Biorefinery of shrimp shell waste via black soldier fly larvae: Larval performance, waste reuse efficiency, and circular economy potential

This Supplementary Material contains:

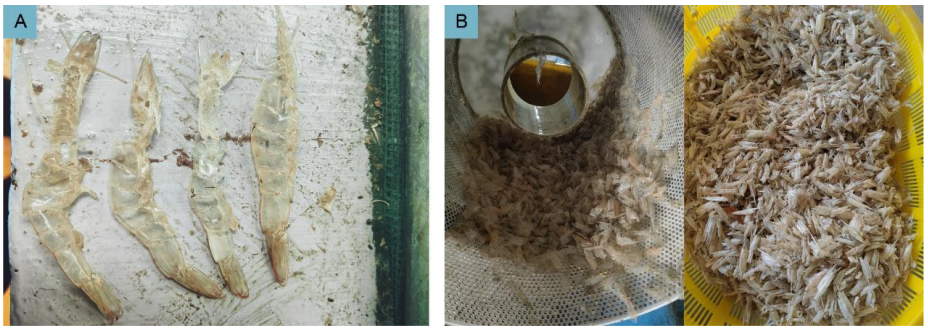

Fig. S1 Naturally molted shrimp shell (A) and the shrimp shell collected from recirculating aquaculture system (B).

Table S1. Composition of wheat bran and shrimp shell used in this study (dry weight).

Data were presented as means ± standard deviation (n = 3).

Parameters	Wheat bran	Shrimp shell
Crude lipid (%)	1.6 ± 0.2	1.3 ± 0.01
Crude ash (%)	5.1 ± 0.1[b]	55.1 ± 1.9[a]
Crude protein (%)	15.7 ± 0.4[b]	22.6 ± 0.8[a]
Nitrogen (%)	2.5 ± 0.1[b]	3.6 ± 0.1[a]
Carbon (%)	36.7 ± 0.9[a]	17.1 ± 0.4[b]
C/N ratio	14.6 ± 0.2[a]	4.7 ± 0.2[b]
Mg (g/kg)	2.9 ± 0.2[b]	28.1 ± 2.3[a]
Ca (g/kg)	1.4 ± 0.2[b]	156.6 ± 4.6[a]
K (g/kg)	10.5 ± 0.1[a]	2.1 ± 0.1[b]

Different letters within lines indicate significant differences at $p < 0.05$.

Human health risk assessment process

The potential human health risk associated with consuming edible products containing bioaccumulated heavy metals (Cr, Mn, Fe, Cu, Sr, Cd, and Pb) was evaluated following established methodologies (Lee et al., 2021; Zhu et al., 2023). The assessment comprised three key components:

1. Heavy metal exposure assessment

The daily intake rate (DIR, mg/kg/day) was calculated to estimate mean daily heavy metal exposure, using Eq. (1):

$$DIR = \frac{C_m \times C_f \times IR}{BW} \quad (1)$$

where:

C_m = heavy metal concentration in the BSFL (mg/kg; fresh weight)

C_f = conversion factor (dry weight/fresh weight, DW/FW)

IR = ingestion rate (0.05 kg/day)

BW = average adult body weight (70 kg)

2. Health risk index (HRI)

The HRI evaluates the toxic potential of noncancerous during a lifetime period, using Eq. (2):

$$HRI = \frac{DIR}{RfD} \quad (2)$$

where RfD (mg/kg/day) is the reference dose set by the U.S. EPA. An HRI < 1 indicates negligible risk, while HRI ≥ 1 suggests potential health concerns.

3. Targeted hazard quotient (THQ)

THQ is the ratio of the pollutant dose to reference dose and is a dimensionless

indicator of the level of noncarcinogenic health risk to humans. The ratio is calculated by eq. (3):

$$THQ = \frac{EF \times IR \times C_m}{365 \times RfD \times BW} \quad (3)$$

where EF is the exposure frequency, the value of 104 days/year was used in this study. THQ < 1 indicates a negligible level of risk of heavy metal exposure through BSFL consumption, whereas 1 < THQ < 5 indicates a certain level of health risk.

Reference

Lee, E., Rout, P.R., Bae, J., 2021. The applicability of anaerobically treated domestic wastewater as a nutrient medium in hydroponic lettuce cultivation: Nitrogen toxicity and health risk assessment. Sci. Total Environ. 780, 146482. https://doi.org/10.1016/j.scitotenv.2021.146482.

