest. The FD + BCH larvae increased Ca, iron, cadmium and lead contents compared to FD. Larvae produced on FD + SSP showed lower lead and higher arsenic concentration than on FD + BCH. Frass of FD + BCH had higher heavy metal concentration than FD + SSP and FD ($P < 0.05$). Except for cadmium and manganese, the larval heavy metal concentration was below the legally permitted upper concentrations for feed. In conclusion, the SSR used could enrich BSFL with Ca and P but at the expense of growth. Due to the accumulation of Cd and Mn, BSFL or products thereof can only be a component of farmed animal feed whereas in BSFL frass heavy metal concentrations remained below the upper limit authorized by EU.

1. Introduction

The major source of phosphorous (P) is rock phosphate, which is a finite resource. Thus, excessive use of P in agri-food systems must be avoided (Van der Kooij et al., 2020). In addition, oversupply of P in animal feed and fertilizers, and P accumulation in the environment increase the risk of P leaching and leads to P losses from the agri-food systems, contributing to environmental pollution (Siddique and Robinson, 2003; Van der Kooij et al., 2020). Thus, P recycling is urgently needed, and part of the solution could be P recovery from sewage sludge as it is a promising source of P (Egle et al., 2016).

Besides its use in plant fertilizers, P is an essential mineral in animal diets (Algren et al., 2022), and the implementation of recycled P in animal feed could reintegrate it into the production cycle. However, due to safety considerations, it is currently prohibited to use waste streams (e.g. sewage sludge recyclates (SSR)) in animal feed (European Commission, 2009). Furthermore, sewage sludge contains heavy metals and organic contaminants, but some of the P recovery technologies extract P from the sewage sludge ash produced by incineration, such as e.g. the Ash2Phos process, which reaches a P recovery of ~90% (Cohen et al.,

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2019), and achieve significant reductions in pollutants and heavy metals in the recyclate products (Egle et al., 2016; Van der Kooij et al., 2020). Recently, carbonization products, known as Biochar (BCH), derived from the thermal processing of sewage sludge has received attention as an efficient P-recovery option (Buss et al., 2022). It was suggested that BCH application in soil mitigates emission of ammonia and nitrite oxide from soil, and improve N use efficiency and crop productivity (Dawar et al., 2021). Besides P, BCH contains other minerals essential to animals, among them Ca, iron (Fe), potassium (K) and magnesium (Mg) (Zhao et al., 2018).

In recent years, there has been increasing interest in black soldier fly (*Hermetia illucens*) larvae (BSFL) as a sustainable protein source in animal feed to replace in part soybean meal and particularly fishmeal, which may contribute to meet the increasing demand for food and feed of a growing global population (Hunter et al., 2017; Raman et al., 2022; United Nations, 2019). It is known that the nutrient content of BSFL can be modified by the nutrient content of their feeding substrates (Diener et al., 2015; Van der Fels-Klerx et al., 2016). Furthermore, concentrations of minerals in the BSFL reflect the concentrations in their feeding substrates (Liland et al., 2017). Many elements can accumulate in BSFL, with Ca, P, and K being the most abundant macro-minerals in the BSFL (Chia et al., 2020; Schmitt et al., 2019). Concurrently, accumulation of certain heavy metals such as cadmium (Cd) in BSFL has been reported, which must be considered from a safety perspective (Proc et al., 2020). In general, the uptake of undesirable substances in animals depends on their concentrations in feed, the duration of exposure, diet composition, and the nutritional and ontogenetic status of the animal. Thus, it is necessary to assess the risk of potential accumulation of hazardous substances in BSFL which ultimately contributes to further development of legislation for safe feeds (Lievens et al., 2021).

Therefore, it was hypothesized that: 1) BSFL modify their mineral content in response to the mineral and heavy metal composition in SSR; and 2) SSR added to BSFL feeding substrates lead to mineral enriched larvae suitable for the implementation in animal feed. Therefore, the objective of this study was to evaluate upcycling of minerals from SSR into agri-food nutrient cycles through BSFL. The results presented in this paper provide novel information on a new sustainable approach to reintegrating recycled P and other minerals from sewage sludge into the agri-food nutrient cycle to reduce P release to the environment.

2. Material and methods

2.1. Animals, sewage sludge recyclates and feeding substrate

A BSFL stock was obtained from Hermetia Baruth GmbH (Baruth, Germany) in the year 2019. Black soldier fly eggs were collected from the 12th and 13th generations of the established colony in the insect facility of the Research Institute for Farm Animal Biology (FBN) in Dummerstorf, Germany. Adult flies were kept in an indoor cage at 27.5 °C and 70% relative humidity with 12 h of artificial light per day (d) from a LED source (daylight spectrum). The flies were provided with 3% (w:v) sugar solution and water. Eggs were harvested daily from the oviposition site made of perforated plastic balls (Bioball, Berlan GmbH, Klingenthal, Germany) and placed above a 200 mL box filled with dead adult flies from the previous generation surrounded by boxes with fermenting chicken starter feed (Trede & von Pein GmbH, Itzehoe, Germany) to stimulate egg laying (Dortmans et al., 2017). Larvae hatched from the egg-laying balls in new hatching boxes with closed lids. Three days after placement of the bioballs in the hatching box, most larvae hatched and were transferred to an open plastic container (30 × 20 × 15 cm) provided with 300 g of chicken starter feed (30% feed: 70% water) on which the larvae were reared up to the fifth d post-hatch.

Two different sources of mineral rich SSR, namely BCH and Single Superphosphate (SSP) were used. The BCH recyclate was derived from pyrolysis of sewage sludge produced by the PYREG-process (PYREG GmbH, Dörth, Germany) as described (Fesharaki and Rath, 2018). The SSP originated from the recycling of sewage sludge produced by incineration using the Ash2Phos process developed by EasyMining Sweden AB (Cohen et al., 2019). The SSP was enriched in P and Ca but had lower heavy metal contents compared to the carbon-rich BCH (Table S1 in the supplementary data). The Ash2Phos process used the residue ash from the incineration of the sewage sludge, while in the PYREG process, the drained and dried sewage sludge was carbonised at around 500–700 °C under oxygen exclusion (Cohen et al., 2019).

A standard fly diet (Hogsette, 1992) with reduced P content was formulated using corn meal, wheat bran, lucerne, and sugar beet pulp (i. e. modified Gainesville fly diet; FD; Table S1 in the supplementary data). The dietary components were analysed by the accredited laboratory LUFA (Landwirtschaftliche Untersuchungs-und Forschungsanstalt der LMS Agrarberatung GmbH), Rostock, Germany. The FD diet was supplemented either with 4% of BCH (FD + BCH) or 3.6% of SSP (FD + SSP) normalized for dry matter (DM) at the expense of wheat bran and corn meal (Table S1 in the supplementary data). Final feeding substrates were prepared with addition of water to obtain a 30% feed: 70% water ratio.

2.2. Experimental setup

On the 5th day after hatching, the larvae were sieved, and weighed, and a larval mass of approximately 8000 individuals was placed in each of 18 pre-weighed containers (40 × 60 × 22 cm). The containers seeded with 5-day-old larvae were allocated to one of the three experimental feeding substrates in each of the two runs (i.e. N = 18 containers/run; total N = 36) (Fig. 1). Six replicate containers were used per each of the feeding substrates per experimental run. The larvae were batch-fed on d 5 (2500 g), 9 (4000 g) and 13 (3500 g) after hatching. The position of each container within the racks was randomized (Mersenne Twister algorithm (Matsumoto and Nishimura, 1998)) and the position within each rack was changed daily. Harvesting took place when approximately 10% of the larvae became prepupae, at which point the feeding experiment was terminated. After an experimental feeding period of 13–15 d, harvesting of larvae took place 18–20 d after hatching. For larvae and frass (i.e. mixture of insect feces, cuticles, parts of dead insects and feeding substrate residuals) sampling, we selected randomly 18 containers from a total of 36 containers (i.e. n = 6 containers per substrate).

