Principal component analysis of nutrient composition in black soldier fly larvae frass leachate from different organic waste substrate

Erick Yuhardi[1*], *Choirul* Umam[1], *Hones* Setia Ningsih[1], *Arinil* Chaq[1]

[1]Program study of Agroecotechnology, Department of Agricultural Science and Technology, Faculty of Agriculture, Universitas Trunodjoyo Madura, Bangkalan, Indonesia

Abstract. The black soldier fly (*Hermetia illucens*, BSFL) larvae can rapidly and efficiently decompose various types of organic waste, producing nutrient-rich solid frass and leachate as valuable by-products. However, the chemical composition of the leachate is highly dependent on the substrate type, which limits its consistency as a liquid biofertilizer. This study aimed to evaluate and compare the nutrient composition of leachate derived from different organic substrates degraded by BSFL and to identify the key factors influencing its variability. The research contributes new insight into how substrate type regulates the balance of macro–micro elements and the distribution of heavy metals within BSFL leachate, providing a theoretical basis for substrate-specific formulation of liquid biofertilizers. Leachate samples obtained from rotten fruit, rotten vegetables, and tofu pulp substrates were analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to determine the concentrations of macro elements (N, P, K, Ca, Mg, Na) and micro elements (Fe, Mn, Zn, Cu, Pb, Cd, Cr). The data were further examined using Principal Component Analysis (PCA) and Spearman correlation to identify elemental clustering patterns. The results revealed distinct separation among substrate groups. Tofu-pulp leachate contained the highest levels of P, Zn, and Cu; vegetable-based leachate was enriched with Ca, Mg, Fe, and Mn; while fruit-based leachate exhibited the highest K concentration. Heavy metals (Pb, Cr, Cd) were detected at trace levels well below international safety limits. These findings highlight that substrate composition plays a decisive role in determining leachate quality and support its potential use as a safe and sustainable liquid biofertilizer.

1 Introduction

Organic waste remains one of the most persistent challenges in achieving sustainable development, particularly in countries such as Indonesia. The large volume of biodegradable waste contributes to environmental degradation, increased greenhouse gas emissions, and the gradual decline of soil and water quality. Data from the National Waste Management Information System (SIPSN) indicate that food residues account for approximately 41% of the total waste generated. When combined with other biodegradable fractions such as crop residues, leaves, and household organics this proportion may exceed 50%. Without proper management, these wastes can intensify methane (CH_4) and nitrous oxide (N_2O) emissions, two potent greenhouse gases that play a major role in global climate change [1][2][3]. Consequently, there is an urgent need for a scientifically grounded, low-emission waste management system that supports circular economy principles [4].

Among the emerging biotechnological approaches, the bioconversion of organic waste using black soldier fly larvae (BSFL) offers a promising and sustainable pathway. BSFL efficiently degrades organic substrates through enzymatic decomposition and bio-oxidation, converting low value residues into high value products. This transformation involves complex biochemical and physicochemical interactions, such as mineralization, pH modulation, and metal ion transformation that drive nutrient release and stabilization within the substrate [5][6][7]. Through this process, BSFL generate two major agronomic products: a nutrient-rich solid residue (frass) and a liquid fraction (leachate) containing soluble macro and micronutrients [8].

Recent studies have demonstrated the strong potential of BSFL frass as an organic soil amendment due to its considerable nitrogen, phosphorus, and potassium content, along with beneficial microbial populations that enhance soil fertility and biological activity [9][10][4]. However, the liquid by product leachate remains far less explored, despite evidence that it contains essential elements such as Ca, Mg, Fe, Zn, and Cu, making it a potential candidate for use as a liquid biofertilizer [11][12]. The nutrient composition of leachate is largely determined by the physicochemical characteristics of the substrate, including its C/N ratio, moisture content, and the biochemical composition of

* Corresponding author: erick.yuhardi@trunojoyo.ac.id

the decomposed materials [13][14]. Understanding these relationships is therefore critical for assessing the agronomic value of BSFL derived leachate.

From a scientific perspective, the decomposition dynamics and nutrient mobility within BSFL systems are governed by several environmental physicochemical mechanisms, including ion diffusion, metal exchange, and colloidal charge variation in the liquid medium. Quantitative analysis of nutrient elements using Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES) provides precise measurements of macro and micronutrients, while Principal Component Analysis (PCA) serves as a robust multivariate statistical approach grounded in mathematical and physical principles. PCA enables visualization of correlations among nutrient elements, allowing the identification of substrate dependent nutrient profiles [10][11]. Beyond its analytical strength, this method provides a conceptual framework for understanding how elemental behavior reflects the underlying complexity of bioconversion systems.

