

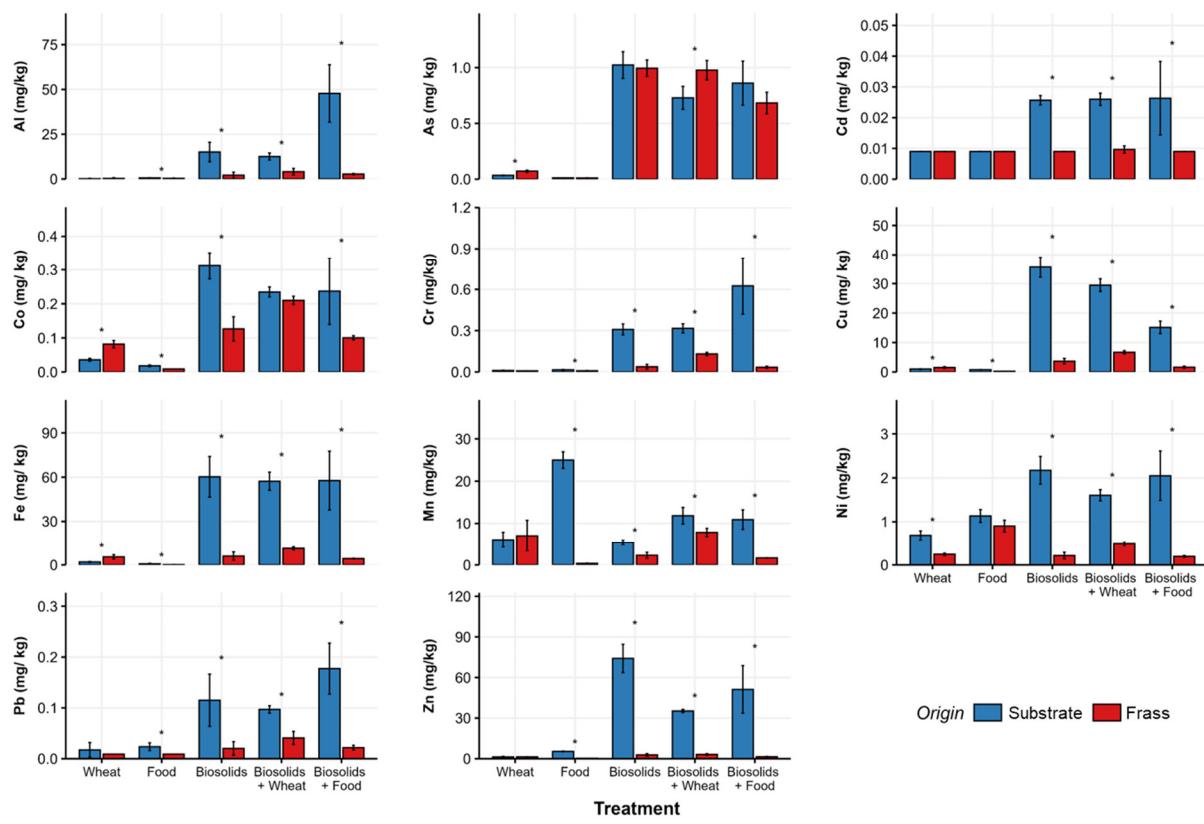
**Supplementary material:**

# **Analysis of Chemical and Phytotoxic Properties of Frass Derived from Black Soldier Fly-Based Bioconversion of Biosolids**