At the end of the experiment, larvae were separated from the feed residue and all of the containers were weighed again to calculate the total amount of frass. The isolated larvae were washed and then dried with paper towels. The total fresh larval mass (LM) was measured for each container. In addition, the average LM (mg per larvae) per each container was calculated by counting and weighing of at least 100 harvested larvae. The larvae were then devitalized by being transferred into liquid nitrogen (N_2); the frozen larvae of each container were mixed, and representative larvae collected in a 200 mL vessel were used for the analysis of heavy metal, mineral and nutrient concentrations. The heavy metal classification was based on atomic weights ranging between 63.5 and 200.6 (Srivastava and Majumder, 2008). Samples were collected from the freshly sieved frass of each container (n = 6 containers per feeding substrate) into 1-L buckets for heavy metal, mineral and nutrient analysis. All samples were stored at −20 °C for further analyses.

2.3. Proximate composition

The contents of Ca, P, Mg, K, sodium (Na) (method of Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten (VDLUFA) III 10.8.2), Mn, Fe, zinc (Zn), copper (Cu), arsenic (As), Cd, lead (Pb) (method VDLUFA III 17.9.1) and mercury (Hg) (VDLUFA III 17.4.3) in the feeding substrate, larvae and the frass were analysed by the accredited feed laboratory LUFA Rostock (Naumann et al., 1997). Additionally, an extended Weende analysis (Van Soest et al., 1991) was performed in frass and the feeding substrates by LUFA Rostock to determine DM, crude ash, crude protein (CP), crude fat,

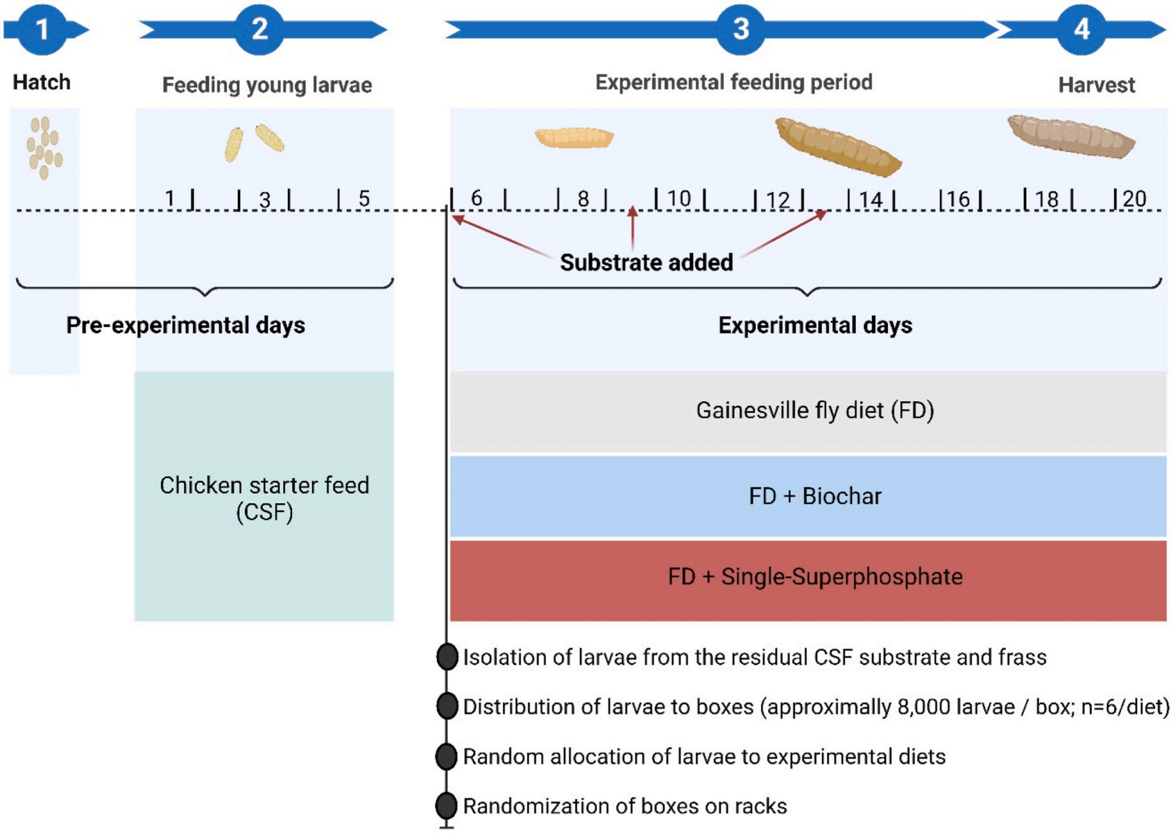

Fig. 1. Schematic diagram representing the pre-experimental and experimental days with the corresponding feeding substrates used after hatch until harvest.

starch, metabolizable energy (ME), aNDFom (neutral detergent fibre after amylase treatment based on organic matter) and ADFom (acid detergent fibre) (Naumann et al., 1997). Gross energy (GE) of the substrates was calculated by multiplying metabolizable energy (ME) with the conversion factor of 1.379 (NRC, 1994). For the larvae, a Weende analysis was performed at the Institute of Animal Nutrition of the Federal Research Institute of Animal Health (Friedrich-Loeffler-Institute) in Braunschweig, Germany. The DM of larvae and frass was analysed using a moisture analyser (Satorius, MA37; Göttingen, Germany). Frozen larvae and frass samples were weighed (2.5 g) and heated gradually to 130 °C until a constant mass was reached. Minerals, nutrients and heavy metal contents of the substrates, larvae and frass were expressed as concentrations (g/kg DM), and absolute amounts (mg or g) per each container. Yield per container was then calculated as the nutrient or mineral concentration in larvae multiplied by the total larval mass on DM basis (i.e. mg or g/container). The N and C contents of dried larvae and substrates were measured as previously described (Seyedalmoosavi et al., 2022a). Content of CP was calculated by N content × 6.25.

2.4. Calculations

Substrate conversion ratio (SCR) (i.e. g substrate provided per g LM gain by BSFL), protein conversion ratio (PCR = g protein supply to gain 100 g LM) and GE conversion ratio (GECR = ME (Mega Joule (MJ)) supply to gain 100 g LM) of the larvae were calculated for each rearing container based on fresh matter. The LM gain was calculated as the difference between the final LM at harvest and the initial LM at start of the experiment. Accounting for developmental time, the specific growth rate (SGR) was calculated (Lugert et al., 2016).

The bioaccumulation factor (BAF) of minerals and heavy metals was calculated according to Eq. (1):

$$BAF = \frac{MClarvae\ at\ harvest}{MCsubstrate} \tag{1}$$

where, MC_{larvae} is the mineral concentration [g/kg DM] in larvae at harvest and $MC_{substrate}$ is the mineral concentration in the substrates fed [g/kg DM]. The BAF is the ratio between the mineral/heavy metal concentration in BSFL and the concentration in the substrate (Walker, 1990); a BAF above 1 indicates mineral enrichment in the larvae, whereas values below 1 imply depletion.

In addition, retention (%) of minerals and heavy metals per container is the total mass [g] of minerals or heavy metals in larvae divided by the total mass [g] of minerals or heavy metals in the feeding substrate and was calculated according to Eq. (2):

$$\text{Retention (\%)} = \frac{Total\ mass\ of\ mineral\ in\ larvae\ at\ harvest}{Total\ mass\ of\ mineral\ in\ substrate} \times 100 \tag{2}$$

2.5. Statistical analysis

The experimental unit was the rearing container (n = 6 replicate rearing containers per treatment group; N = 18). Data were analysed by analysis of variance using the general linear model (PROC GLM) of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). The statistical model included the fixed effects of substrate treatments (FD, FD + BCH, FD + SSP) and the blocking effect of the runs (1 and 2). Since the interaction treatment × block was not significant, it was omitted from the model. Treatment group differences were separated by Tukey-Kramer test at $P < 0.05$. The significance level was $P < 0.05$, and a tendency was declared at $0.05 < P \leq 0.10$. All data are presented as LSMeans and their SE. In order to investigate overall changes in mineral and heavy metal concentrations of BSFL due to differences in feeding substrates, a linear discriminant analysis was performed using JMP 15 (SAS Institute Inc.). For this purpose, minerals (Ca, P, Mg, Na, K) or heavy metal (Mn, Fe, Zn, Cu, As, Cd and Pb) concentrations of BSFL were used as covariates and the feeding substrate as the categorical variable in two discriminant analyses separately. Measurement units of all the covariates were normalized to mg/kg DM.