Zhu, Z., Yogev, U., Keesman, K.J., Rachmilevitch, S., Gross, A., 2023. Integrated hydroponics systems with anaerobic supernatant and aquaculture effluent in desert regions: Nutrient recovery and benefit analysis. Sci. Total Environ. 904, 166867. https://doi.org/10.1016/j.scitotenv.2023.16686.

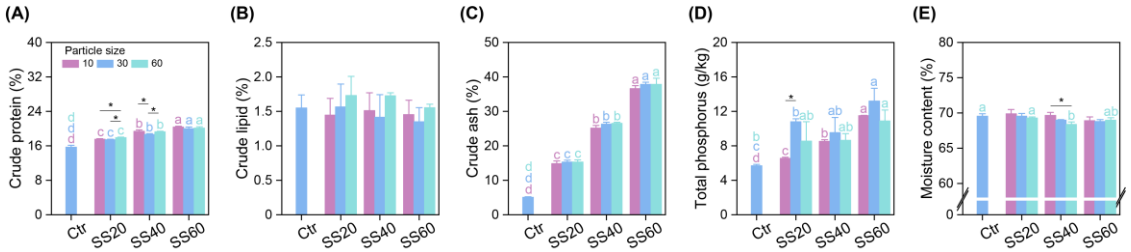

Fig. S2 Proximate component of the food substrates (mean ± standard; n = 3). (A) Crude protein. (B) Crude lipid. (C) Crude ash. (D) Total phosphorus. (E) Moisture content. Ctr (control): substrate composed of 100% wheat bran, SS20: 80% wheat bran, 20% shrimp shell, SS40: 60% wheat bran, 40% shrimp shell, SS60: 40% wheat bran, 60% shrimp shell. In the bar chart, different letters of the same color indicate significant differences among groups with varying shrimp shell ratios ($p < 0.05$, Tukey test). The * represent significant difference among same shrimp shell ratio with different mesh number ($p < 0.05$, Tukey test).

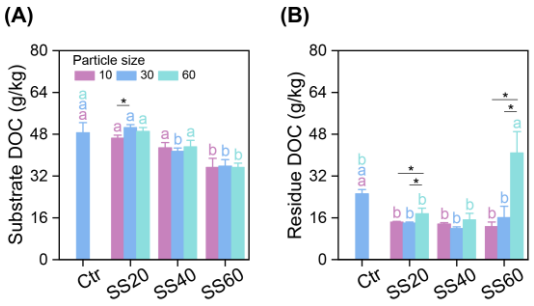

Fig. S3 Dissolved organic carbon (DOC) of substrate and residue. Ctr (control): substrate composed of 100% wheat bran, SS20: 80% wheat bran, 20% shrimp shell, SS40: 60% wheat bran, 40% shrimp shell, SS60: 40% wheat bran, 60% shrimp shell.