Although several studies have explored the agronomic potential of BSFL frass, systematic research linking leachate chemistry, substrate variability, and multivariate statistical interpretation remains limited. Such investigations are vital not only for advancing sustainable agriculture but also for enriching interdisciplinary fields such as environmental science, biophysics, and organic materials technology. These studies contribute to a more integrated understanding of nutrient dynamics and ionic interactions in biological systems involving energy and mass transformation processes [5][6][15].

The present study contributes to this growing body of knowledge by applying Principal Component Analysis (PCA) to identify and compare the patterns of macro and micronutrient composition in leachate derived from BSFL bioconversion of various organic waste substrates. By integrating ICP–OES measurements with a statistically grounded modeling approach, this research aims to (i) explain the chemical variability among substrate derived leachates, (ii) determine the dominant elements driving these differences, and (iii) assess the potential of BSFL leachate as a sustainable liquid biofertilizer. Ultimately, this study establishes a scientific framework connecting the physicochemical transformations occurring during BSFL bioconversion with the resulting nutrient profiles, thereby providing new insight into waste valorization and sustainable nutrient recycling within modern organic waste management systems [4][3].

2 Research method

2.1 Location location and time

The research was conducted from June to December 2025. BSF maggot cultivation was carried out in the soil and land resources laboratory, Agroecotechnology Study Program, Faculty of Agriculture, Trunodjoyo University, Madura. Meanwhile, testing of maggot leachate nutrients was carried out in the energy and environment laboratory, ITS, Surabaya.

2.2 Experimental design

2.2.1 Substrate Preparation

Three types of organic waste were used as substrates for bioconversion: tofu residue, spoiled vegetables, and overripe fruits. Each type of waste was collected separately and shredded into smaller particles to accelerate the decomposition process. The prepared substrates were stored in closed containers for 24 hours prior to use to stabilize moisture content and minimize the risk of external microbial contamination.

2.2.2 BSFL Rearing and Bioconversion Process

Seven-day-old BSFL were used in this study. For each substrate type, larvae were introduced at a ratio of 5 g of larvae per 2 kg of organic material. The bioconversion process was conducted for 14 days under controlled environmental conditions, including temperature, humidity, and aeration. Gentle mixing was carried out periodically to maintain substrate homogeneity and ensure uniform oxygen distribution. The process was terminated when the larvae reached the prepupal stage, indicating that most of the organic matter had been biologically degraded.

2.2.3 Leachate Collection and Handling

Upon completion of the bioconversion process, the solid residue (frass) and liquid fraction (leachate) were separated. This study focused on the characterization of leachate as the primary liquid product of BSFL bioconversion. The leachate was collected from the bottom section of the rearing containers using a sealed drainage system to prevent evaporation and cross-contamination with the solid fraction. The collected leachate was filtered using Whatman No. 42 filter paper (pore size 2.5 µm) to remove suspended solids and fine organic residues. The clarified liquid samples were transferred into tightly sealed polyethylene bottles and stored at 4 °C to preserve nutrient stability prior to analysis. All collection procedures were conducted in a clean laboratory environment to minimize the risk of trace metal contamination from air or equipment.

2.2.4 Nutrient Analysis Using ICP–OES

The nutrient composition of the leachate was determined using Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES). Before analysis, samples were diluted to appropriate concentrations within the detection range of the instrument. The analyzed elements included macronutrients (N, P, K, Ca, Mg), micronutrients (Fe, Zn, Cu, Mn, Na), and heavy metals (Cu, Pb, Cd, Cr). Results were expressed in mg L^{-1}. Concentration data obtained from ICP–OES were used for multivariate statistical analysis to identify relationships among elements and assess nutrient composition differences across substrate types. Each treatment was analyzed in triplicate to ensure data reliability. The results were presented as mean ± standard deviation (SD) to indicate variability among replicates. The mean values were subsequently used as inputs for multivariate analyses, including Principal Component Analysis (PCA) and Spearman correlation analysis, to explore nutrient composition patterns and inter-element associations within the leachate.