3. Results and discussion

3.1. Growth, nutrient utilization efficiencies and body composition

The LM did not differ among the substrate groups, however the LM of FD + SSP larvae tended to be lower than in larvae grown on FD substrate (Table 1; $P = 0.066$). The LM observed in the FD group was of the same magnitude as reported by Tomberlin et al. (2002), who used Gainesville diet to feed BSFL (average LM; 104 mg). Broeckx et al. (2021) even reported lower LM in BSFL fed Gainesville diet (83.8 mg). In contrast, Miranda et al. (2020) observed a higher LM (173 mg) for BSFL fed Gainesville diet. Because in these studies a similar substrate composition for feeding the larvae was used, the differences in LM across the studies might be largely related to different rearing conditions such as larval density, humidity and temperature, as well as BSFL genetic variation (Barragan-Fonseca et al., 2018; Chia et al., 2018; Sandrock et al., 2022). The SGR in the FD + SSP larvae was lower compared with those in FD and FD + BCH (Table 1; $P < 0.05$), which was due to a longer development time of FD + SSP larvae compared to FD and FD + BCH larvae (20 d vs. 18 d, respectively). The SCR, PCR, and GECR values did not differ among the groups (Table 1; $P > 0.10$), and the SCR ranged between 3.38 and 3.64 which is similar to that reported by Broeckx et al. (2021) for larvae fed Gainesville diet (SCR = 3.43).

The FD + SSP larvae had a lower C and N content and a lower C/N ratio than those larvae fed on FD and FD + BCH (Table 1; $P < 0.05$). This could be explained by the fact that in FD + SSP larvae the C content was 3.7% lower while the N content was only 0.3% lower compared to the FD fed larvae. In a review, Makkar et al. (2014) reported the chemical composition and mineral content of BSFL reared on different substrates and showed that depending on the lipid and energy content of substrates, the lipid content of BSFL varies between 11% and 58% of DM. In the current study, the lipid content of FD larvae was similar to that reported in previous studies (17–28% DM), where Gainesville diet was fed to BSFL (Arabzadeh et al., 2022; Arnone et al., 2022; Pliantiangtam et al., 2021). Although energy and crude fat content of the substrates in the present study were approximately similar (Table S1 in the supplementary data), the FD + SSP larvae had a lower crude fat content (Table 2; $P < 0.05$) and yield (Table 3; $P < 0.05$) (by 33% and 27%, respectively) than FD + BCH and FD larvae, presumably reflecting the lower C content of FD + SSP larvae (Table 1). The lower crude fat content in FD + SSP larvae might be linked to a higher accumulation of heavy metals such as Fe, As, Cd, and Pb than in FD and FD + BCH larvae (Figs. 4 and 5). Schmitt et al. (2019) discussed that a decreased fat content in BSFL might be related to high accumulation of heavy metals such as Cd, Cu and Zn in the larvae. Also a high concentration of Cd

Table 1

Growth, efficiency indicators and carbon/nitrogen ratio in black soldier fly (*Hermetia illucens*) larvae fed on a fly diet (FD) supplemented with or without two different sewage sludge recyclates (FD + BCH, FD + SSP).

	Feeding substrates[a]				P-values[b], ≤	
	FD	FD + BCH	FD + SSP	SE	T	B
LM, mg	$103.6^{\dagger}$	99.5	$89.7^{\dagger}$	4.0	0.066	0.044
LDM/container, g	254.5	250.0	230.9	9.6	0.207	0.595
SGR, %	50.22^{a}	50.02^{a}	45.32^{b}	1.24	0.021	0.908
SCR	3.38	3.58	3.64	0.16	0.514	0.849
PCR	44.33	45.14	45.88	2.07	0.868	0.835
GECR	3.14	3.11	3.16	0.14	0.968	0.826
C, %	49.12^{a}	48.34^{a}	45.45^{b}	0.39	0.001	0.001
N, %	7.37^{a}	$7.28^{ab\dagger}$	$7.07^{b\dagger}$	0.06	0.013	0.003
C/N	6.66^{a}	6.64^{a}	6.43^{b}	0.05	0.004	0.837

a-c: Values in a row that are marked with different letters differ significantly (Tukey, $P < 0.05$). The symbol † in a row indicates a tendency of two treatments to differ (Tukey, $0.05 < P \leq 0.10$).

Abbreviations: LM: larval mass; LDM/container: larval dry mass per container; SGR: specific growth rate; SCR: substrate conversion ratio; PCR: protein conversion ratio; GECR: gross energy conversion ratio; C: carbon; N: nitrogen.

SGR = (ln (harvest weight in g) – ln (initial weight at d5 in g))/fattening period (d) × 100 (Lugert et al., 2016).

SCR = Substrate supply (g)/body weight gain (g).

PCR = Crude protein supply (g)/100 g body weight gain.

GECR = Gross energy supply (MJ GE)/100 g body weight gain.

[a] **FD**: modified Gainesville fly diet; **FD + BCH**: Pyrolysis product of sewage sludge (Biochar; BCH) added to FD (4%); **FD + SSP**: Single Superphosphate (SSP) added to FD (3.6%). Total number of observations used for statistical analyses, N = 18 (i.e., n = 6 replicate rearing containers per treatment group over 2 experimental runs).

[b] **T**: treatment effect; **B** = block effect (experimental run (2 runs) considered as block).

Table 2

Nutrient, macro-mineral and heavy metal concentrations and bioaccumulation factor (BAF) of macro-minerals in black soldier fly (*Hermetia illucens)* larvae fed on a fly diet (FD) supplemented with or without two different sewage sludge recyclates (FD + BCH, FD + SSP).

	Feeding substrates[a]				P-values[b], ≤	
	FD	FD + BCH	FD + SSP	SE	T	B
Dry matter, g/kg FM	285.7	292.6	278.5	0.66	0.339	0.095
Nutrients, g/kg DM						
Crude protein[c]	$469.9^{\dagger}$	459.1	$455.5^{\dagger}$	4.0	0.057	0.141
Crude fat	224.8^{a}	215.7^{a}	172.5^{b}	7.0	0.001	0.007
Crude fibre	65.7	68.1	74.0	3.8	0.300	0.126
aNDF	119.8	128.4	135.1	7.7	0.385	0.702
ADF	86.5	90.1	95.5	5.1	0.471	0.629
Crude ash	147.3^{c}	165.7^{b}	192.1^{a}	1.8	0.001	0.001
Macrominerals, g/kg DM						
Ca	45.77^{c}	53.24^{b}	64.59^{a}	1.22	0.001	0.022
P	7.14^{b}	7.24^{b}	10.89^{a}	0.39	0.001	0.035
Mg	3.31^{b}	3.63^{ab}	3.96^{a}	0.12	0.005	0.015
Na	0.94^{b}	0.94^{b}	1.27^{a}	0.03	0.001	0.212
K	10.89^{b}	10.01^{b}	14.46^{a}	0.74	0.005	0.071
Heavy metals, mg/kg DM						
Mn	285	304	303	7.46	0.167	0.108
Fe	150^{b}	557^{a}	339^{ab}	74.4	0.006	0.248
Zn	$95.8^{\dagger}$	$107.4^{\dagger}$	98.5	3.6	0.087	0.047
Cu	$9.92^{\dagger}$	$12.22^{\dagger}$	10.14	0.67	0.052	0.085
As	0.047^{b}	0.066^{b}	0.115^{a}	0.006	0.001	0.340
Cd	0.63^{b}	0.77^{a}	0.74^{ab}	0.037	0.045	0.147
Pb	0.23^{c}	1.16^{a}	0.54^{b}	0.050	0.001	0.201
Hg	0.000	0.000	0.001	–	–	–
BAF[d]						
Ca	$5.70^{a\dagger}$	$5.31^{a\dagger}$	3.81^{b}	0.11	0.001	0.010
P	1.48^{a}	1.03^{b}	1.32^{ab}	0.06	0.001	0.067
Mg	1.26^{b}	1.31^{b}	1.54^{a}	0.04	0.001	0.015
Na	1.99^{a}	1.70^{b}	1.16^{c}	0.06	0.001	0.409
K	0.730^{b}	0.754^{b}	0.995^{a}	0.050	0.001	0.135

a-c: Values in a row that are marked with different letters differ significantly (Tukey, $P < 0.05$). The symbol † in a row indicates a tendency of two treatments to differ (Tukey, $0.05 < P \leq 0.10$).