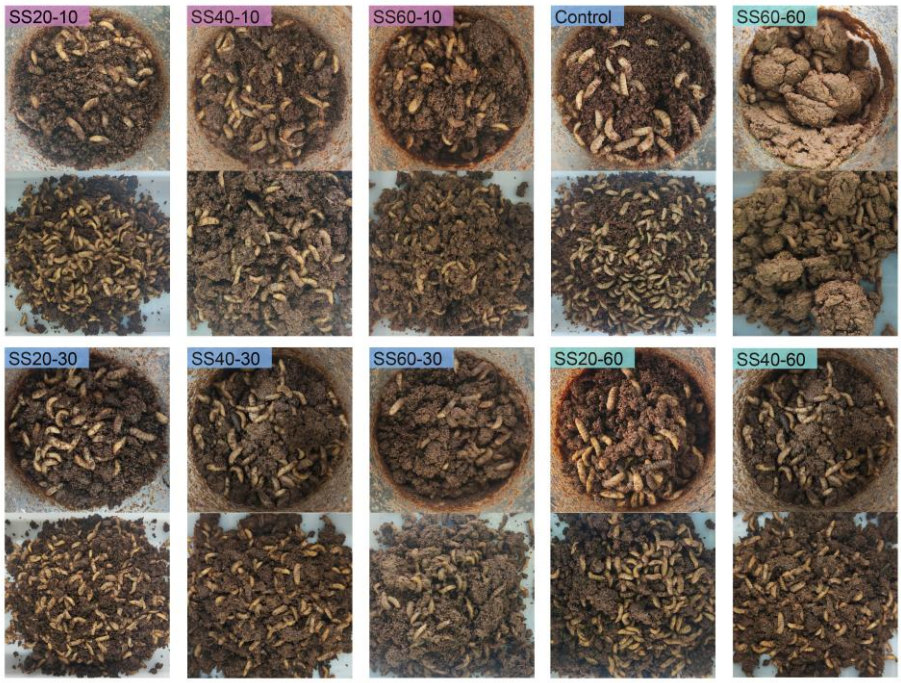

Fig. S4 Black soldier fliy larvae rearing on substrate after 10 days bioconversion. Ctr (control): substrate composed of 100% wheat bran, SS20: 80% wheat bran, 20% shrimp shell, SS40: 60% wheat bran, 40% shrimp shell, SS60: 40% wheat bran, 60% shrimp shell. SS-20-10 represents shrimp shells with a particle size of 10 mesh, constituting 20% of the substrate, while SS-60-60 represents shrimp shells with a particle size of 60 mesh at a proportion of 60%.

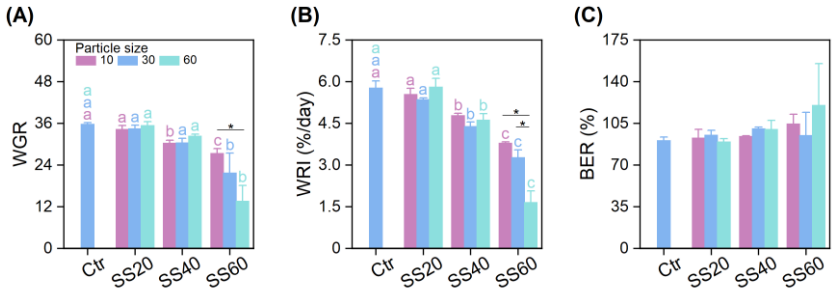

Fig. S5 Weight gain rate (WGR; A), waste reduction rate index (WRI; B), and bioconversion efficiency corrected for residue (BER; C) of the black soldier fly larvae when fed different food substrates (mean ± standard; n = 3). Ctr (control): substrate composed of 100% wheat bran, SS20: 80% wheat bran, 20% shrimp shell, SS40: 60% wheat bran, 40% shrimp shell, SS60: 40% wheat bran, 60% shrimp shell. In the bar chart, different letters of the same color indicate significant differences among groups with varying shrimp shell ratios ($p < 0.05$, Tukey test). The * represent significant difference among same shrimp shell ratio with different mesh number ($p < 0.05$, Tukey test).

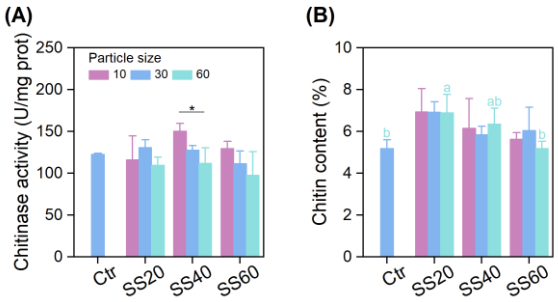

Fig. S6 Chitinase activity (A) and chitin content (B) of the black soldier fly larvae when fed different food substrates (mean ± standard; n = 3). Ctr (control): substrate composed of 100% wheat bran, SS20: 80% wheat bran, 20% shrimp shell, SS40: 60% wheat bran, 40% shrimp shell, SS60: 40% wheat bran, 60% shrimp shell. In the bar chart, different letters of the same color indicate significant differences among groups with varying shrimp shell ratios ($p < 0.05$, Tukey's test). The * represent significant difference among same shrimp shell ratio with different mesh number ($p < 0.05$, Tukey test).

