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

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Abstract: Black soldier fly (BSF)-based bioconversion can reduce significant volumes of biosolids and other organic waste while generating high-value BSF larvae (BSFL) and frass. While the mass of frass is greater than the BSFL biomass, its end use is less explored, especially when the bioprocessed waste, such as biosolids, contains high concentrations of contaminants. We assessed the potential to use frass from bioconverted biosolids as fertiliser by analysing chemical parameters and conducting phytotoxicity germination tests. We included frass from bioconverted food waste and wheat bran as comparisons. The chemical composition and phytotoxicity of the frass was related to the type of feedstock. Frass originating from biosolids and from wheat bran had the highest phytotoxicity, which was correlated with increased NH_4^+-N and EC. Initially, these feedstocks had significantly higher total N compared to food waste. Frass derived from food waste showed the lowest phytotoxicity, which was related to low NH_4^+-N and EC. This study demonstrates that frass from BSF-based bioconversion could be used as fertiliser; however, the original feedstock will dictate how this by-product has to be used. In this study, frass from food waste was most suitable as fertiliser. Frass originating from bioconverted biosolids needs to be applied at similar rates as unconverted biosolids to avoid phytotoxicity.

Keywords: black soldier fly; bioconversion; organic waste; biosolids; frass; phytotoxicity; seed germination test; fertilizer

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1. Introduction

Globally, around two billion tonnes of solid waste is produced annually, of which food and green waste make up 44% [1]. Similarly, some 30 million tonnes (dry matter equivalent) of sewage sludge is produced [2]. Most organic waste is disposed of in landfills (37%), open dumps (33%) or in the ocean [1]. These practices constitute a threat to the environment and, consequently, public health due to greenhouse gas emissions and leaching of high concentrations of nutrients and toxic compounds into water and soils [3]. Currently, poor financial outcomes are associated with organic waste recovery, as costs of collecting and treating often exceed revenue from generated products [4]. Therefore, there is little incentive to work towards achieving the UN Sustainable Development Goals, including "Clean Water and Sanitation", "Sustainable Cities and Communities", and "Climate Action and Sustainable" [5].

An emerging organic waste management option that applies the principles of a circular bioeconomy [5] is black soldier fly (BSF)-based bioconversion. This insect-based technology converts organic waste into two valuable products: mature, protein-rich larvae and processing residues (also called frass). Frass is a mixture of larval excrement, undigested organic waste and shed exoskeletons. Compared to conventional composting, BSF-based bioconversion is reported to be more economically viable [6,7], quicker in reducing large amounts of waste (up to 80% in two weeks [8] compared to a 10% reduction in eight weeks with (vermi-)composting [9]) and more sustainable in terms of greenhouse gas and ammonia emissions [10–12].

Mature larvae from BSF-based bioconversion are commonly marketed as high-value animal feed, but larvae products could also be used for the development of other high-value products [13]. While the literature is mainly focused on the improvement of larvae growth and biomass composition [14], there is scant research on the beneficial use of the frass, even though it is produced in larger masses than the BSFL biomass [5]. Some studies have indicated that frass can be viable as soil conditioner or organic fertiliser [5,15]. However, the question is if that is always the case, especially when the original feedstock contains high concentrations of contaminants like heavy metals that could be toxic to plants.

Bohm et al. [16] demonstrated that BSF-based bioconversion could be a promising technology to reduce significant masses (<40%) of biosolids (i.e., treated sewage sludge [17]) in a short period of time (20 days). In addition, they showed that this technology can generate high-value BSFL biomass with low heavy metal concentrations from this type of emerging organic waste. However, in order to suggest BSF-based bioconversion as a sustainable waste management option for the treatment of biosolids, it is important to also explore potential end uses of the generated frass, which can accumulate with more than four times higher mass than the mature BSFL [15].

This study aimed to determine whether frass from BSF-based bioconversion of biosolids or biosolid blends could be used as fertiliser. The chemical composition of the frass was analysed and phytotoxicity tests with lettuce and radish were performed. Wheat bran and food waste were the organic wastes used as controls because they are commonly used as feedstocks for BSFL [14]. We sought to identify chemical parameters related to causing phytotoxicity or plant growth promotion and relate these to the chemistry of the feedstocks.

2. Material and Methods

2.1. Origin of BSFL Feedstock and Frass

The frass and feedstock used in this research were obtained from the bioconversion experiments with BSFL performed by Bohm et al. [16]. The frass was the product of 20 days of incubation of wheat bran (wheat), food waste (food), dewatered sludge (biosolids), dewatered sludge mixed with wheat bran (biosolids + wheat) and food waste (biosolids + food) in 50:50 ratios (w/w, based on fresh weight). The chemical composition of the feedstocks was described previously [16].

2.2. Chemical Analysis of BSFL Feedstocks and Frass

Concentrations of organic matter, recoverable phosphorus, total carbon and nitrogen in the frass were analysed as described by Bohm et al. [16]. Electrical conductivity (EC) and pH were measured for the extracts of the BSFL feedstocks or frass according to Bohm et al. [16].