Abbreviations: **DM**: Dry matter; a**NDF**: Neutral detergent fibre after amylase treatment; **ADF**: Acid detergent fibre.

[a] **FD:** modified Gainesville fly diet**; FD + BCH:** Pyrolysis product of sewage sludge (Biochar; BCH) added to FD (4%); **FD + SSP:** Single Superphosphate (SSP) added to FD (3.6%). Total number of observations used for statistical analyses, N = 18 (i.e., n = 6 replicate rearing containers per treatment group within 2 experimental runs).

[b] **T**: treatment effect; **B** = block effect (experimental run (2 runs) considered as block).

[c] Calculated as N × 6.25.

[d] BAF values > 1 refer to an accumulation of minerals in BSFL and values < 1 refer to a relative depletion of minerals in BSFL during the experimental period.

Table 3
Nutrient, macro-mineral and heavy metal yield (per container) in black soldier fly (*Hermetia illucens*) larvae fed on a fly diet supplemented with or without two different sewage sludge recyclates (BCH, SSP).

	Feeding substrates[a]				P-values[b], ≤	
	FD	FD + BCH	FD + SSP	SE	T	B
Nutrients, g						
Crude protein[c]	120	115	105	4.67	0.117	0.419
Crude fat	57.35^{a}	53.93^{a}	39.92^{b}	2.75	0.001	0.032
Crude fibre	16.69	16.98	16.97	0.93	0.969	0.287
aNDF	30.38	32.14	31.00	1.87	0.795	0.963
ADF	21.98	22.50	21.93	1.30	0.941	0.992
Crude ash	37.42^{b}	41.40^{ab}	44.34^{a}	1.65	0.031	0.585
Macrominerals, g						
Ca	11.63^{b}	13.32^{ab}	14.93^{a}	0.68	0.013	0.441
P	1.81^{b}	1.80^{b}	2.52^{a}	0.12	<0.001	0.158
Mg	0.84	0.91	0.91	0.04	0.436	0.177
Na	0.24^{b}	0.23^{b}	0.29^{a}	0.01	0.007	0.717
K	2.76	2.74	3.34	0.21	0.107	0.173
Heavy metals, µg						
Mn	72,459	75,721	69,970	3,446	0.501	0.637
Fe	36,968^{b}	137,745^{a†}	78,472^{ab†}	18,451	0.006	0.257
Zn	24,356	26,759	22,759	1,283	0.113	0.278
Cu	2525	3,043†	2,346†	202	0.066	0.231
As	12.0^{b†}	16.3^{b†}	26.4^{a}	1.35	<0.001	0.474
Cd	161	192	170	11.23	0.154	0.391
Pb	58.7^{c}	288.6^{a}	123.6^{b}	15.4	<0.001	0.318
Hg	0.10	0.04	0.19	0.14	0.744	0.172

a-c: Values in a row that are marked with different letters differ significantly (Tukey, $P < 0.05$). The symbol † in a row indicates a trend for a difference between two treatments (Tukey, $0.05 < P \leq 0.10$).

Abbreviations: **DM**: Dry matter; a**NDF**: Neutral detergent fibre after amylase treatment; **ADF**: Acid detergent fibre.

[a] **FD:** modified Gainesville fly diet**; FD + BCH:** Pyrolysis product of sewage sludge (Biochar; BCH) added to FD (4%); **FD + SSP:** Single Superphosphate (SSP) added to FD (3.6%). Total number of observations used for statistical analyses, N = 18 (i.e., n = 6 replicate rearing containers per treatment group within 2 experimental runs).

[b] **T**: treatment effect; **B** = block effect (experimental run (2 runs) considered as block).

[c] Calculated as N × 6.25.

(20–40 mg) in the diet of *Galleria mellonella* larvae reduced lipid content in the larval body (Emre et al., 2013). The authors suggested that due to the high heavy metal content, a large proportion of larval lipids could be metabolized to provide the required energy under the stress caused by heavy metals (Emre et al., 2013). In addition, the lower intake of heavy metal-contaminated substrates by *Folsomia candida* larvae may indicate an attempt by the larvae to counteract an oversupply of heavy metals, resulting in lower energy intake and thus lower body fat (Fountain and Hopkin, 2001).

The CP content of the BSFL ranged between 46 and 47% of DM in the larval body with no difference among the three groups (Table 2). However, FD + SSP fed BSFL tended to have a lower CP content than BSFL fed on FD substrate (Table 2; $P = 0.055$). In previous studies, larval CP values ranging from 38 to 51% DM were found in BSFL fed Gainesville diet (Arabzadeh et al., 2022; Arnone et al., 2022; Pliantiangtam et al., 2021), which was the same order of magnitude as the values found in our study.

There was no difference among the groups for BSFL crude fibre, aNDFom, and ADFom concentrations or for their yields (Tables 2 and 3; $P > 0.10$). In plant material, aNDFom is composed of cellulose, lignin, and hemicellulose while ADFom is composed of cellulose and lignin (Van Soest and Robertson, 1977). However, the components of ADFom and aNDFom in insects are not fully known. Fibre content in the body of edible insects such as BSFL is mainly composed of chitin (linear polymer of b-(1–4) N-acetyl-D-glucosamine units) (Soetemans et al., 2020). Besides chitin, ADF could contain also amino acids probably representing cuticular proteins (Finke, 2007).

Larvae fed substrates containing either SSR had a higher crude ash content than larvae grown on FD substrate (Table 2; $P < 0.05$). The crude ash concentration of FD + SSP larvae was even higher than that of FD + BCH larvae, whereas the crude ash yield was higher in the FD + SSP larvae than in the FD control (Tables 2 and 3; $P < 0.05$). Depending on the substrate, the larvae in the present study contained 14–19% DM of crude ash which was similar to that reported earlier (Pliantiangtam et al., 2021) for BSFL fed Gainesville diet (~15% DM) or various other diets (9–28% DM) as reviewed by Makkar et al. (2014).

Overall, the inclusion of BCH and SSP recyclates in the BSFL feeding substrates was associated with differences in larval body nutrient contents in terms of CP, fat and crude ash, but did not affect larval feed, protein and nutrient utilization efficiencies.

3.2. *Macro-mineral contents, bioaccumulation, yield and retention*

Recycled minerals in both SSP and BCH may be reintegrated into the nutrient cycle through the use as feed components for BSFL, implying a potential upcycling of valuable minerals. The SSP recyclate contained higher Ca, P and Na levels, but was lower in Mg and K concentration than BCH (Table S1 in the supplementary data). In order to evaluate the efficiency of mineral incorporation in BSFL, concentration, yield, BAF, and retention were calculated as the most relevant parameters (Daş et al., 2023) for each mineral and heavy metal. The BAF is an estimate of the efficiency of larvae to accumulate certain minerals from substrate per body mass unit (i.e. on concentration basis) without considering larval growth (Walker, 1990). Yield and retention represent the efficiency of total substrate minerals accumulated in total larval body mass in absolute (i.e. g) or relative (%) terms, respectively. Yield and retention can reflect element enrichment in the larvae not only due to concentration changes in the larvae as compared to the feeding substrate, but additionally due to larval growth (i.e. total larval mass).

According to Makkar et al. (2014) the Ca content of BSFL ranges between 50.0 and 86.3 g/kg DM which is similar to what was observed in our study (46–64 g/kg DM). Likely, as a consequence of the mineralized exoskeleton in BSFL which is high in Ca (Finke, 2013), Ca BAF values were greater than 1 in all groups, indicating a Ca enrichment in the larvae relative to the Ca content in the substrate (Table 2). The FD + SSP and FD + BCH larvae had a higher Ca concentration than those in the FD group (Table 2; $P < 0.05$), whereas among the SSR-fed groups, the FD + SSP larvae had the highest Ca concentration ($P < 0.05$). This reflects the highest Ca concentration among the three feeding substrates, followed by FD + BCH and FD substrates (Table S1 in the supplementary data). Nonetheless, FD + SSP larvae had a lower Ca BAF than those of the FD + BCH and FD groups (Table 2; $P < 0.05$), which may suggest a less efficient utilization of Ca by BSFL in response to the higher Ca availability in the FD + SSP substrate. In line with the lower Ca BAF, FD + SSP larvae had a lower Ca retention than those in the FD + BCH and FD groups (Table 4; $P < 0.05$). Nevertheless total Ca yield per container was higher in FD + SSP than in FD larvae (Table 3; $P < 0.05$), because of the higher Ca concentration in FD + SSP larvae, even though larval size tended to be higher in FD larvae.