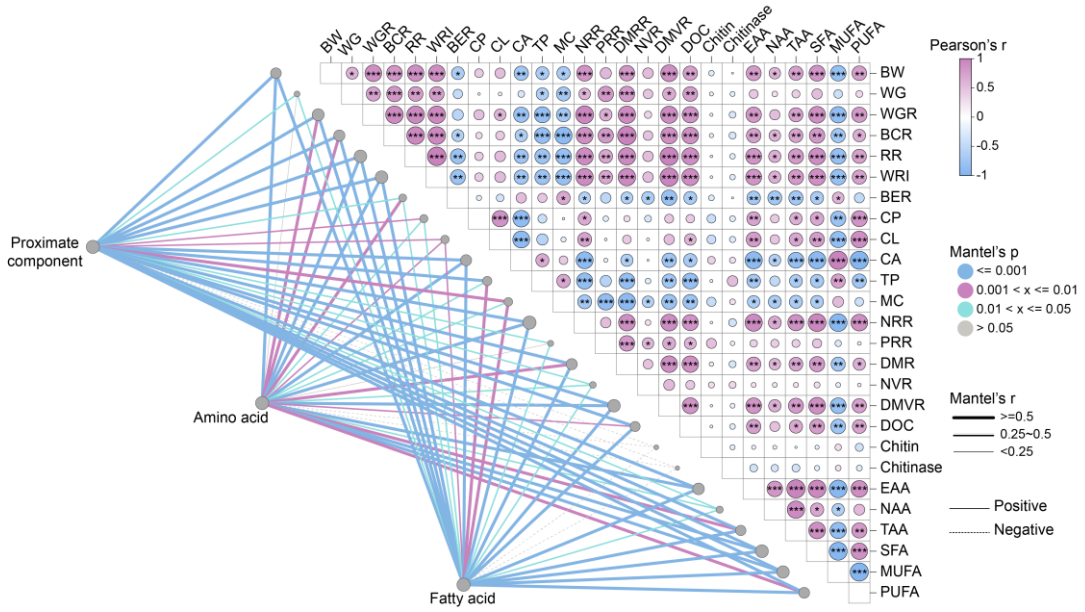

Fig. S7 Mantel teste between substrate nutrition component and black soldier fly larvae parameters. Abbreviation: BW = body weight; WG = weight gain; WGR = weight gain rate; BCR = biomass conversion rate; RR = reduction rate; WRI = waste reduction rate index; BER = bioconversion efficiency corrected for residue; CP = crude protein; CL = crude lipid; CA = crude ash; TP = total phosphorus; MC = moisture content; NRR = N recycle rate; PRR = N recycle rate; DMR = dry matter recycle rate; NVR = N volatilization rate; DMVR = dry matter volatilization rate; DOC = dissolved organic carbon; EAA = essential amino acid; NAA = non-essential amino acid; TAA = total amino acid; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

Fig. S8 Food substrates consist of 80 % shrimp shell (60-mesh; < 0.28 mm) and 20 % wheat bran.

Table S2 Amino acid composition of food substrates in each group (mean ± standard; n = 3). Ctr (control): substrate composed of 100% wheat bran, SS20: 80% wheat bran, 20% shrimp shell, SS40: 60% wheat bran, 40% shrimp shell, SS60: 40% wheat bran, 60% shrimp shell, and SS100: 100% shrimp shell.

Amino acid	Ctr	SS20	SS40	SS60	SS100
Asp	1.0 ± 0.01^{a}	1.2 ± 0.1^{b}	1.3 ± 0.04^{b}	1.6 ± 0.03^{c}	1.9 ± 0.3
Thr*	0.5 ± 0.0^{a}	0.5 ± 0.05^{b}	0.5 ± 0.01^{b}	0.6 ± 0.02^{c}	0.8 ± 0.05
Ser	0.5 ± 0.0^{d}	0.7 ± 0.06^{c}	0.8 ± 0.02^{b}	0.9 ± 0.01^{a}	1.0 ± 0.1
Glu	2.9 ± 0.03	2.9 ± 0.3	2.7 ± 0.05	2.8 ± 0.05	2.7 ± 0.4
Gly	0.8 ± 0.01^{c}	1.0 ± 0.09^{b}	1.0 ± 0.03^{b}	1.2 ± 0.03^{a}	1.5 ± 0.2
Ala	0.7 ± 0.01^{c}	0.8 ± 0.08^{b}	0.9 ± 0.03^{b}	1.0 ± 0.02^{a}	1.3 ± 0.2
Cys	0.2 ± 0.0^{a}	0.2 ± 0.02^{a}	0.1 ± 0.01^{b}	0.1 ± 0.01^{b}	0.1 ± 0.01
Val*	0.7 ± 0.01^{b}	0.8 ± 0.08^{b}	0.8 ± 0.02^{b}	0.9 ± 0.02^{a}	1.2 ± 0.1
Met*	0.2 ± 0.0^{d}	0.8 ± 0.05^{c}	1.4 ± 0.1^{b}	2.5 ± 0.02^{a}	2.9 ± 0.8
Ile*	0.5 ± 0.0^{b}	0.5 ± 0.06^{ab}	0.5 ± 0.01^{ab}	0.6 ± 0.02^{a}	0.8 ± 0.05
Leu*	1.0 ± 0.01	1.0 ± 0.1	0.9 ± 0.03	0.9 ± 0.03	1.0 ± 0.07
Tyr	0.4 ± 0.02^{b}	0.5 ± 0.07^{a}	0.5 ± 0.05^{a}	0.6 ± 0.03^{a}	0.8 ± 0.2
Phe*	0.7 ± 0.01^{c}	0.9 ± 0.09^{b}	0.9 ± 0.04^{b}	1.0 ± 0.04^{a}	1.2 ± 0.2
Lys*	0.6 ± 0.0	0.6 ± 0.07	0.6 ± 0.02	0.6 ± 0.02	0.7 ± 0.05
His*	0.4 ± 0.0	0.4 ± 0.04	0.4 ± 0.01	0.4 ± 0.0	0.4 ± 0.07
Arg*	1.0 ± 0.03	1.0 ± 0.1	0.9 ± 0.03	1.0 ± 0.02	1.0 ± 0.2
Pro	1.0 ± 0.01	1.1 ± 0.1	1.0 ± 0.03	1.1 ± 0.03	1.0 ± 0.13
EAA	5.3 ± 0.06^{c}	6.5 ± 0.7^{b}	6.9 ± 0.2^{b}	8.5 ± 0.1^{a}	9.9 ± 1.6
NAA	7.5 ± 0.06^{b}	8.4 ± 0.8^{ab}	8.3 ± 0.2^{ab}	9.2 ± 0.2^{a}	10.3 ± 1.4
EAA/NAA	0.7 ± 0.01^{d}	0.8 ± 0.01^{c}	0.8 ± 0.01^{b}	0.9 ± 0.01^{a}	1.0 ± 0.02
TAA	12.8 ± 0.1^{c}	14.9 ± 2.2^{b}	15.2 ± 2.0^{b}	17.7 ± 1.5^{a}	20.2 ± 3.0