Total extractable concentrations of trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn), as well as macronutrients (N, Ca, Mg, Na, K, S), were analysed previously for both the feedstock and frass [16]. Here, concentrations of the $Ca(NO_3)_2$-extractable fraction of the trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn) for the BSFL feedstocks and frass were obtained according to the method described by Blakemore et al. [18]. In brief, 2.5 g of dried sample material was mixed with 15 mL 0.05 M $Ca(NO_3)_2$ solution and shaken for 120 min on a GFL Overhead Rotator 3040 (Lauda, Berlin, Germany). Subsequently, the solution was centrifuged (5 min at 3500 rpm) and filtered using 42 Whatman paper (Sigma Aldrich, Sydney, Australia). Then, 0.5 mL of filtered extract was digested with 10 mL 2% HNO_3 and analysed using an Agilent 8900 Triple Quadrupole ICP-MS (Agilent, Santa Clara, CA, USA).

To determine the concentration of nitrate nitrogen (NO_3^--N) in the BSFL feedstocks and frass, a colorimetric method described by Blakemore et al. [18] and Miranda et al. [19] was used. The concentration of ammonium nitrogen (NH_4^+-N) in BSFL feedstocks and frass was determined following the method described by Mulvaney [20]. For both NO_3^--N and NH_4^+-N analyses, a KCl extract was prepared first: 40 mL 2 M KCl was added to 4 g of sample and mixed for 1 h at 22 rpm; the KCl extracts were filtered through 42 Whatman filter paper.

2.3. Seed Germination Assay with Extracts from BSFL Feedstock and Frass

Seed germination tests were performed for BSFL feedstocks and frass. A seed germination test is a common way to evaluate compost maturity and to detect any phytotoxic effects [21,22]. The seed germination test procedure consists of three steps: (1) preparation of the aqueous extract of the plant breeding material, (2) incubation of the seeds with the extract and (3) measuring and calculating the relevant indicators, including relative seed germination (RSG), root growth and relative radicle growth (RGG), as well as the seed germination index (GI) [21]. We selected lettuce (*Lactuca sativa*, cultivar Great Lakes) and radish (*Raphanus sativus*, cultivar French Breakfast) for the seed germination test. These two crops are commonly used to identify the phytotoxicity of compost or soils [21].

Extracts of BSFL feedstocks and frass were prepared by mixing the frozen sample with demi-water in a 1:10 ratio (*w*/*v*) and shaking for 1 h at 20 °C and 250 rpm. The solid fraction was separated by 15 min of centrifugation at 20 °C and 4700 rpm. The extracts were filter-sterilized through a 0.22 µm syringe filter (Millex®GP, Thermo Fisher Scientific, Scoresby, Australia) and stored at 4 °C until use. For the seed germination test, seeds were surface sterilized following Armas et al. [23]. Then, 2 mL extract solution or 2 mL sterile deionized water (i.e., control) were added on autoclaved filter paper in sterile Petri dishes, and ten seeds per replicate were placed on top. For each treatment, three replicates were set up. Petri dishes were sealed with parafilm and incubated in a growth chamber at 25 °C and a light intensity of 350 µmol PAR $m^{-2}s^{-1}$ with a 12 h dark–light cycle. After one week of incubation, the germination rate was measured, and germinated seedlings were harvested to measure the length of roots and shoots. The dry weight of the seedlings was determined after 24 h drying at 60 °C. RSG, RRG and GI were calculated according to Luo et al. [21] (Equations (1)–(3)):

$$\text{RSG} = (\text{SGS}/\text{SGC}) \times 100\% \quad (1)$$

where SG_S is the number of germinated seeds for the treatment and SG_C is the number of germinated seeds for the control;

$$\text{RRG} = (RG_S/RG_C) \times 100\% \quad (2)$$

where RG_S is the total radicle length of the germinated seeds for the treatment and RG_C is the total radicle length of the germinated seeds for the control;

$$\text{GI} = \text{RSG} \times \text{RGG} \times 100\% \quad (3)$$

2.4. Statistical Analysis

Differences in the compositions of the $Ca(NO_3)_2$-extractable fractions of trace elements for the feedstock and frass and the different treatments were analysed using two-way ANOVA followed by a Tukey HSD test [16]. A paired T-test with Bonferroni correction using the R package *rstatix* [24] was applied to compare differences in the RSG, RRG, GI, germination success, root or shoot length and concentrations of $Ca(NO_3)_2$-extractable fractions of heavy metals and trace elements for the BSFL feedstock and frass within each treatment. Values for the germination success were arcsin transformed before performing the T-test. Kruskal–Wallis and Dunn's tests were conducted to compare differences in the chemical parameters per treatment for frass and the feedstocks, as well as to compare differences in the GI for different dilutions of the frass extracts per treatment. Spearman's rank correlation analysis was performed with R package *corx* [25] to test for potential relationships between RSG, RRG or GI and the chemical composition of the feedstocks and frass. Results were visualized using *ggcorrplot* [26]. All statistical tests and graphical interpretations were performed with RStudio 1.4.17 based on R 4.1.0 [27].

3. Results

3.1. Analysis of the Chemical Composition of BSFL Frass and Feedstocks

The chemical composition of the frass (e.g., total C, N and P; organic matter; EC) differed significantly between the treatments, which was associated with differences in the chemical composition of the original feedstocks fed to the BSFL (Figure 1, Table S1). Generally, BSF-based bioconversion led to significant reductions in total C, total N (except for food waste) and organic matter, while concentrations of total P and NH_4^+-N and EC increased significantly (Table 1, Figure 1). Frass of the biosolids treatments had significantly higher total N (4–5% compared to 2% for food waste and wheat bran) and NH_4^+-N concentrations (6.8 g/kg) compared to the control treatments (1–2 g/kg for food waste and wheat bran) (Figure 1). Similarly, the originating feedstocks for the biosolids treatments had higher concentrations of total N compared to wheat bran and food waste and the C/N ratio was correspondingly lower (Figure 1, Table S1). There were no significant differences between the NO_3^--N concentrations of the BSFL feedstocks and frass except for the food waste. The NO_3^--N concentration in the food waste was about three times higher than the other BSFL feedstocks. BSF-based bioconversion of food waste resulted in a decrease in NO_3^--N (Figure 1). For the food waste and biosolids blended with food waste, EC significantly decreased from around 2 mS/cm to 1.3–1.6 mS/cm, while the EC for the other treatments, especially for the biosolids and biosolids blended with wheat bran, was significantly higher in the frass (<2 times) after the bioconversion process.