The P concentration in larvae ranged between 7 and 11 g/kg DM, which was similar to previous reports summarized by Makkar et al. (2014) (6–15 g/kg DM). The P concentration was higher in FD + SSP larvae than that in FD + BCH and FD larvae (Table 2; $P < 0.05$), which was linked to a higher P concentration in the FD + SSP substrate than in FD + BCH and FD (Table S1 in the supplementary data). Phosphorus accumulated in all groups (BAF >1), however, the FD + BCH larvae had a lower P accumulation than those in the FD group (Table 2; $P < 0.05$), suggesting a lower P accumulation efficiency from the substrate with BCH recyclate. The FD + SSP larvae had a higher P yield than those larvae fed on FD + BCH and FD (Table 3; $P < 0.05$). Nevertheless, both FD + SSP and FD + BCH larvae had a lower P retention than those in FD (Table 4; $P < 0.05$), suggesting a lower efficiency of total P retention.

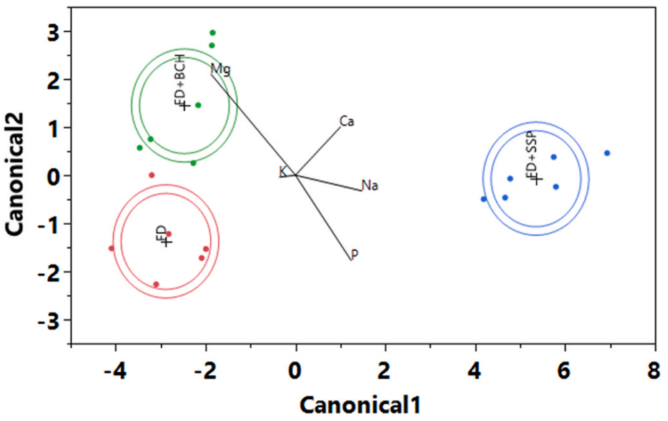

Fig. 2. Canonical plot of points and means (+) from linear discriminant analysis of larval concentrations of the macro-minerals calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na) and potassium (K) with substrate groups defined as FD: modified Gainesville diet; FD + BCH: pyrolysis product of sewage sludge (BCH) added to FD (4%); FD + SSP: single superphosphate (SSP) added to FD (3.6%). All variables had the same units of measurement (i.e. mg/kg DM). Each dot represents the overall response of one container of BSFL fed on a specific substrate. The inner circles represent the 95% confidence region for containing the true overall mean of the group, and the outer circles are the 50% contours. Rays with covariates names show the coordinate directions in the canonical space.

A linear discriminant analysis of the larval mineral concentration was performed (Fig. 2) to visualize the overall differentiation in mineral composition of the BSFL induced by different feeding substrates, and to identify minerals most responsible for the differentiation. The overall mineral composition of BSFL showed three distinct and non-overlapping clusters that are clearly attributable to the feeding substrates. Phosphorous and Mg associated most strongly with the separation of groups on both the first and second canonical axes, while Ca and Na were more associated with the separation of groups on the second canonical axis (Fig. 2). As shown in Fig. 2, the FD and the FD + BCH groups are closer together on the first canonical axis as compared to the FD + SSP group. The main reason for the separation of the FD + SSP larvae from the other two groups were their higher Ca, P and Na concentrations. In summary, our study shows that the Ca and P content of FD + SSP larvae increased in response to a higher Ca and P content of the SSP recyclate. In line with our results, previous studies reported plasticity of mineral composition in BSFL depending on the mineral contents in the substrate (Liland et al., 2017; Spranghers et al., 2017). To date, knowledge on the regulation of Ca and P homeostasis in BSFL is limited but the involvement of the Malpighian tubules in homeostasis and storage of minerals in insects such as *Drosophila melanogaster* (Browne and O'Donnell, 2016; Rose et al., 2019) is likely to be present in BSFL, too.

Table 4
Macro-mineral and heavy metal retention (%) in black soldier fly (*Hermetia illucens*) larvae fed on a fly diet supplemented with or without two different sewage sludge recyclates (BCH, SSP).

	Feeding substrates[a]				P-values[b], ≤	
	FD	FD + BCH	FD + SSP	SE	T	B
Macrominerals, %						
Ca	55.4^{a}	50.9^{a}	33.9^{b}	2.35	0.001	0.379
P	14.7^{a}	10.0^{b}	11.9^{b}	0.69	0.001	0.241
Mg	12.5	12.7	13.9	0.64	0.296	0.176
Na	19.8^{a}	16.6^{b}	10.5^{c}	0.83	0.001	0.927
K	7.2	7.3	9.0	0.57	0.080	0.171
Heavy metals, %						
Mn	42.9^{a}	35.8^{b}	41.5^{ab}	1.84	0.028	0.737
Fe	4.0^{b}	0.9^{c}	8.7^{a}	0.43	0.001	0.329
Zn	24.2^{a}	10.3^{b}	23.4^{a}	0.87	0.001	0.608
Cu	10.1^{a}	4.3^{b}	9.5^{a}	0.47	0.001	0.391
As	3.9^{b†}	2.7^{b†}	6.0^{a}	0.32	0.001	0.673
Cd	48.2	54.6	51.7	3.30	0.400	0.410
Pb	8.4^{b}	3.1^{c}	14.7^{a}	0.91	0.001	0.303
Hg	0.2	0.1	0.3	0.24	0.743	0.173

a-c: Values in a row that are marked with different letters differ significantly (Tukey, $P < 0.05$). The symbol † in a row indicates a trend for a difference between two treatments (Tukey, $0.05 < P \leq 0.10$).

[a] **FD:** modified Gainesville fly diet; **FD + BCH:** Pyrolysis product of sewage sludge (Biochar; BCH) added to FD (4%); **FD + SSP:** Single Superphosphate (SSP) added to FD (3.6%). Total number of observations used for statistical analyses, N = 18 (i.e., n = 6 replicate rearing containers per group within 2 experimental runs).

[b] **T:** treatment effect; **B** = block effect (experimental run (2 runs) considered as block).

Compared to BSFL fed on FD substrate, FD + SSP larvae had a higher Mg BAF in spite of similar Mg concentrations in the feeding substrates (i.e. 2.6–28 g/kg DM), suggesting a greater bioaccumulation efficiency of Mg due to recyclate supplementation.

With a concentration range of 0.9–1.3 g/kg, larvae in the current study contained less Na than reported by Zulkifli et al. (2022) and Shumo et al. (2019) at 2–5 g/kg for BSFL fed various residues. The FD + SSP larvae had a higher Na concentration and Na yield than larvae in the FD + BCH and FD groups (Table 2; $P < 0.05$, Table 3; $P < 0.01$). The Na BAF showed that larvae fed on both FD + SSP or FD + BCH substrates had a lower efficiency for Na accumulation than when fed FD substrate only (Table 2; $P < 0.05$). Larvae of the FD + SSP group had the lowest BAF and retention for Na (Table 2; $P < 0.05$; Table 4; $P < 0.001$), which could be due to the higher Na content in the SSP recyclate (Table S1 in the supplementary data).

The BAF of K shows that this mineral was not accumulated (Table 2; BAF <1) in the larval body on any of the substrates. Nevertheless, FD + SSP larvae had a higher K BAF than those in the FD + BCH and FD groups ($P < 0.05$), suggesting that K was less depleted when larvae were fed on FD + SSP than on FD or FD + BCH substrates. No difference was found among the groups for K concentration, yield and retention ($P > 0.10$).

Overall, our results suggest differences in mineral accumulation patterns in BSFL that are strongly dependent on the source of minerals (SSP or BCH). The responsible mechanisms for mineral incorporation in BSFL are poorly understood (Seyedalmoosavi et al., 2022b), but it is possibly related to the chemical form, ionization and oxidation status of the mineral, and the mineral tolerance of the intestinal microbiota (Wu et al., 2021).