Notes: Essential amino acid (EAA, *), nonessential amino acid (NAA), and total amino acid (TAA). Different letters within lines indicate significant differences at $p < 0.05$.

Table S3 Fatty acid profile of food substrates in each group (mean ± standard; n = 3). Ctr (control): substrate composed of 100% wheat bran, SS20: 80% wheat bran, 20% shrimp shell, SS40: 60% wheat bran, 40% shrimp shell, SS60: 40% wheat bran, 60% shrimp shell, and SS100: 100% shrimp shell.

Fatty acid	Ctr	SS20	SS40	SS60	SS100
C10:0	< LOQ	< LOQ	< LOQ	0.1 ± 0.01	0.3 ± 0.04
C12:0	< LOQ	< LOQ	1.0 ± 0.06^{b}	1.3 ± 0.01^{a}	1.6 ± 0.1
C14:0	< LOQ	< LOQ	0.6 ± 0.06^{b}	1.1 ± 0.2^{a}	3.4 ± 0.2
C15:0	< LOQ	< LOQ	0.6 ± 0.03^{b}	1.0 ± 0.06^{a}	1.3 ± 0.03
C16:0	18.0 ± 0.6^{d}	20.0 ± 1.0^{c}	23.3 ± 0.06^{b}	26.6 ± 0.4^{a}	32.9 ± 0.4
C16:1	< LOQ	1.7 ± 0.03^{c}	3.2 ± 0.1^{b}	4.6 ± 0.5^{a}	6.4 ± 0.3
C17:0	< LOQ	< LOQ	0.5 ± 0.04^{b}	1.1 ± 0.02^{a}	2.3 ± 0.05
C18:0	1.5 ± 0.05^{d}	2.6 ± 0.3^{c}	3.5 ± 0.4^{b}	4.6 ± 0.1^{a}	6.8 ± 0.3
C18:1n9	20.6 ± 0.8^{b}	21.9 ± 1.3^{ab}	23.4 ± 0.4^{a}	24.0 ± 0.3^{a}	28.2 ± 0.3
C18:2n6	55.4 ± 0.8^{a}	46.8 ± 0.6^{b}	37.4 ± 0.4^{c}	27.8 ± 0.8^{d}	8.5 ± 0.7
C20:1n9	3.6 ± 0.05^{a}	3.2 ± 0.1^{b}	2.6 ± 0.3^{c}	1.9 ± 0.1^{d}	0.3 ± 0.01
C18:3n3	0.9 ± 0.05	0.9 ± 0.07	0.8 ± 0.05	0.8 ± 0.1	0.8 ± 0.1
C20:2	< LOQ	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.0
C20:3n3	< LOQ	0.1 ± 0.01^{c}	0.2 ± 0.02^{b}	0.2 ± 0.0^{a}	0.4 ± 0.02
C24:0	< LOQ	2.6 ± 0.4^{b}	2.9 ± 0.7^{b}	4.6 ± 0.2^{a}	6.7 ± 0.3
SFA	19.6 ± 0.6^{d}	25.3 ± 0.8^{c}	32.3 ± 0.4^{b}	40.5 ± 0.5^{a}	55.3 ± 0.4
MUFA	24.2 ± 0.9^{c}	26.8 ± 1.4^{b}	29.2 ± 0.3^{a}	30.5 ± 0.4^{a}	34.9 ± 0.3
n-6	55.4 ± 0.8^{a}	46.8 ± 0.6^{b}	37.4 ± 0.4^{c}	27.8 ± 0.8^{d}	8.5 ± 0.7
n-3	0.9 ± 0.05	1.0 ± 0.08	0.9 ± 0.07	1.0 ± 0.05	1.1 ± 0.1
PUFA	56.2 ± 0.8^{a}	47.8 ± 0.6^{b}	38.4 ± 0.3^{c}	28.9 ± 0.8^{d}	9.8 ± 0.7