2.2.5 Data Processing and PCA Analysis

Elemental data obtained from ICP–OES were processed using Principal Component Analysis (PCA) to evaluate inter-element relationships and characterize leachate composition across different substrate treatments. Prior to analysis, all variables were standardized using z-score transformation to ensure equal weighting of each element in the PCA model. The PCA results were visualized through biplots to illustrate the distribution of nutrient elements and the clustering of treatments. In addition, Spearman correlation coefficients were calculated and visualized as a heatmap to reinforce the interpretation of nutrient interactions and associations identified by PCA.

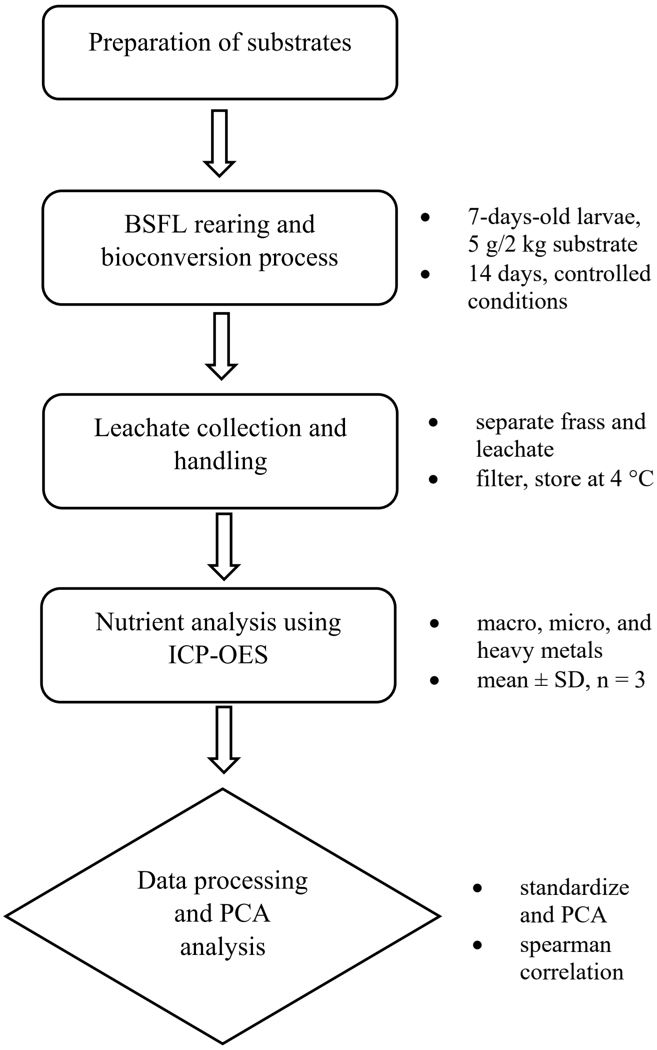

Fig. 1. Flowchart of the experimental procedure for BSFL leachate preparation and analysis.

3 Result and discussion

3.1 Results

The ICP–OES analysis of macro and micronutrients in the frass leachate derived from three substrate types fruit waste, vegetable waste, and tofu pulp revealed significant variations in nutrient composition (**Table 1**).

Table 1. Element concentrations in various substrates (mean ± SD, n=3).

Substrate	N	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	Pb	Cd	Cr
Rotten fruit	3483.33 ±35.12	451.67± 10.41	7233.33 ±125.83	793.33 ±15.28	148.33± 7.64	46.37± 0.58	24.9± 0.4	5.2± 0.1	9.93± 0.15	1.9± 0.22	0.21 ± 0.01	0.05 ± 0.01	0.14 ± 0.15
Rotten vegetables	4186.67 ±40.41	591.67± 12.58	5346.67 ± 70.24	1480.0 ± 20.0	293.33± 7.64	38.39± 0.46	45.16 ± 0.93	11.95 ±0.36	18.05 ± 0.49	3.3± 0.1	0.24 ± 0.01	0.08 ± 0.01	0.38 ± 0.01
Tofu pulp	881.67± 12.58	2973.33 ± 50.33	1173.33 ±15.28	215.0± 5.0	64.02± 0.82	64.02± 0.82	28.47 ±0.66	7.93± 0.15	24.9± 0.56	6.0± 0.16	0.15 ± 0.01	0.04 ± 0.01	0.35 ± 0.01