3.3. Heavy metal content, bioaccumulation, yield and retention

Transition-metal ions (iron, copper, manganese, and zinc) are important in various physiological processes in insects, including immunity and interactions with microbes (Hrdina and Iatsenko, 2022). However, little is known on mechanisms of metal metabolism in *Hermetia illucens*. Thus, we focus here on the accumulation of metal ions in BSFL due to its environmental relevance. The SSP recyclate had a lower heavy metal content (Mn, Fe, Zn, Cu, As, and Pb) than BCH, resulting in different mineral and heavy metal contents in the feeding substrates. The concentration of both Cd and Hg in SSR were lower than the lower limit of quantification of 0.02 mg/kg DM (Table S1 in the supplementary data).

The linear discriminant analysis of the heavy metal (Fig. 3) concentration showed a full separation of the three feeding substrates in their overall effects on heavy metal composition of BSFL. Copper and Fe were mainly responsible for the separation of groups along the second canonical axis, i.e. particularly separating FD and FD + SSP diets from each other. On the other hand, Pb and Mn had the highest association with the separation of groups on the first canonical axis, i.e. leading to differentiation of FD + BCH from the other two substrates in their overall effects on heavy metal concentration of BSFL (Fig. 3). Indeed, particularly the Pb content in the FD + BCH larvae (Table 2) was highest among the three substrates.

In the current experiment, the range of larval Mn concentrations (285–304 mg/kg DM) did not differ among the groups (Table 2; $P < 0.05$), and was similar to the values reported by Spranghers et al. (2017) (220–380 mg/kg DM) in BSFL fed different substrates (chicken feed, digestate and vegetable waste). However, the larval Mn concentration observed, even in the group not supplemented by SSR, exceeded the maximal Mn concentration in complete feed legally prescribed by the EU Commission (150 mg/kg DM; Table S2 in the supplementary data) (European Commission, 2003, 2006). The Mn BAF value was lower in FD + BCH larvae than in those fed on FD + SSP and FD substrates (Fig. 4; $P < 0.05$), which was possibly linked to the high Mn concentration found in the FD + BCH feeding substrate (Table S1 in the supplementary data). The yield for larval Mn did not differ among the groups (Table 3; $P < 0.05$), whereas retention of Mn was lower in FD + BCH larvae than of those in the FD group (Table 4; $P < 0.05$). Carbon-coated minerals on the surface of BCH effectively reduce the bioavailability of heavy metals in soil (Joseph et al., 2021). Moreover, mineral bioavailability in BCH depends on several factors such as pH of the environment, feedstock source and pyrolytic temperature (Ding et al., 2016). In addition, bioavailability of minerals and heavy metals for BSFL may depend on the pH in the substrate and their gastrointestinal tract. The pH (acidic to alkaline) values vary in different intestiBonelli et al., 2019nal compartments of BSFL () which may affect mineral uptake. Therefore, although the concentration of certain minerals such as Mn was higher in BCH than in the SSP recyclate, it does not mean that all of it was bioavailable to the larvae.

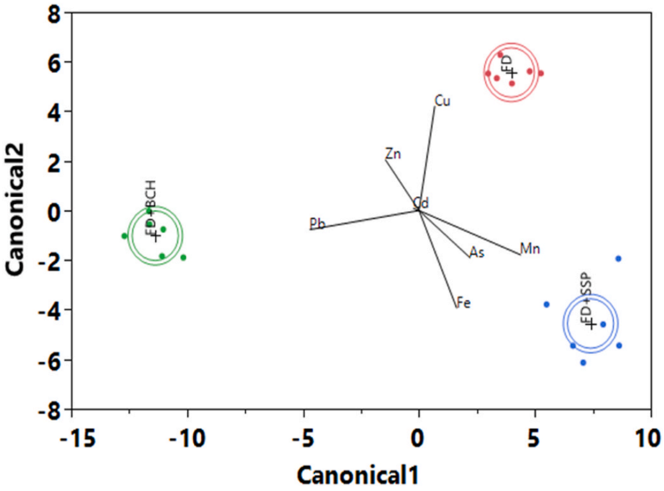

Fig. 3. Canonical plot of points and means (+) from linear discriminant analysis of larval concentrations of the heavy metals manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), arsenic (As), cadmium (Cd) and lead (Pb) with substrate groups defined as FD: modified Gainesville diet; FD + BCH: pyrolysis product of sewage sludge (BCH) added to FD (4%); FD + SSP: single superphosphate (SSP) added to FD (3.6%). All variables had the same units of measurement (i.e. mg/kg DM). Each dot represents the overall response of one container of BSFL fed on a specific substrate. The inner circles represent the 95% confidence region for containing the true overall mean of the group, and the outer circles are the 50% contours. Rays with covariates names show the coordinate directions in the canonical space.

Insects such as *Drosophila melanogaster* are highly sensitive to excessive Mn concentrations, which negatively affect feeding behaviour and brain functions (Ben-Shahar, 2018; Søvik et al., 2017). This is comparable to neurotoxic effects of Mn in certain animal models and humans (Ben-Shahar, 2018), as discussed by Bessa et al. (2021). Therefore, when BSFL should be used as food or feed, care must be taken, not to exceed the recommendations for Mn in human food or animal feed (Broom et al., 2021; DGE, 2021).

The iron concentration was higher in FD + BCH larvae than those fed FD (Table 2; $P < 0.05$) which is linked to the exceedingly high Fe concentrations in the BCH recyclate (328 times higher than in SSP) and consequently also in the respective feeding substrate (Table S1 in the supplementary data). Larval Fe concentration in the present study was comparable to BSFL fed on chicken manure and brewer's spent grain (Shumo et al., 2019). The iron content in BSFL fed BCH supplemented substrates, along with Mn concentration, was the highest heavy metal content of all elements measured. Interestingly, the BAF results show that BSFL did not accumulate Fe from any of the feeding substrates (i.e. BAF <1; Fig. 4). However, FD + SSP larvae had the highest BAF for Fe, which was several times higher than in FD + BCH and FD larvae, respectively (Fig. 4; $P < 0.05$). The lowest BAF in FD + BCH larvae suggests depletion of Fe in BSFL to prevent excessively high and perhaps toxic Fe concentrations. As a function of Fe concentration and larval mass, the Fe yield was higher in FD + BCH larvae than in those of the FD group (Table 3; $P < 0.05$), while the Fe retention was lowest in FD + BCH larvae (Table 4; $P < 0.05$). Knowledge regarding the Fe homeostasis in BSFL is limited (Gorman, 2023). For *Drosophila melanogaster*, it was discussed that non-heme Fe absorption and storage is linked to ferritin and the (acidic) pH in the midgut, which is known as the 'iron region' (Gorman, 2023; Mandilaras et al., 2013). In a recent study on BSFL, Bonelli et al. (2020) noted that parts of the midgut appear to be the main site for Fe absorption and storage as highest level of ferritin RNA transcripts was observed in the anterior and posterior midgut.

The reported Zn content in BSFL was 108 mg/kg DM (Makkar et al., 2014), which is within the range of values observed in our study (96–107 mg/kg DM). The Zn concentration in FD + BCH larvae tended to be higher than those in the FD group (Table 2; $P = 0.087$). Moreover, BAF results showed that Zn accumulated in all substrate groups (BAF >1). However, the Zn BAF was lower in FD + BCH larvae than those in FD + SSP and FD (Fig. 4; $P < 0.05$). Similar to our results, bio-accumulative potential for Cu and Zn in BSFL was also reported (Proc et al., 2020). However, in contrast, Wu et al. (2021) found a Zn BAF for BSFL of 0.80, suggesting it was not accumulated in larvae. This inconsistency for Zn BAF among the studies might be associated with different Zn levels and forms in the feeding substrates (Wu et al., 2021). The Zn yield was not different among dietary groups (Table 3; $P > 0.10$), and Zn retention was lower in FD + BCH larvae compared with that in FD + SSP and FD larvae (Table 4; $P < 0.05$). Zinc is necessary for the reproduction of insects such as *Drosophila melanogaster*, however, high Zn concentration can disturb protein function (Cardoso-Jaime et al.,