LOQ = limit of qualification. SFA = saturated fatty acid. MUFA = monounsaturated fatty acid. PUFA = polyunsaturated fatty acid. Different letters within lines indicate significant differences at $p < 0.05$.

Table S4 Concentration of minerals composition of black soldier fly larvae in each group (mean ± standard; n = 3). Ctr (control): substrate composed of 100% wheat bran, SS20: 80% wheat bran, 20% shrimp shell, SS40: 60% wheat bran, 40% shrimp shell, SS60: 40% wheat bran, 60% shrimp shell.

Minerals	Ctr	SS20	SS40	SS60
Al	21.9 ± 8.6	21.4 ± 2.8	14.9 ± 0.7	22.3 ± 3.3
V	0.4 ± 0.3^{ab}	0.6 ± 0.2^{a}	0.1 ± 0.02^{b}	0.1 ± 0.04^{b}
Cr	33.9 ± 6.6^{a}	17.5 ± 4.0^{b}	9.8 ± 3.3^{bc}	3.2 ± 1.1^{c}
Mn	861.7 ± 196.4^{a}	724.8 ± 162.0^{a}	251.6 ± 5.8^{b}	152.6 ± 2.9^{b}
Fe	370.8 ± 26.8^{a}	211.7 ± 31.8^{b}	223.5 ± 23.0^{b}	239.7 ± 12.6^{b}
Co	0.2 ± 0.1^{ab}	0.3 ± 0.1^{a}	0.1 ± 0.01^{ab}	0.08 ± 0.01^{b}
Ni	1.9 ± 1.0	4.5 ± 3.6	4.2 ± 2.1	1.4 ± 0.7
Cu	17.9 ± 3.3^{a}	14.3 ± 3.7^{a}	6.3 ± 0.1^{a}	5.8 ± 0.6^{a}
Zn	145.8 ± 22.5^{a}	136.5 ± 29.0^{a}	50.8 ± 5.6^{b}	45.2 ± 6.4^{b}
Ga	0.05 ± 0.03	0.06 ± 0.02	0.02 ± 0.0	0.03 ± 0.01
Rb	13.3 ± 2.5	12.3 ± 2.7	8.2 ± 0.3	8.4 ± 2.3
Mo	1.6 ± 0.5^{a}	1.5 ± 0.3^{a}	0.5 ± 0.2^{b}	0.4 ± 0.05^{b}
Ag	0.02 ± 0.0	0.01 ± 0.0	0.03 ± 0.03	0.03 ± 0.03
Cd	0.7 ± 0.1^{a}	0.6 ± 0.1^{a}	0.3 ± 0.02^{b}	0.3 ± 0.06^{b}
Cs	0.03 ± 0.01	0.03 ± 0.02	0.01 ± 0.0	0.01 ± 0.01
Ba	35.7 ± 8.2^{b}	90.8 ± 18.8^{a}	39.6 ± 2.7^{b}	35.0 ± 9.7^{b}

Values reported as mg/kg. Different letters within lines indicate significant differences at $p < 0.05$.

Table S5 Concentration of minerals composition of substrate in each group (mean ± standard; n = 3). Results are in mg/kg unless otherwise stated. Ctr (control): substrate composed of 100% wheat bran, SS20: 80% wheat bran, 20% shrimp shell, SS40: 60% wheat bran, 40% shrimp shell, SS60: 40% wheat bran, 60% shrimp shell, and SS100: 100% shrimp shell.