The tofu pulp substrate produced the highest concentrations of phosphorus (P), zinc (Zn), and copper (Cu), while the vegetable substrate showed higher levels of calcium (Ca), magnesium (Mg), iron (Fe), and manganese (Mn). In contrast, the leachate derived from fruit substrates contained the highest potassium (K) concentration. These results indicate that the chemical characteristics of the initial organic materials significantly influence the final nutrient profile of the leachate frass produced by BSFL. In addition to these nutrient elements, the ICP–OES analysis also detected the presence of heavy metals such as lead (Pb), chromium (Cr), and cadmium (Cd) in all leachate samples (**Table 1**). However, the concentrations of these metals were very low and remained far below the maximum limits for organic fertilizers recommended by the FAO and the European Union. The leachate derived from the tofu dreg substrate exhibited slightly higher Pb concentrations than the other two substrates, but still within safe limits. This finding suggests that the BSFL bioconversion process does not lead to significant accumulation of toxic metals, and that the resulting leachate can be considered safe for use as an organic liquid fertilizer.

The results of the *Principal Component Analysis* (PCA) revealed a clear separation among the substrate (Fig. 2).

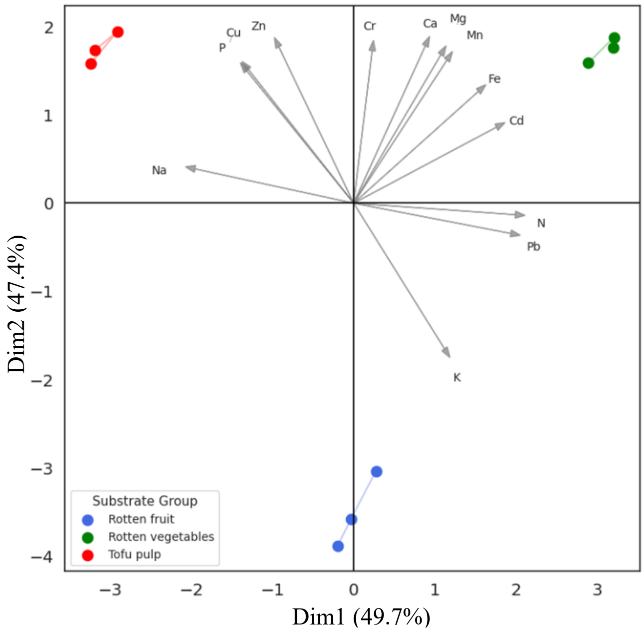

Fig. 2. PCA biplot of BSFL frass leachate nutrient composition.

Two principal components, PC1 and PC2, cumulatively explained the majority of the data variation. The tofu dreg substrate occupied a distinct position due to the strong contribution of phosphorus (P) and zinc (Zn), whereas leachate from fruit and vegetable substrates formed separate clusters characterized by different distribution patterns of potassium (K), calcium (Ca), and magnesium (Mg). The Spearman correlation analysis (Fig. 3) revealed a strong positive relationship (r > 0.9) among Ca, Mg, Fe, and Mn, indicating a close association in the mobilization of these elements during substrate decomposition by the larvae.

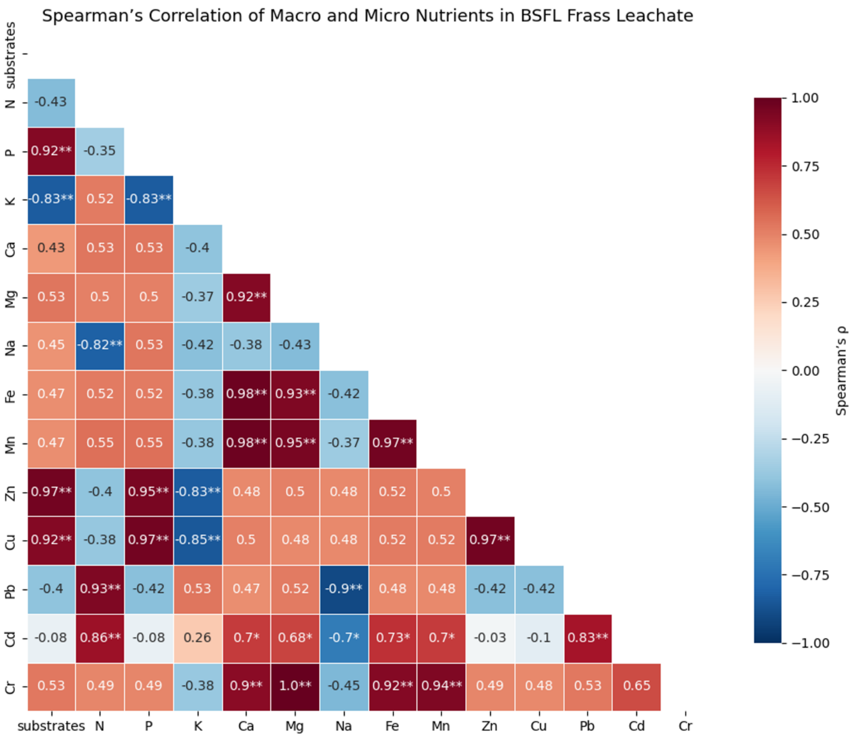

Fig. 3. Clustered heatmap of Spearman's correlation between macro and micro nutrients in BSFL frass leachate.