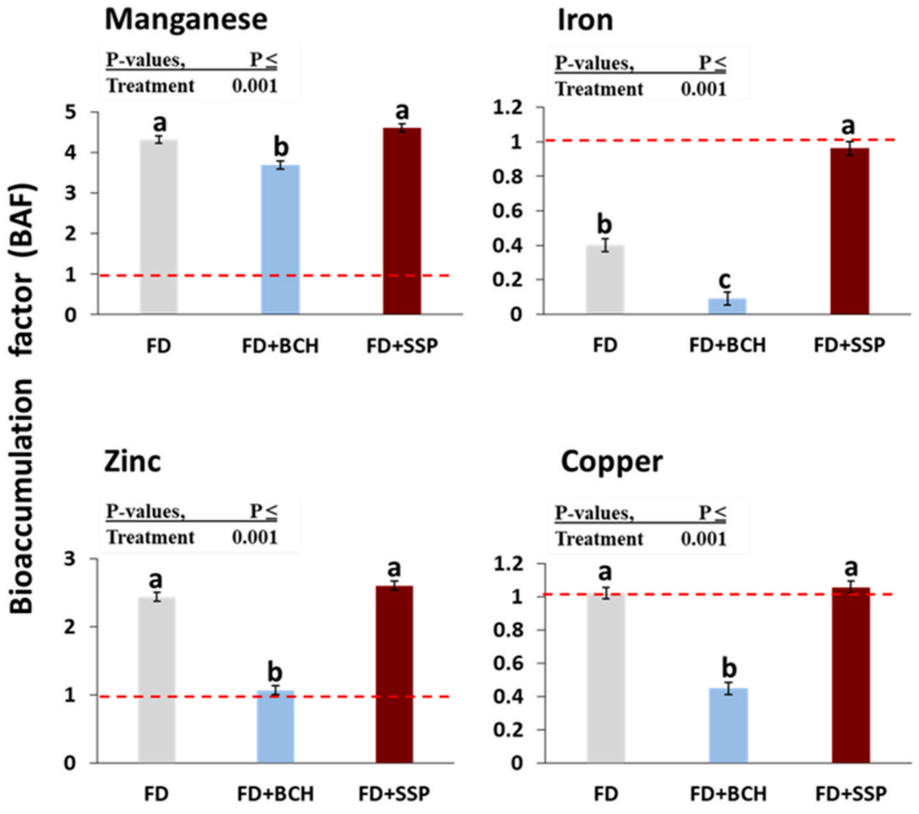

Fig. 4. Bioaccumulation factor (BAF) of manganese, iron, zinc and copper in black soldier fly (*Hermetia illucens*) larvae fed on a fly diet (FD) supplemented with or without two different sewage sludge recyclates (FD + BCH and FD + SSP). Values are LSM with their SE. a–c: Values denoted with different letters within each panel differ significantly (Tukey, $P < 0.05$). BAF >1 refers to accumulation of minerals in BSFL and BAF <1 refers to depletion of minerals from larval body during the experimental period. FD: modified Gainesville diet; FD + BCH: pyrolysis product of sewage sludge (BCH) added to FD (4%); FD + SSP: single superphosphate (SSP) added to FD (3.6%). Total number of observations used for statistical analysis, N = 18 (i.e. n = 6 replicate rearing containers per group over 2 experiment runs).

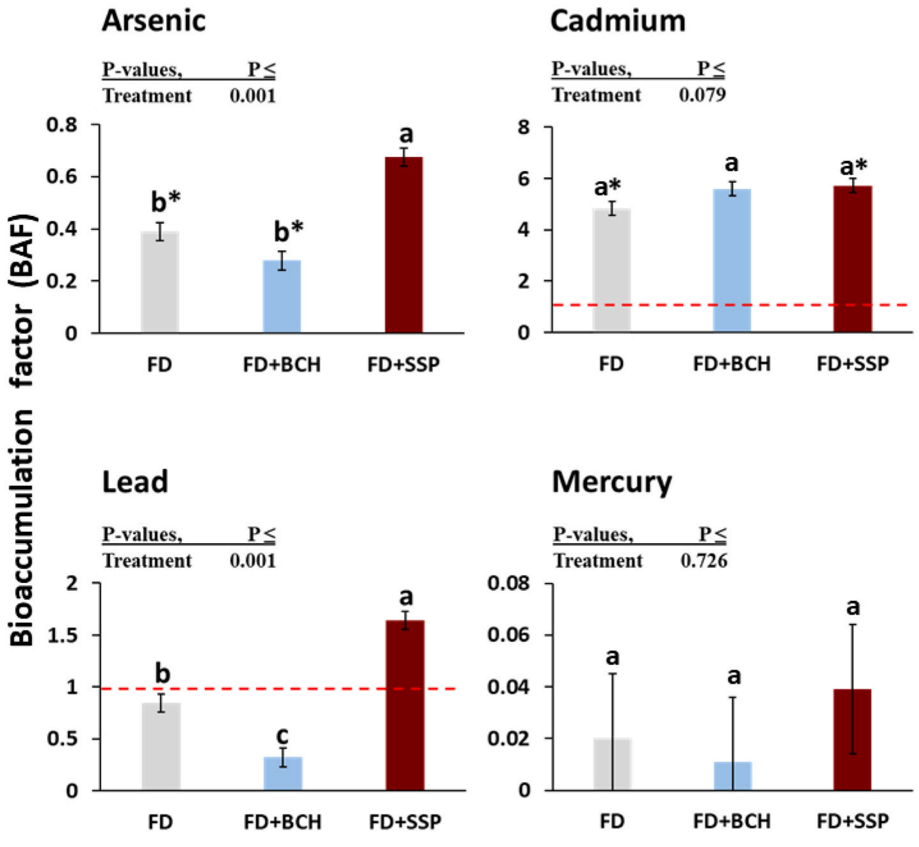

Fig. 5. Bioaccumulation factor (BAF) of arsenic, cadmium, lead and mercury in black soldier fly (*Hermetia illucens*) larvae fed on a fly diet (FD) supplemented with or without two different sewage sludge recyclates (FD + BCH and FD + SSP). Values are LSM with their SE. a–c: Values denoted with different letters within each panel differ significantly (Tukey, $P < 0.05$). The symbol * indicates a tendency of two treatments to differ (Tukey, $0.05 < P \leq 0.10$). BAF >1 refers to accumulation of minerals in BSFL and BAF <1 refers to depletion of minerals from larval body during the experimental period. FD: modified Gainesville diet; FD + BCH: pyrolysis product of sewage sludge (BCH) added to FD (4%); FD + SSP: single superphosphate (SSP) added to FD (3.6%). Total number of observations used for statistical analysis, N = 18 (i.e. n = 6 replicate rearing containers per group over 2 experiment runs).

2022).

Larval Cu concentrations observed (10–12 mg/kg DM) were comparable to those previously reported (Ferrari et al., 2022) (13 mg/kg DM) for BSFL fed Gainesville diet. The Cu concentration tended to be higher in FD + BCH larvae compared to those fed on FD (Table 2; $P = 0.052$). Copper BAF was approximately 1 in FD and FD + SSP, whereas it was not accumulated in FD + BCH (BAF <1) (Fig. 4; $P < 0.05$) presumably as a result of the high Cu-level in the feeding substrate (Table S1 in the supplementary data). A comparably low Cu BAF of 0.69 was interpreted as a self-protection mechanism (e.g. Cu excretion) in larvae exposed to a high Cu concentration in the feeding substrate (Wu et al., 2020). The Cu yield tended to be higher in FD + BCH compared to the FD + SSP larvae (Table 3; $P = 0.066$). Moreover, FD + BCH larvae had a lower Cu retention than those larvae fed on FD and FD + SSP substrates (Table 4; $P < 0.05$).

In the present study, the larval concentration of As ranged from 0.05 to 0.12 mg/kg DM (Table 2) which was lower than reported by Ferrari et al. (2022) for BSFL fed Gainesville diet (0.28 mg/kg). Arsenic concentration in FD + SSP larvae was higher than in FD + BCH and FD larvae (Table 2). The As BAF indicates that As was not accumulated in the larval body (BAF <1) of any substrate group (Fig. 5), while larvae fed FD + SSP substrate showed a relatively higher As BAF compared to FD + BCH and FD larvae ($P < 0.05$). Similar to our results, Van der Fels-Klerx et al. (2016) and Van der Fels-Klerx et al. (2018) observed that As was not accumulated in BSFL and rather the majority of consumed As was not taken up (BAF <1). Moreover, FD + SSP larvae had a higher As yield and retention than those in FD + BCH and FD (Tables 3 and 4 respectively; $P < 0.05$).