Minerals	Ctr	SS20	SS40	SS60	SS100
Mg[1]	2.9 ± 0.2^{c}	8.3 ± 1.4^{b}	12.8 ± 3.3^{ab}	16.1 ± 2.6^{a}	25.8 ± 1.4
Al	9.2 ± 1.2^{c}	29.0 ± 5.5^{bc}	42.7 ± 11.3^{b}	72.1 ± 9.1^{a}	174.6 ± 11.5
K[1]	10.5 ± 0.1^{a}	9.1 ± 0.2^{b}	7.7 ± 0.08^{c}	7.1 ± 0.1^{d}	2.1 ± 0.1
Ca[1]	1.4 ± 0.2^{d}	20.1 ± 0.2^{c}	63.9 ± 0.4^{b}	80.3 ± 2.1^{a}	156.6 ± 4.6
Ti	60.1 ± 15.4^{ab}	67.1 ± 10.6^{a}	43.1 ± 10.1^{ab}	33.2 ± 12.0^{b}	15.0 ± 1.4
V	0.09 ± 0.0	0.3 ± 0.3	0.4 ± 0.2	0.3 ± 0.02	0.4 ± 0.08
Cr	1.3 ± 0.3^{b}	4.5 ± 2.1^{ab}	7.0 ± 0.6^{a}	7.6 ± 2.1^{a}	11.5 ± 1.1
Mn	148.1 ± 10.2	141.1 ± 27.2	123.7 ± 16.4	115.7 ± 17.5	97.0 ± 4.1
Fe	19.5 ± 3.8^{c}	53.2 ± 22.6^{bc}	95.6 ± 39.8^{b}	170.4 ± 17.7^{a}	371.2 ± 77.7
Co	0.1 ± 0.01	0.2 ± 0.04	0.2 ± 0.1	0.2 ± 0.02	0.2 ± 0.05
Ni	2.4 ± 0.2	2.4 ± 0.4	3.1 ± 0.2	2.4 ± 0.4	2.3 ± 0.06
Cu	9.8 ± 0.3^{ab}	12.1 ± 1.4^{a}	10.7 ± 1.7^{ab}	8.3 ± 1.2^{b}	6.4 ± 0.2
Zn	51.8 ± 3.8	68.5 ± 17.4	66.0 ± 19.5	56.9 ± 6.2	55.6 ± 2.4
Ga	0.02 ± 0.0^{b}	0.03 ± 0.0^{ab}	0.04 ± 0.02^{ab}	0.05 ± 0.0^{a}	0.05 ± 0.01
Rb	11.2 ± 0.9^{ab}	14.7 ± 2.1^{a}	12.2 ± 2.9^{ab}	7.7 ± 1.0^{b}	5.5 ± 0.5
Sr	0.01 ± 0.0^{c}	0.5 ± 0.1^{b}	1.1 ± 0.2^{a}	1.5 ± 0.2^{a}	2.9 ± 0.4
Mo	0.5 ± 0.1	0.8 ± 0.5	0.3 ± 0.2	0.5 ± 0.4	0.3 ± 0.04
Ag	0.003 ± 0.0^{b}	0.01 ± 0.0^{ab}	0.01 ± 0.0^{a}	0.01 ± 0.0^{ab}	0.01 ± 0.0
Cd	0.08 ± 0.0	0.1 ± 0.01	0.1 ± 0.05	0.2 ± 0.2	0.6 ± 0.12
Cs	0.02 ± 0.0	0.03 ± 0.01	0.03 ± 0.02	0.02 ± 0.0	0.02 ± 0.01
Ba	14.7 ± 1.2^{c}	38.1 ± 7.7^{b}	48.7 ± 3.9^{ab}	59.7 ± 6.3^{a}	89.3 ± 4.3
Pb	1.9 ± 0.09^{a}	0.6 ± 0.3^{b}	0.4 ± 0.1^{b}	0.3 ± 0.08^{b}	0.2 ± 0.05

Different letters within lines indicate significant differences at $p < 0.05$.

Table S6 Bioaccumulation factor of black soldier fly larvae reared on different feeding substrates (mean ± standard; n = 3). Ctr (control): substrate composed of 100% wheat bran, SS20: 80% wheat bran, 20% shrimp shell, SS40: 60% wheat bran, 40% shrimp shell, SS60: 40% wheat bran, 60% shrimp shell.