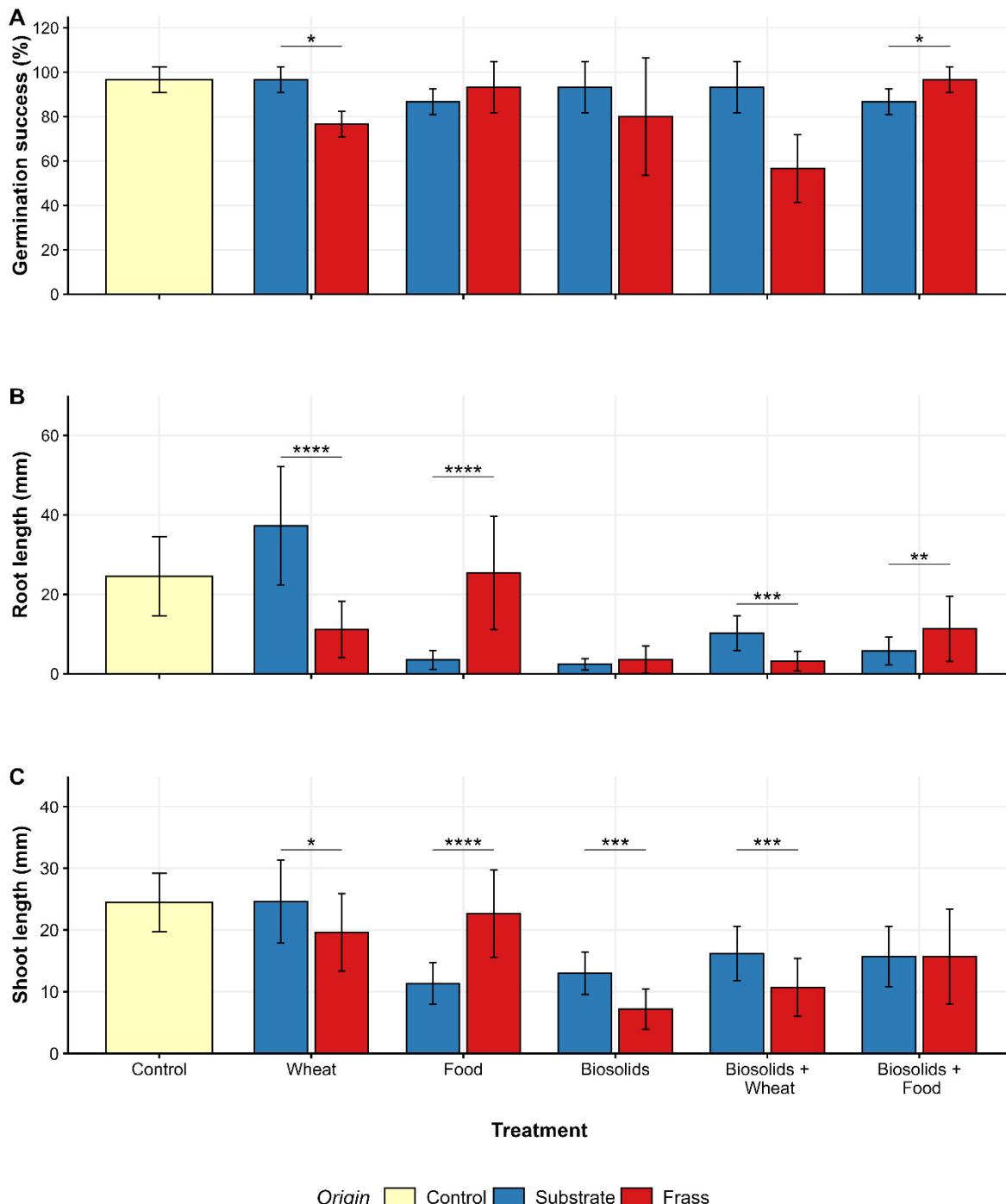
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**Table S1.** Kruskal-Wallis test results to compare differences between the type of feedstock processed by BSFL per chemical parameter as well as its origin (feedstock or frass). Significance codes: < 0.001 ‘\*\*\*’; < 0.01 ‘\*\*’; < 0.05 ‘\*’.