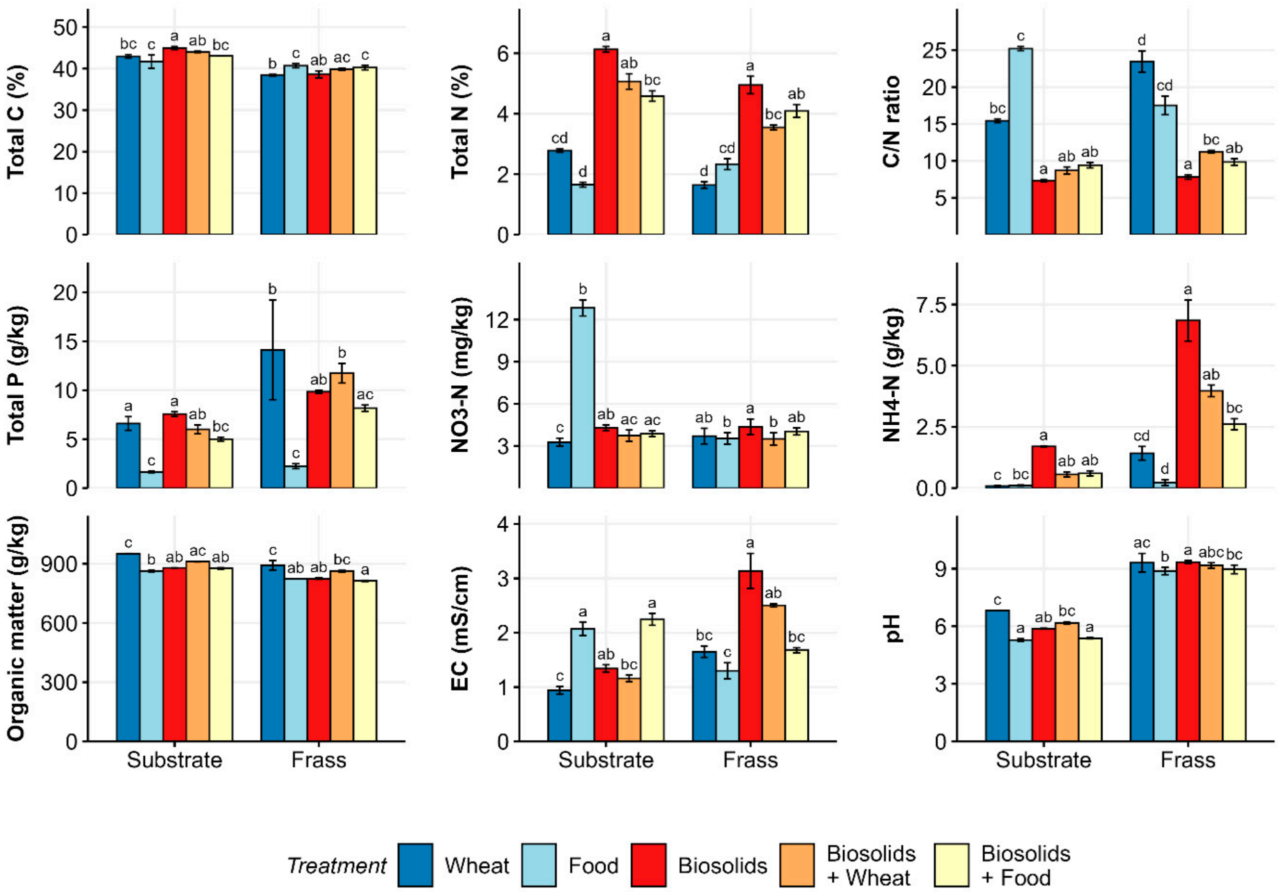

Figure 1. Chemical composition of feeding feedstocks and frass. Mean values with standard deviation (n = 3) are shown. Different letters represent significant differences in the respective parameters between treatments per origin (feedstock or frass). Treatments: wheat bran (wheat), food waste (food), dewatered sludge (biosolids), dewatered sludge blended with food waste in 50:50 ratio (biosolids + food), dewatered sludge blended with wheat bran in 50:50 ratio (biosolids + wheat).

Table 1. Kruskal–Wallis test results for tested differences between the feedstock and frass per chemical parameter. Significance codes: <0.0001 "****"; <0.001 "***"; <0.01 "**"; <0.05 "*".