Minerals	Ctr	SS20	SS40	SS60
Mg	1.2 ± 0.1^{a}	0.6 ± 0.1^{b}	0.2 ± 0.03^{c}	0.9 ± 0.03^{c}
Al	2.4 ± 1.1^{a}	0.8 ± 0.3^{b}	0.4 ± 0.08^{b}	0.3 ± 0.06^{b}
K	1.0 ± 0.04^{d}	1.2 ± 0.08^{c}	1.4 ± 0.02^{b}	1.6 ± 0.03^{a}
Ca	4.3 ± 0.8^{a}	2.1 ± 0.05^{b}	1.2 ± 0.01^{b}	1.3 ± 0.04^{b}
Ti	1.5 ± 0.6^{b}	2.7 ± 0.9^{b}	6.0 ± 1.3^{ab}	13.0 ± 5.8^{a}
V	4.2 ± 3.1	2.7 ± 1.3	0.4 ± 0.3	0.5 ± 0.1
Cr	26.7 ± 9.8^{a}	5.1 ± 3.9^{b}	1.4 ± 0.4^{b}	0.5 ± 0.3^{b}
Mn	5.8 ± 0.9^{a}	5.1 ± 0.6^{a}	2.1 ± 0.3^{b}	1.3 ± 0.2^{b}
Fe	19.5 ± 3.7^{a}	4.7 ± 2.6^{b}	2.5 ± 0.8^{b}	1.4 ± 0.2
Co	1.5 ± 0.7	1.9 ± 1.2	0.5 ± 0.2	0.4 ± 0.02
Ni	0.8 ± 0.5	2.1 ± 1.9	1.4 ± 0.7	0.6 ± 0.4
Cu	1.8 ± 0.3^{a}	1.2 ± 0.4^{ab}	0.6 ± 0.1^{b}	0.7 ± 0.2^{b}
Zn	2.8 ± 0.4^{a}	2.2 ± 1.0^{ab}	0.8 ± 0.1^{b}	0.8 ± 0.2^{b}
Ga	2.6 ± 1.8	2.0 ± 0.8	0.6 ± 0.2	0.6 ± 0.1
Rb	1.2 ± 0.1	0.9 ± 0.3	0.7 ± 0.1	1.1 ± 0.2
Sr	6.7 ± 2.7^{a}	3.1 ± 1.1^{ab}	0.8 ± 0.1^{b}	0.6 ± 0.04^{b}
Mo	3.5 ± 1.7	2.5 ± 1.0	5.2 ± 7.2	0.9 ± 0.6
Ag	5.3 ± 0.7	1.5 ± 0.8	3.0 ± 4.0	3.9 ± 5.1
Cd	9.4 ± 1.6^{a}	5.5 ± 1.0^{b}	2.5 ± 0.7^{bc}	2.1 ± 1.5^{c}
Cs	1.7 ± 0.4^{a}	1.0 ± 0.7^{ab}	0.3 ± 0.07^{b}	0.5 ± 0.2^{b}
Ba	2.4 ± 0.4^{a}	2.5 ± 0.9^{a}	0.8 ± 0.01^{b}	0.6 ± 0.1^{b}
Pb	0.3 ± 0.2^{b}	3.6 ± 2.3^{ab}	5.3 ± 1.3^{a}	7.6 ± 2.4^{a}

Different letters within lines indicate significant differences at $p < 0.05$.

Table S7. Heavy-metal health-risk assessment: heavy-metal concentrations, health-risk index (HRI), and targeted hazard quotient (THQ).

Parameter	Treatment	Cr	Mn	Fe	Cu	Sr	Cd	Pb
Concentration (mg/kg fresh weight)	Ctr	9.7	246.0	105.9	5.1	21.9	0.2	0.2
	SS20	5.2	215.8	63.0	4.2	450.4	0.2	0.5
	SS40	2.7	69.9	62.1	1.8	239.3	0.08	0.6
	SS60	0.8	39.9	62.7	1.5	247.9	0.07	0.6
Reference doses[1]		1.5	0.1	0.7	0.04	0.005	0.001	0.004
HRI[2]	Ctr	0.002	0.5	0.04	0.04	1.2	0.06	0.01
	SS20	0.001	0.5	0.03	0.03	27.3	0.06	0.04
	SS40	0.0005	0.1	0.02	0.01	13.2	0.02	0.05
	SS60	0.0001	0.07	0.02	0.01	12.5	0.02	0.04
THQ[3]	Ctr	0.001	0.4	0.03	0.03	0.9	0.04	0.01
	SS20	0.0007	0.3	0.02	0.02	18.3	0.04	0.03
	SS40	0.0004	0.1	0.02	0.009	9.7	0.02	0.03
	SS60	0.0001	0.06	0.02	0.008	10.1	0.01	0.03

1 United States Environment Protection Agency (USEPA); Unit: mg/kg/day.

2 Daily intake of vegetables (IR) and body weight (BW) were assumed to be 0.05 kg/day and 70 kg, respectively.

3 Exposure frequency (EF), exposure duration (ED), and average exposure time (AT) were assumed to be 104 days/year, 67 years, and ED × 365 days/year, respectively