The concentration of Cd in BSFL grown on Gainesville diet reported (0.45 mg/kg) (Ferrari et al., 2022) was somewhat lower than observed in the current study (0.63–0.74 mg/kg DM). Cadmium concentration was higher in FD + BCH than in FD larvae (Table 2; $P < 0.05$). Since the maximal Cd concentration in complete feed legally prescribed by the EU Commission is 0.5 mg/kg DM (European Commission, 2002) (Table S2 in the supplementary data), the larval Cd contents observed are above this limit. It is of note however, that the SSR supplementation did not contribute much to the larval accumulation because the Cd content in larvae fed on the control substrate FD was already above the authorized limit suggesting that BSFL are prone to Cd accumulation. This confirms earlier observations of a Cd BAF of 6–8 for control diets and 6 to 10 when the substrates were spiked with extra Cd at 0.5–2 fold the authorized limit for Cd content in total feed (Van der Fels-Klerx et al., 2016). Cadmium had the highest BAF among all measured heavy metals with a tendency for a higher BAF in FD + SSP compared to the FD larvae (Fig. 5; $P = 0.080$). Cadmium yield and retention did not differ among the substrate groups (Tables 3 and 4 respectively; $P > 0.10$). It is known that BSFL accumulate high amounts of Cd (Proc et al., 2020), which is highly toxic and may constitute serious health risks for animal and humans (Truzzi et al., 2019). The Cd toxicity is dependent on oxidation level and methylation grade (Bolan et al., 2014; Egorova and Ananikov, 2017), which was not analysed in our study. In general, little information is available on transport mechanisms of minerals in BSFL. However, it has been suggested that high BAF for Cd in BSFL could be attributed to the large number of Ca^{2+} channels in their gut, which facilitate the Cd transport by means of heat shock proteins, resulting in high Cd accumulation compared to other heavy metals (Bessa et al., 2021; Van der Fels-Klerx et al., 2016).

Ferrari et al. (2022) reported that Pb concentration in BSFL fed Gainesville diet was 0.33 mg/kg which is similar to the Pb content of FD larvae in the present study. The FD + BCH larvae had the highest Pb concentration among the substrate groups, followed by larvae fed FD + SSP (Table 2; $P < 0.05$). Moreover, BAF showed that Pb was only accumulated in FD + SSP larvae (Fig. 5; BAF >1), while Pb was not stored in larvae of the FD + BCH and FD groups. Nevertheless, the yield for Pb in FD + BCH larvae was almost 5 times higher than that of FD larvae, whereas the Pb yield of the FD + SSP group was twice of that found in FD (Table 3; $P < 0.05$). The Pb retention resulted in the highest and lowest values in the FD + SSP and FD + BCH groups, respectively (Table 4; $P < 0.05$). The lack of Pb accumulation in the FD + BCH larvae despite its high concentration in the FD + BCH substrate (11 times higher in FD + BCH than in FD + SSP) could be explained by physico-chemical properties of BCH which may affect Pb release (Yang et al., 2018). It has been suggested that the exoskeleton of the BSFL is the storage site for Pb (Diener et al., 2015; Van der Fels-Klerx et al., 2016). Therefore, during processing of larvae, the Pb content in the final larval meal product could be reduced.

The concentration of Hg in BSFL was below the limit of quantification, which is in line with a low Hg content in BSFL fed Gainesville diet (0.007 mg/kg) as reported (Ferrari et al., 2022). In addition, the BAF result for Hg showed no accumulation (Fig. 5; $P > 0.10$).

Recently, the use of processed animal protein derived from insects to partly replace soybean meal and fishmeal to feed aquaculture animals, pigs, and poultry has been authorized by the European Commission (2021). However, nutrient recycling by using organic residues, which are currently defined as waste, still poses health risk that need to be averted for the future (Salemdeeb et al., 2017). Our data show that Fe, Zn, Cu, As, Pb, and Hg contents in BSFL did not exceed the currently EU authorized maximum concentration in complete feed (Table S2 in the supplementary data). However, the Mn and Cd contents of BSFL were above the authorized maximum concentration (Table S2 in the supplementary data).

There are potential limitations to our study. Since BSFL guts were not fully emptied by starvation at harvest, the results obtained on mineral and heavy metal concentrations in BSFL bodies might have been affected to a certain degree by gut filling. However, gut filling does not necessarily imply an elevated concentration of minerals in BSFL. As BAF indicated clear accumulation of certain minerals in BSFL, potential substrate residues in the gut content might have even diluted the true accumulation levels at least for elements shown to have high recovery (e.g. Ca, Mn and Cd). Nevertheless, since all BSFL groups were treated in the same way, potential gut filling effects may be considered negligible.

3.4. Nutrients, minerals and heavy metal composition of frass

Crude fibre, aNDFom, and ADFom contents in frass of FD + SSP were lower than those in the FD group (Table S3 in the supplementary data; $P < 0.05$), which might suggest an increased fibre degradation by FD + SSP larvae or microbiota and fungi in the substrate. Although in the current study the fibre degrading characteristics were not determined in the BSFL, fibre degrading enzymes such as ligninases and cellulases are known to be present in the larval gut but their pattern depends on the microbiota in the substrate and the substrate composition (Müller et al., 2017). It has been suggested that cellulase activity produced by the fungi *Rhizopus oryzae* isolated from soil was inhibited by heavy metals such as Cu, Zn, Co or Pb (Murashima et al., 2002), but it remains to be determined if the heavy metal concentration pattern in the BSFL feeding substrate affects the activity of fibre degrading enzymes in bacteria and fungi. Frass of both FD + SSP and FD + BCH groups had a higher Ca, P and Na concentration than FD frass (Table S3 in the supplementary data; $P < 0.05$) and among the recyclate supplemented groups, FD + SSP had the highest Ca, P and Na concentration ($P < 0.05$). Moreover, FD + BCH had the highest Mn, Fe, Zn, Cu, As, Cd, and Pb content in frass among the groups ($P < 0.05$). However, heavy metal content in frass of all groups was below the currently EU authorized maximum concentration for organic fertilizers (Table S4 in the supplementary data) (European Commission, 2019). Frass of BSFL has been suggested as a sustainable and environmentally safe fertilizer (Beesigamukama et al., 2020). In summary, according to our results, frass of FD + SSP contains higher amounts of CP, ash and macro-minerals while heavy metal content was of the same magnitude as in FD frass which suggest that FD + SSP frass might be a better fertilizer.

4. Conclusions

This study provides information on the impact of using sewage sludge recyclates as a mineral-rich supplement to BSFL substrates with the aim of testing the reintegration of minerals from currently legally prohibited wastes and residues into the nutrient cycle. Dietary inclusion of SSP recyclate negatively affected the growth performance of BSFL. The concentrations of the minerals found in the larvae confirm that the micronutrient profile of BSFL depends on the initial mineral concentrations in the substrate. Both BCH and SSP supplements increased Ca content in BSFL, whereas only SSP increased P content. Inclusion of SSP was associated with lower heavy metal content in larvae and frass as compared to BCH which reflects the lower concentrations of heavy metals in SSP due to the technological reduction process used. Nevertheless, BSFL enriched with minerals from both SSR containing substrates (i.e. SSP, BCH) do not exceed the current EU authorized maximum concentration in complete feed for Fe, Zn, Cu, As, and Hg. However, the EU thresholds for Mn and Cd concentrations are exceeded even for BSFL reared on the control feeding substrate FD without SSR supplementation. Because of the toxicity of excessive Mn and Cd in feed or food, limits on the BSFL incorporation level in animal feed need to be established depending on the SSR and BSFL processing technology. Apart from nutrient recycling and legal considerations in agriculture, supplementation of larval substrate with SSP is not economical and should be used directly as fertilizer. However, lower quality and less expensive SSR processed in an agricultural context could be further considered for a potential re-integration in agri-food nutrient cycles, provided that the heavy metal concentration is already reduced during wastewater treatment.

Credit authorstatement

MMS: data collection, formal analysis, writing original draft, writing-review and editing; MM: data collection, methodology, writing-review and editing; KS: formal analysis; SG: formal analysis; PW: supervision, funding acquisition; JT: Resources; LH: formal analysis; SD: writing-review and editing; GD: conceptualization, formal analysis, methodology, writing-review and editing; CCM: conceptualization, funding acquisition, supervision, methodology, writing-review and editing. All authors read the article and approved the submitted version.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Prof. Dr. Cornelia C. Metges reports financial support was provided by Leibniz ScienceCampus Phosphorus Research Rostock.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.118695.

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