Parameter	n	Statistic	df	*p*-Value
Total C (%)	30	19.5	1	1.0×10^{-5} ****
Total N (%)	30	2.29	1	0.13
C/N ratio	30	0.795	1	0.373
Total P (g/kg)	30	10.1	1	0.0015 **
NO_3^--N (mg/kg)	30	0.876	1	0.349
NH_4^+-N (mg/kg)	30	10.9	1	0.00097 ***
Organic matter (g/kg)	30	13.5	1	0.00024 ***
EC (mS/cm)	30	4.56	1	0.0327 *
pH	30	21.8	1	3.1×10^{-6} ****

Although concentrations of the $Ca(NO_3)_2$-extractable fraction of trace elements were significantly higher for the biosolids containing feedstocks, the concentrations of most of these elements (except for As) decreased significantly after the bioconversion process and were not significantly higher for the frass compared to wheat bran or food waste (Table 2 and Figure S1 in the Supplementary Material).

3.2. Phytotoxicity of BSFL Frass and Feedstocks against Lettuce and Radish

Some of the changes induced in the chemical composition of the feedstocks by BSF-based bioconversion may improve plant germination or seedling growth. Of particular interest was frass obtained from biosolids treatments. Seed germination tests showed that the germination (RSG), root growth (RGG) and shoot growth of lettuce and radish were significantly affected by the type of frass (Figures 2, S2 and S3 and Tables S2 and S3). However, larger differences between the frass originating from the different feedstocks were observed for RGG but not RSG (Figure 2A,B,D,E). Moreover, germination of lettuce was more affected by the type of frass compared to radish (Figure 2). The mean GI over all treatments of radish was significantly higher compared to lettuce ($p < 0.0001$). The frass that produced the highest RGG—and, accordingly, seed germination index (GI)—for lettuce originated from bioconverted food waste; the average GI was around 100% (Figure 2B,C). Furthermore, the GIs for frass derived from bioconverted wheat bran and all three biosolids containing feedstocks were below 50%. In the case of radish, the highest RGG and GI were observed for the frass originating from wheat bran and food waste; the average GI for these frass types were above 100% (Figure 2E,F). Germination of radish was inhibited by frass from the biosolids treatments, resulting in a GI of 35% to 79% (Figure 2F).

Interestingly, BSF-based bioconversion of the original feedstocks significantly affected the phytotoxicity of the resulting frass (Figure 2, Table 3). For lettuce, the GIs for the frass from the wheat bran and biosolids + wheat treatments were about 3 to 10 times lower compared to the feedstocks, while the GI for the frass originating from food waste was about 10 times higher compared to the feedstocks (Figure 2A). A similar trend was observed for radish; however, the reduction in the GI for the wheat bran treatment was less pronounced and the GI for the frass from the biosolids treatment was significantly lower (about three times) compared to the original feedstock (Table 3).

Table 2. Concentrations of the $Ca(NO_3)_2$-extractable fractions of trace elements (in mg/kg based on dry weight) in BSFL feeding feedstocks and frass. Values are means ± SEM (n = 3) per treatment group. Means in a row without a common letter differ ($p < 0.05$), as analysed by two-way ANOVA and the Tukey test.

Trace Element	Feedstock					Frass					*p*-Value		
	Wheat	Food	Biosolids 1	Biosolids 1 + Wheat	Biosolids 1 + Food	Wheat	Food	Biosolids 1	Biosolids 1 + Wheat	Biosolids 1 + Food	Origin [1]	Treatment	O × T [2]
Al	0.14 ± 0.13 d	0.60 ± 0.07 d	15.1 ± 3.13 cd	12.60 ± 1.13 cd	47.80 ± 9.24 ab	0.32 ± 0.24 d	0.37 ± 0.08 d	2.14 ± 0.97 d	4.07 ± 1.05 d	2.75 ± 0.18 d	<0.001	<0.001	<0.001
Cr	0.010 ± 0.002 d	0.020 ± 0.002 d	0.31 ± 0.02 bc	0.32 ± 0.02 b	0.63 ± 0.12 a	0.01 ± 0.00 d	0.01 ± 0.00 d	0.04 ± 0.01 d	0.13 ± 0.01 bd	0.040 ± 0.004 d	<0.001	<0.001	<0.001
Mn	6.10 ± 1.04 bd	25.00 ± 1.13 a	5.40 ± 0.35 bd	11.80 ± 1.13 b	10.90 ± 1.34 b	7.07 ± 2.12 bd	0.36 ± 0.04 d	2.28 ± 0.41 cd	7.87 ± 0.56 bc	1.65 ± 0.03 cd	<0.001	<0.001	<0.001
Fe	2.0 ± 0.2 b	0.75 ± 0.15 b	60.30 ± 7.92 a	57.20 ± 3.55 a	57.7 ± 11.5 a	5.51 ± 0.86 b	0.21 ± 0.03 b	6.03 ± 1.64 b	11.20 ± 0.59 b	4.34 ± 0.11 b	<0.001	<0.001	<0.001
Co	0.04 ± 0.002 ef	0.020 ± 0.002 f	0.31 ± 0.02 a	0.23 ± 0.01 ab	0.24 ± 0.06 ab	0.08 ± 0.01 df	0.01 ± 0.00 f	0.13 ± 0.02 cde	0.21 ± 0.01 bc	0.100 ± 0.004 df	0.001	<0.001	<0.001
Ni	0.69 ± 0.06 cd	1.13 ± 0.08 bc	2.17 ± 0.18 a	1.60 ± 0.10 ab	2.05 ± 0.33 a	0.24 ± 0.02 d	0.91 ± 0.08 c	0.22 ± 0.04 d	0.49 ± 0.03 cd	0.20 ± 0.02 d	<0.001	<0.001	<0.001
Cu	0.98 ± 0.06 f	0.77 ± 0.03 f	35.6 ± 2.0 a	29.40 ± 1.24 b	15.10 ± 1.23 c	1.54 ± 0.16 ef	0.24 ± 0.03 f	3.69 ± 0.53 df	6.70 ± 0.33 d	1.68 ± 0.18 ef	<0.001	<0.001	<0.001
Zn	1.31 ± 0.23 c	5.41 ± 0.11 c	74.10 ± 6.06 a	35.20 ± 0.66 b	51.2 ± 10.1 b	1.37 ± 0.06 c	0.31 ± 0.02 c	2.83 ± 0.58 c	3.24 ± 0.32 c	1.52 ± 0.08 c	<0.001	<0.001	<0.001
As	0.030 ± 0.001 d	0.01 ± 0.00 d	1.02 ± 0.07 a	0.73 ± 0.06 bc	0.86 ± 0.11 ac	0.07 ± 0.01 d	0.010 ± 0.001 d	0.99 ± 0.04 a	0.97 ± 0.05 ab	0.68 ± 0.06 c	0.535	<0.001	0.01
Cd	0.009 ± 0.000 b	0.009 ± 0.000 b	0.030 ± 0.001 a	0.030 ± 0.001 a	0.03 ± 0.01 a	0.01 ± 0.00 b	0.01 ± 0.00 b	0.01 ± 0.00 b	0.010 ± 0.001 b	0.01 ± 0.00 b	<0.001	<0.001	<0.001
Pb	0.02 ± 0.01 d	0.020 ± 0.004 d	0.12 ± 0.03 ab	0.097 ± 0.004 bc	0.18 ± 0.03 a	0.01 ± 0.00 d	0.01 ± 0.00 d	0.02 ± 0.01 d	0.04 ± 0.01 cd	0.020 ± 0.003 d	<0.001	<0.001	<0.001