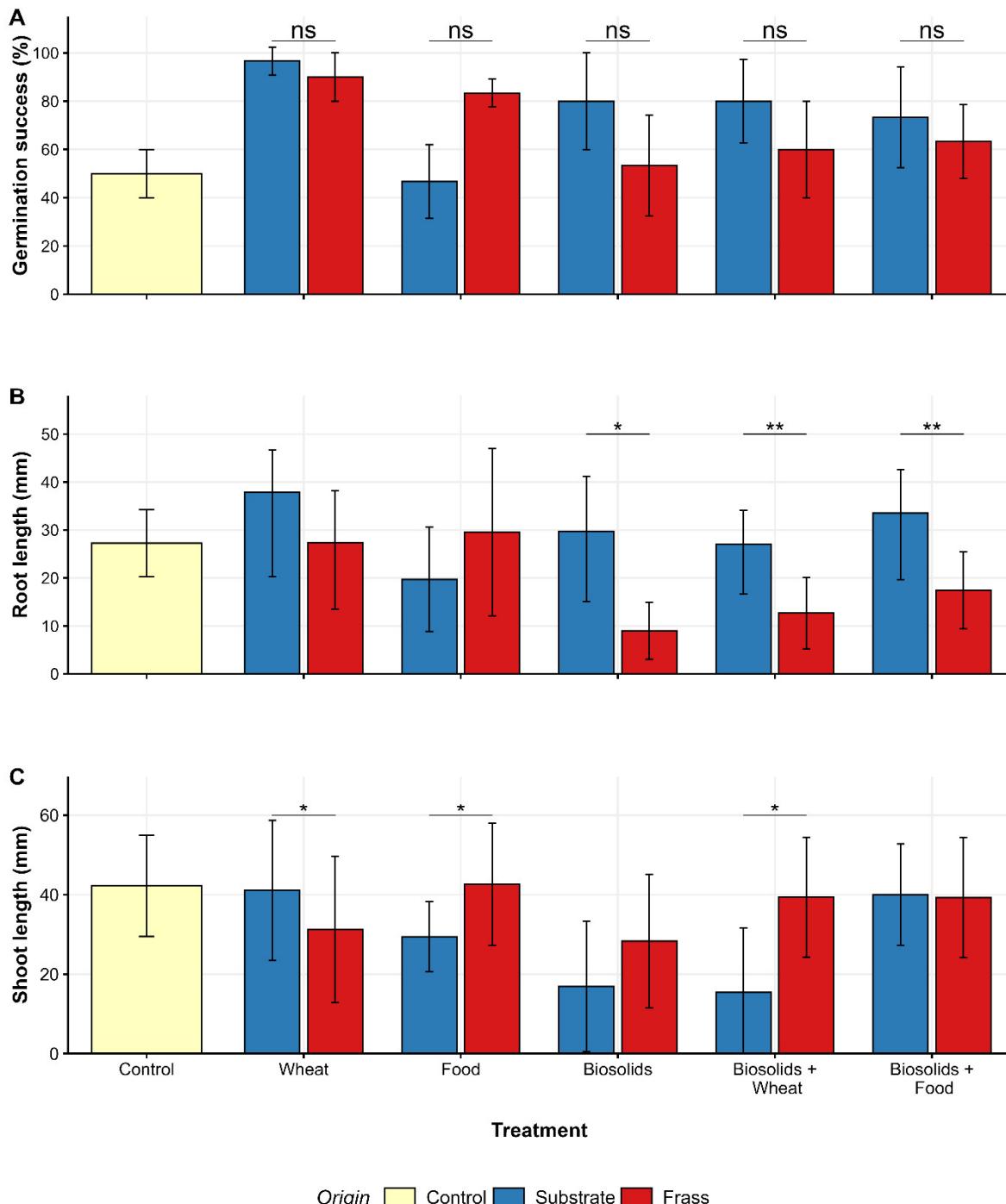
Origin	Parameter	n	statistic	df	p
Feedstock	Total C (%)	15	12.3	4	0.0156*
Feedstock	Total N (%)	15	13.5	4	0.00907**
Feedstock	C/N ratio	15	13	4	0.0111*
Feedstock	Total P (g/kg)	15	13	4	0.0111*
Feedstock	NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	15	11.6	4	0.0207*
Feedstock	NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	15	12.4	4	0.0147*
Feedstock	Organic matter (g/kg)	15	12.9	4	0.0115*
Feedstock	EC (mS/cm)	15	13.2	4	0.0102*
Feedstock	pH	15	13.2	4	0.0105*
Frass	Total C (%)	15	11.2	4	0.0241*
Frass	Total N (%)	15	13.5	4	0.00907**
Frass	C/N ratio	15	13.5	4	0.00907**
Frass	Total P (g/kg)	15	12.8	4	0.0121*
Frass	NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	15	5.6	4	0.231
Frass	NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	15	13.5	4	0.00907**
Frass	Organic matter (g/kg)	15	12.7	4	0.013*
Frass	EC (mS/cm)	15	12.9	4	0.012*
Frass	pH	15	6.73	4	0.151



**Figure S1.** Concentrations of bioavailable fraction of trace elements in BSFL feedstock (blue) and frass (red). Asterisks indicate significant differences between feedstock and frass per treatment ( $p < 0.05$ ). Missing asterisks indicate non-significance.