[1] Origin: feedstock versus frass; [2] O × T = origin × treatment interaction effect.

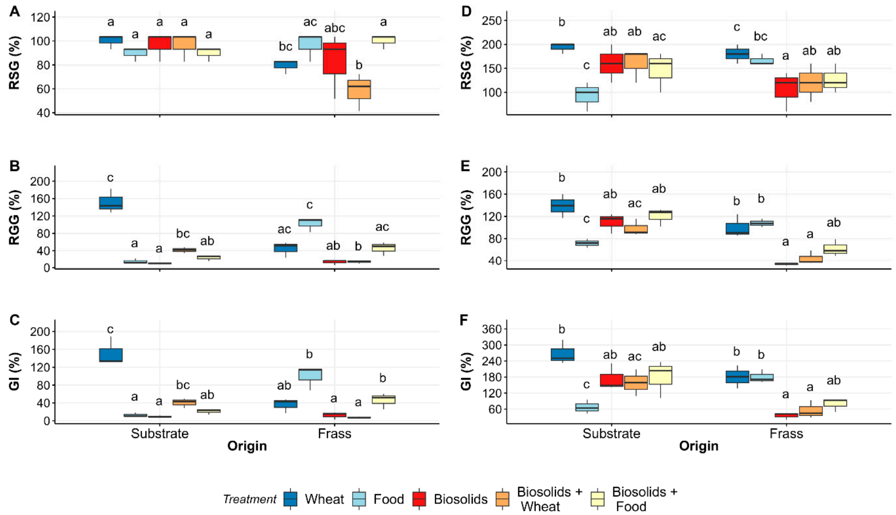

Figure 2. Relative seed germination (RSG), relative radicle growth (RGG) and seed germination index (GI) as indications for toxicity of BSFL feedstock or frass against lettuce (**A**–**C**) or radish (**D**–**F**). BSFL treatments: wheat bran (wheat), food waste (food), dewatered sludge (biosolids), dewatered sludge blended with food waste in 50:50 ratio (biosolids + food), dewatered sludge blended with wheat bran in 50:50 ratio (biosolids + wheat). Different letters indicate significant differences amongst each group (feedstock or frass).

Table 3. T-test results for tested differences in GI between the feedstock (n1) and frass (n2) per treatment: wheat bran (wheat), food waste (food), dewatered sludge (biosolids), dewatered sludge blended with food waste in 50:50 ratio (biosolids + food), dewatered sludge blended with wheat bran in 50:50 ratio (biosolids + wheat). Significance codes: <0.01 "**"; <0.05 "*".

Plant Species	Treatment	n1	n2	Statistic	df	*p*-Value
Lettuce	Wheat	3	3	5.52	2.97	0.012 *
Lettuce	Food	3	3	−5.5	2.12	0.028 *
Lettuce	Biosolids	3	3	−0.552	2.24	0.631
Lettuce	Biosolids + wheat	3	3	5.24	2.07	0.032 *
Lettuce	Biosolids + food	3	3	−2.31	2.44	0.124
Radish	Wheat	3	3	2.43	3.99	0.072
Radish	Food	3	3	−5.43	3.99	0.006 **
Radish	Biosolids	3	3	4.66	2.26	0.034 *
Radish	Biosolids + wheat	3	3	2.98	3.54	0.048 *
Radish	Biosolids + food	3	3	−2.35	2.54	0.116

Phytotoxicity tests of dilutions from the BSFL frass extracts derived from the biosolids treatments were performed to test if the inhibition of seed germination and root growth could be minimized by diluting out toxic elements. For lettuce, the GI of the diluted frass extracts was significantly higher compared to the non-diluted extract (Figure 3A). There were linear increases in the GIs for the biosolids and biosolids + wheat treatments from around 20% to 120% and 90%, respectively. The highest GI scores for lettuce occurred with the highest dilutions of the frass extracts (25%; i.e., fourfold dilution) for all treatments (Figure 3A). With regard to radish, GIs for the dilutions of the frass extracts of the biosolids and biosolids + wheat treatments increased significantly, reaching around 130% and 170% with the highest dilution, respectively (Figure 3B). Diluting the frass extract of the biosolids + food treatment had no significant impact on the germination.