**Figure S2.** Germination success (A), root (B) and shoot length (C) of lettuce. Seeds were incubated on filter paper with water (control) or extracts of feedstocks and frass of BSFL feeding experiments of different treatments: Wheat bran (Wheat), Food waste (Food), dewatered sludge (Biosolids), dewatered sludge blended with Food waste in ratio 50:50 (Biosolids + Food), dewatered sludge blended with Wheat bran in the ratio 50:50 (Biosolids + Wheat). Asterisks represent significant differences of the respective parameter among feedstock and frass per treatment (Significance codes: < 0.001 ‘\*\*\*’; < 0.01 ‘\*\*’; < 0.05 ‘\*’). Missing asterisks indicate non-significance (ns). Mean values with standard deviations are displayed ( $n = 30$  [3 x 10 seeds]).



**Figure S3.** Germination success (A), root (B) and shoot length (C) of radish grown on water (control) or extracts of feedstocks or frass of BSFL feeding experiments of different treatments: Wheat bran (Wheat), Food waste (Food), dewatered sludge (Biosolids), dewatered sludge blended with Food waste in ratio 50:50 (Biosolids + Food), dewatered sludge blended with Wheat bran in the ratio 50:50 (Biosolids + Wheat). Asterisks represent significant differences of the respective parameter among feedstock and frass per treatment (Significance codes: < 0.001 ‘\*\*\*’; < 0.01 ‘\*\*’; < 0.05 ‘\*’). Missing asterisks indicate non-significance (ns). Mean values with standard deviations are displayed (n = 30 [3 x 10 seeds]).

**Table S2.** T-test results for differences of germination success (n = 3), seedling root or shoot length (n = 30) for extracts obtained from BSFL feedstock or frass compared to the control (water). Plant species tested was lettuce.

Variable tested	Group 1	Group 2	Origin	Statistic	df	P value <sup>1</sup>
Germination success	Control	Wheat	Feedstock	0.00	4.00	1.00
Germination success	Control	Food	Feedstock	2.23	2.75	0.60
Germination success	Control	Biosolids	Feedstock	0.25	3.56	1.00
Germination success	Control	Biosolids + Wheat	Feedstock	0.25	3.56	1.00
Germination success	Control	Biosolids + Food	Feedstock	2.23	2.75	0.60
Root length	Control	Wheat	Feedstock	11.80	29.20	6.15E-12****
Root length	Control	Food	Feedstock	9.49	35.60	1.39E-10****
Root length	Control	Biosolids	Feedstock	7.03	38.60	1.03E-07****
Root length	Control	Biosolids + Wheat	Feedstock	11.00	31.50	1.24E-11****
Root length	Control	Biosolids + Food	Feedstock	-3.82	48.90	0.002**
Shoot length	Control	Wheat	Feedstock	10.40	51.20	1.36E-13****
Shoot length	Control	Food	Feedstock	6.71	51.90	7.10E-08****
Shoot length	Control	Biosolids	Feedstock	6.79	54.90	4.13E-08****
Shoot length	Control	Biosolids + Wheat	Feedstock	11.90	50.60	1.47E-15****
Shoot length	Control	Biosolids + Food	Feedstock	-0.09	50.30	0.93
Germination success	Control	Wheat	Frass	3.47	2.51	0.053
Germination success	Control	Food	Frass	0.25	3.56	0.82
Germination success	Control	Biosolids	Frass	1.04	2.84	0.38
Germination success	Control	Biosolids + Wheat	Frass	4.36	3.88	0.013**
Germination success	Control	Biosolids + Food	Frass	0.00	4.00	1.00
Root length	Control	Wheat	Frass	15.60	49.60	4.83E-20****
Root length	Control	Food	Frass	5.21	46.70	2.08E-05****
Root length	Control	Biosolids	Frass	9.56	34.00	1.86E-10****
Root length	Control	Biosolids + Wheat	Frass	1.13	47.10	1.00
Root length	Control	Biosolids + Food	Frass	3.07	40.00	0.019*
Shoot length	Control	Wheat	Frass	15.60	49.60	4.83E-20****
Shoot length	Control	Food	Frass	5.21	46.70	2.08E-05****
Shoot length	Control	Biosolids	Frass	9.56	34.00	1.86E-10****
Shoot length	Control	Biosolids + Wheat	Frass	1.13	47.10	1.00
Shoot length	Control	Biosolids + Food	Frass	3.07	40.00	0.019*

<sup>1</sup>Significance codes: < 0.0001 '\*\*\*\*'; < 0.001 '\*\*\*'; < 0.01 '\*\*'; < 0.05 '\*'

**Table S3.** T-test results for differences of germination success ( $n = 3$ ), seedling root or shoot length ( $n = 30$ ) for extracts obtained from BSFL feedstock or frass compared to the control (water). Plant species tested was radish.

Variable tested	Group 1	Group 2	Origin	Statistic	df	P value <sup>1</sup>
Germination success	Control	Wheat	Feedstock	-5.56	3.08	0.011*
Germination success	Control	Food	Feedstock	0.53	2.8	0.64
Germination success	Control	Biosolids	Feedstock	-1.92	2.33	0.18
Germination success	Control	Biosolids + Wheat	Feedstock	-2.55	2.88	0.087
Germination success	Control	Biosolids + Food	Feedstock	-1.76	2.7	0.19
Root length	Control	Wheat	Feedstock	-2.97	40.8	0.025*
Root length	Control	Food	Feedstock	2.21	21.9	0.188
Root length	Control	Biosolids	Feedstock	-0.68	34.9	1
Root length	Control	Biosolids + Wheat	Feedstock	0.06	36.8	1
Root length	Control	Biosolids + Food	Feedstock	-1.57	24.7	0.65
Shoot length	Control	Wheat	Feedstock	0.04	36.1	1
Shoot length	Control	Food	Feedstock	4.29	22.8	0.001**
Shoot length	Control	Biosolids	Feedstock	6.87	28.4	8.50E-07****
Shoot length	Control	Biosolids + Wheat	Feedstock	5.85	35.6	5.75E-06****
Shoot length	Control	Biosolids + Food	Feedstock	1.15	34.5	1
Germination success	Control	Wheat	Frass	-3.51	2.7	0.046*
Germination success	Control	Food	Frass	-4.92	3.84	0.009**
Germination success	Control	Biosolids	Frass	-0.25	2.85	0.82
Germination success	Control	Biosolids + Wheat	Frass	-0.79	2.86	0.49
Germination success	Control	Biosolids + Food	Frass	-0.79	2.86	0.49
Root length	Control	Wheat	Frass	-0.02	39.5	1
Root length	Control	Food	Frass	-0.57	34.2	1
Root length	Control	Biosolids	Frass	7.84	27.7	8.35E-08****
Root length	Control	Biosolids + Wheat	Frass	5.82	30.6	1.08E-05****
Root length	Control	Biosolids + Food	Frass	4.02	33	0.002**
Shoot length	Control	Wheat	Frass	2.16	38.7	0.19
Shoot length	Control	Food	Frass	-0.1	34	1
Shoot length	Control	Biosolids	Frass	1.67	26.7	0.54
Shoot length	Control	Biosolids + Wheat	Frass	0.08	30.4	1
Shoot length	Control	Biosolids + Food	Frass	0.48	29.9	1

<sup>1</sup>Significance codes: < 0.0001 ‘\*\*\*\*’; < 0.001 ‘\*\*\*’; < 0.01 ‘\*\*’; < 0.05 ‘\*’

**Table S4.** Spearman's rank correlation results for germination parameters in relation to chemical composition of BSFL feedstocks and frass. Plant species tested was lettuce.