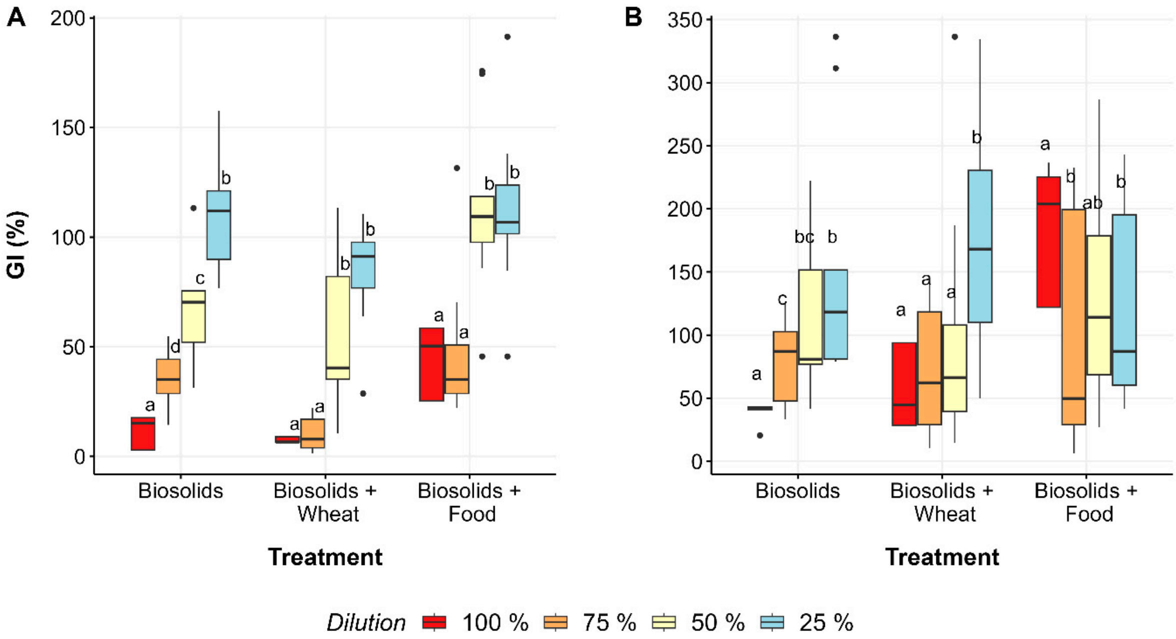

Figure 3. Seed germination index (GI) for germination assays of diluted frass extracts with lettuce (**A**) and radish (**B**). The extracts were obtained from the following treatments: dewatered sludge (biosolids), dewatered sludge blended with food waste in 50:50 ratio (biosolids + food) and dewatered sludge blended with wheat bran in 50:50 ratio (biosolids + wheat). The dilution percentages indicate the concentration of original extract present in the dilution. Different letters display significant differences ($p < 0.05$) between treatments.

3.3. Chemical Parameters Related to Germination Success and Seedling Growth

Figure 4 shows the Spearman rank correlation analysis of the RSG, RGG and GI and the chemical parameters of all BSFL feedstocks and frass. Only a few parameters were significantly positively correlated with the RSG, RGG or GI. These were, in the case of lettuce, the C/N ratio and concentrations of K and, in the case of radish, organic matter, pH and Mg concentrations. For both plant species, the RSG, RGG and GI were significantly negatively correlated with EC and NO_3^--N. In the case of lettuce, significant negative correlations with the RGG and GI were also obtained for NH_4^+-N, Na, S and the $Ca(NO_3)_2$-extractable fraction of the trace elements Al, As, Co, Cr, Cu, Fe, Pb and Zn (Figure 4A). Significant negative correlations for Ca, total macro-elements and $Ca(NO_3)_2$-extractable Mn with the RSG, RGG or GI were additionally obtained for radish (Figure 4B). Overall, more chemical parameters were significantly correlated with the RSG compared to the RGG. This indicates that the radicle extension (RGG) was more sensitive to toxic elements or unfavourable conditions compared to the overall germination rate (RSG). Furthermore, a higher number of chemical parameters were significantly correlated with the RSG, RGG or GI (16 in total) in the case of lettuce (Figure 4A) compared to radish (8 significant correlations in total; Figure 4B).

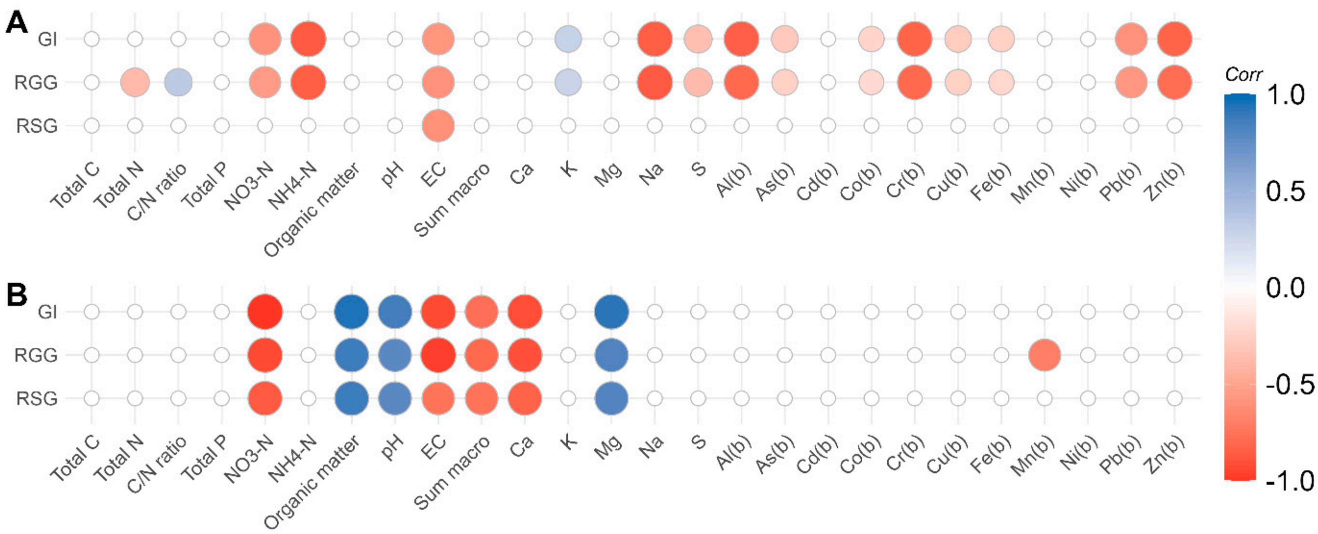

Figure 4. Spearman rank correlations for germination parameters (relative seed germination (RSG), relative radicle growth (RGG) and seed germination index (GI)) measured for lettuce (**A**) and radish (**B**) against chemical parameters of feedstock and frass extracts obtained from BSF feeding experiments with different treatments (see Figures 1 and 2). Only significant correlations ($p < 0.05$) are displayed (coloured circles). The fill colour of the circles represents the correlation coefficient (Corr), with white to blue being positive and white to red being negative.

4. Discussion

Here, we investigated if frass from bioconverted biosolids and blends of biosolids can be potentially used as fertiliser and compared results from seed germination tests and chemical analysis with those for the frass originating from wheat bran or food waste. For the seed germination tests, we observed that root growth was generally more affected by the different types of frass compared to the germination rate. Moreover, germination of lettuce was significantly lower compared to radish, indicating that lettuce seemed to be more sensitive to the presence of phytotoxins. This aligns well with a study by Di Salvatore et al. [28] where lettuce was more sensitive than other crops, including radish, to elevated concentrations of trace elements in the growth medium. They also observed that, overall, the germination rate was less affected by the type of medium and concentration of trace elements compared to the root growth. Therefore, the GI, combining the RSG and

RGG, which can both reflect phytotoxicity, is widely adopted to measure the quality of compost [29] and even included in regulations for compost commercialization [30].

A GI >80% indicates that the germination medium is safe to use [31]. In the case of lettuce, frass obtained from BSF-based bioconversion of biosolids containing feedstocks and from wheat bran resulted in GI levels below 60%. For radish, GI levels of frass originating from the biosolids treatments were below 80% to 50%. Frass from the biosolids containing feedstocks became richer in NH_4^+-N and total P. However, concentrations of the macro-elements Na, K, Mg and S [16] also increased, which might have been correlated with elevated EC levels (i.e., salinity) in these types of frass. These observations align with a previous BSF feeding study on sewage sludge [32]. In the case of bioconverted wheat bran, the frass showed significantly elevated NH_4^+-N, total P and EC. Spearman rank correlation analysis showed that all these factors, including elevated levels of S and salts, were significantly negatively correlated with the GI. Increases in NH_4^+-N and EC were also reported by other BSF studies; e.g., using sewage sludge, wheat bran, chicken manure and grass cuttings [32–35]. Liu et al. [36] attributed high EC and a high concentration of NH_4^+-N to the lack of maturity of chicken manure frass and a negative correlation between the GI and NH_4^+-N or EC, and similar results as in this study have been reported for biosolids and composted pig manure [37–39]. Moreover, negative effects of high S concentrations and salinity on plant growth have been reported as well [40,41]. NH_4^+-N production in BSFL is driven by protein metabolism [42], which is why BSF-based bioconversion of feedstocks with higher protein and N content, like wheat bran or biosolids, generate frass with high levels of NH_4^+-N. A high concentration of NH_4^+-N can cause damage in plants and inhibit germination [43]. It has been suggested that composting, as subsequent step in BSF-based bioconversion, could reduce high concentrations of NH_4^+-N and the resulting phytotoxicity of the frass [44]. For example, a study by Sanchez-Monedero et al. [45] showed that NH_4^+-N levels in composted sewage sludge and municipal solid waste decreased gradually during the composting process, and Zubillaga and Lavado [39] demonstrated that composting of biosolids increased the germination success for ryegrass.