Parameter	RSG	RGG	GI
Total carbon	0.30	-0.05	0.03
Total nitrogen	0.20	<b>-0.38**</b>	-0.32
C/N ratio	-0.21	<b>0.37*</b>	0.33
NO <sub>3</sub> -N	-0.13	<b>-0.58**</b>	<b>-0.48**</b>
NH <sub>4</sub> -N	-0.31	<b>-0.49**</b>	<b>-0.54**</b>
Total recoverable P	-0.32	-0.16	-0.24
Organic matter	0.03	0.19	0.19
pH	-0.21	0.15	0.06
EC	<b>-0.53**</b>	<b>-0.63***</b>	<b>-0.69***</b>
Sum of macro elements	-0.02	0.13	0.14
Ca	0.25	-0.07	0.01
K	-0.05	<b>0.54**</b>	<b>0.51**</b>
Mg	-0.22	0.07	-0.02
Na	0.01	<b>-0.55**</b>	<b>-0.53**</b>
S	-0.12	<b>-0.53**</b>	<b>-0.54**</b>
Al (bioavailable)	-0.03	<b>-0.47*</b>	<b>-0.46*</b>
As (bioavailable)	-0.24	<b>-0.57**</b>	<b>-0.60***</b>
Cd (bioavailable)	0.06	-0.29	-0.25
Co (bioavailable)	-0.10	<b>-0.45*</b>	<b>-0.46**</b>
Cr (bioavailable)	-0.04	<b>-0.49**</b>	<b>-0.47**</b>
Cu (bioavailable)	-0.06	<b>-0.48**</b>	<b>-0.47**</b>
Fe (bioavailable)	-0.11	<b>-0.38*</b>	<b>-0.39*</b>
Mn (bioavailable)	-0.25	-0.29	-0.29
Ni (bioavailable)	0.18	-0.16	-0.10
Pb (bioavailable)	-0.05	<b>-0.40*</b>	<b>-0.38*</b>
Zn (bioavailable)	0.04	<b>-0.63*</b>	<b>-0.57*</b>

**Table S5.** Spearman's rank correlation results for germination parameters in relation to chemical composition of BSFL feedstocks and frass. Plant species tested was radish.

Parameter	RSG	RGG	GI
Total carbon	0.25	0.27	0.13
Total nitrogen	0.43	0.57	0.42
C/N ratio	-0.48	-0.54	-0.43
NO <sub>3</sub> -N	<b>-0.93***</b>	<b>-0.87**</b>	<b>-1.00***</b>
NH <sub>4</sub> -N	-0.22	-0.06	-0.3
Total recoverable P	0.61	0.43	0.47
Organic matter	<b>0.87**</b>	<b>0.87**</b>	<b>0.94***</b>
pH	<b>0.78*</b>	<b>0.78*</b>	<b>0.86**</b>
EC	<b>-0.97****</b>	<b>-0.74*</b>	<b>-0.93***</b>
Sum of macro elements	<b>-0.80**</b>	<b>-0.75*</b>	<b>-0.77*</b>
Ca	<b>-0.90***</b>	<b>-0.83**</b>	<b>-0.92***</b>
K	-0.27	-0.31	-0.18
Mg	<b>0.82**</b>	<b>0.81**</b>	<b>0.92***</b>
Na	-0.43	-0.26	-0.47
S	0.43	0.51	0.42
Al (bioavailable)	-0.43	-0.36	-0.54
As (bioavailable)	0.53	0.43	0.44
Cd (bioavailable)	0.03	0.17	-0.02
Co (bioavailable)	0.47	0.54	0.43
Cr (bioavailable)	-0.41	-0.21	-0.46
Cu (bioavailable)	0.48	0.52	0.42
Fe (bioavailable)	0.4	0.43	0.37
Mn (bioavailable)	-0.70*	-0.54	-0.63
Ni (bioavailable)	-0.4	-0.29	-0.48
Pb (bioavailable)	-0.24	-0.04	-0.28
Zn (bioavailable)	-0.42	-0.34	-0.53