The highest GIs for both lettuce and radish were observed for frass from bioconverted food waste. The GI ranged from around 100% to 150%, while that for frass from biosolids treatments was 20–50%. This aligns with the results of Liu et al. [32], who observed GIs of 82% and 50% for frass obtained from BSF treatments with food waste and sewage sludge, respectively. The authors also reported an increase in total N and decrease in NO_3^--N concentrations for the food waste treatment. Similarly, Green and Popa [33] reported a reduction in NO_3^--N concentrations for the bioconversion of food waste using BSF. A reduction in NO_3^--N concentration may be beneficial if the frass is to be used as a soil amendment because high NO_3^--N concentration may result in excessive NO_3^--N leaching [46]. Moreover, high NO_3^--N can cause phytotoxicity [47,48]. Due to elevated concentrations of NO_3^--N and volatile organic acids (indicated by a low pH of 5) and higher EC of around 2 mS/cm before the BSF-based bioconversion, the phytotoxicity of unprocessed food waste was significantly higher compared to the generated frass. Besides the elevation in total N, pH and concentrations of P and K also increased for the bioconversion with food waste [16], which aligns with observations from previous BSF studies [32,49]. Overall, frass obtained from food waste seemed to be more mature and less toxic compared to frass from processed biosolids and biosolid blends.

Due to the nature of BSFL's conversion of available N into NH_4^+-N, BSFL feedstocks with high N content—and, accordingly, low C/N ratios—might not produce frass that can be directly used as fertiliser. Therefore, Beesigamukama et al. [50] suggested that feedstocks for BSFL growth should have C/N ratios above 15 to 30 in order to generate non-phytotoxic frass. C/N ratios above 15 were only reached for the BSFL food waste and not the biosolids containing feedstocks or wheat bran. Besides the N content, initial concentrations of minerals and salts in the BSFL feedstock are also factors affecting the outcome in terms of frass quality. Bohm et al. [16] showed that BSF-based bioconversion led to partitioning of macro-nutrients like Na and S from the feedstock into the frass. Therefore, the germination rates of the resulting frass from wheat bran, biosolids or biosolids blended with wheat bran were significantly lower compared to the unprocessed waste. Interestingly, BSF-based bioconversion of the biosolids feedstocks resulted in a significant decrease in the $Ca(NO_3)_2$-extractable (i.e., phytoavailable [51]) fraction of trace elements (except for As), and concentrations were at similar levels for frass originating from biosolids as for frass from food waste and wheat bran. Decreases in the bioavailability of trace elements compared to the total fraction [16] in these feedstocks were likely correlated with the rise in pH [52]. The high initial concentrations of trace elements in the BSFL feedstocks may have been less related to the decreased germination rate in the resulting frass than the actual high N and salt content.

Additional germination tests with diluted frass extracts from the biosolids treatments were performed to determine whether dilution of potential phytotoxins would improve germination. Indeed, the GI of lettuce increased significantly from below 30–50% to up to 120% for the highest frass extract dilutions (25%; i.e., a fourfold dilution). A similar trend was observed for radish and the biosolids and biosolids + wheat treatments, reaching a GI of up to 170%. Different potting experiments with BSFL frass originating from various feedstocks demonstrated that lower application rates would stimulate plant growth rather than inhibiting growth or causing wilting [5]. In this regard, some studies have identified a frass/soil mixture of 10% as optimal to promote plant growth in lettuce, basil, tomato and *Brassica rapa* [35,53,54]. Considering the concentration of inorganic N (i.e., the sum of NO_3^--N and NH_4^+-N), which was >6000 mg/kg (Figure 1) for frass derived from bioconverted biosolids, the results of our study suggest that a frass/soil mixture of 1% would provide benefits to plant growth. This is 10-fold lower than mentioned in the literature above and is supported by previous observations [55]. In this regard, a 1% application rate would correspond to about 70 mg/kg inorganic N, which is similar to the application rate for biosolids and soil recommended by Gutierrez-Gines et al. [55].

Besides a lower application rate for fresh BSFL frass, thermophilic composting of frass in order to increase its stability and decrease phytotoxic NH_4^+-N concentrations and for sanitation is recommended [5]. The composting process with BSFL is typically fast (up to two weeks) and the resulting frass compost is mostly immature and biologically unstable [35]. Song et al. [56] showed that composted BSFL frass can be applied with higher application rates (40%) compared to fresh frass (10%) without causing any plant growth inhibition. Another factor that might be limiting when using fresh BSFL frass from processed biosolids as fertiliser without further treatment is the relatively low C/N ratio of 8–12. A C/N ratio between 20 and 40 is recommended to avoid the possible immobilization of mineral nutrients in the soil, which can cause poor plant growth but also nutrient leaching, impacting the surrounding environment [57–59]. Therefore, mixing the frass from biosolids with other organic waste rich in carbon before composting or application to the field could level out the initial low C/N ratio.

5. Conclusions

This study demonstrated that the maturity (i.e., low phytotoxicity) of the frass resulting from BSF-based bioconversion strongly depends on the initial chemical composition of the organic waste fed to the BSFL. Fresh frass obtained from BSF-based bioconversion of biosolids, as well as that obtained from wheat bran, was not suitable for use as fertiliser directly, unlike the frass obtained from bioconverted food waste. An additional transformation step (e.g., thermophilic composting), a lower application rate and blending to reduce NH_4^+-N availability and EC may improve the maturity and stability of frass originating from biosolids and from high N feedstocks. However, further pot and field trials with low application rates for frass from bioconverted biosolids would be needed to quantify impacts on the soil carbon and nutrient cycle, the soil microbial community and the overall growth performance of older seedlings.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su151511526/s1. The Supplementary Materials comprise Figures S1–S3, as well as Tables S1–S5, which support the results described in the manuscript.

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