3.2 Discussion

3.1.1 Main findings of the present study

The findings of this study demonstrate that the chemical composition of *Hermetia illucens* leachate frass is strongly influenced by the type of organic substrate used during the bioconversion process. The tofu dreg substrate, rich in protein and phosphate, produced leachate with the highest concentrations of phosphorus (P), zinc (Zn), and copper (Cu). In contrast, the vegetable substrate enriched the leachate with basic cations such as calcium (Ca), magnesium (Mg), iron (Fe), and manganese (Mn), while the fruit substrate contributed the highest potassium (K) content. These variations reflect differences in mineralization pathways and chemical reactions that occur during the decomposition of organic materials with varying C/N ratios.

3.1.2 Comparison with other studies

The results are consistent with the findings of Gärttling and Schulz [11], who reported that the N, P, K ratio and the distribution of micronutrients in BSFL frass largely depend on the chemical characteristics of the feedstock. Similarly, Salomon et al. [11] found that frass derived from protein-rich substrates contained higher levels of phosphorus and nitrogen than frass produced from vegetable substrates, which tend to enrich basic elements such as Ca and Mg. [16] [17] also confirmed that high-protein substrates, such as soybean residue and kitchen waste, generate frass with greater P and Zn concentrations compared with substrates characterized by a higher C/N ratio. Beasley et al. [18] reported that BSFL frass not only enhances nitrogen and potassium content in the growing medium but also reduces nitrogen loss through leaching, supporting the present finding that fruit-based leachate exhibited elevated K levels. Differences among studies may be attributed to variations in larval-to-substrate ratios, harvest time, and post-treatment procedures such as drying or leachate filtration [20]. Nevertheless, the overall pattern linking substrate type and nutrient composition remains consistent with observations in previous studies [15][11][21].

3.1.3 Implication and explanation of findings

The macro and micronutrient contents of the leachate reflect the mineralization dynamics occurring during bioconversion. The tofu dreg substrate, which is rich in nitrogenous and phosphate compounds, promotes the release of soluble organic phosphate and complex Zn species. Conversely, the vegetable substrate, characterized by a high C/N ratio, facilitates the formation of carbonate and basic phosphate compounds, thereby increasing Ca and Mg levels. The elevated Fe and Mn concentrations are likely associated with natural redox reactions during substrate degradation. Meanwhile, the low concentrations of heavy metals suggest that the initial organic materials were free from inorganic contaminants and that the BSFL bioconversion process contributes to metal stabilization through ionic binding within the organic matrix.

3.1.4 Strengths and Limitations

The primary strength of this study lies in its analytical approach, which integrates Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES) with *Principal Component Analysis* (PCA) and Spearman correlation to identify statistical relationships among nutrient elements. This combined methodology provides a robust quantitative understanding of how substrate type influences the chemical characteristics of the leachate. However, the study is limited to a single leachate sampling time, which may not fully capture temporal variations in nutrient content during the bioconversion process. Future research involving long-term monitoring and dissolved fraction analysis is recommended to better elucidate the dynamics of elemental transformation under different bioconversion conditions.

4 Conclusions

The findings of this study demonstrate that the type of organic substrate exerts a significant influence on the chemical composition of Hermetia illucens (BSFL) frass leachate. Results obtained through ICP–OES, PCA, and Spearman correlation analyses revealed distinct elemental distribution patterns among the different substrate types. Leachate derived from tofu pulp exhibited the highest concentrations of phosphorus (P), zinc (Zn), and copper (Cu); vegetable-based substrates were enriched with calcium (Ca), magnesium (Mg), iron (Fe), and manganese (Mn); whereas fruit-based substrates contained the greatest potassium (K) levels. These patterns emphasize the pivotal role of the initial chemical characteristics of organic feedstocks in governing mineralization processes and nutrient distribution during bioconversion. Moreover, trace analysis indicated that heavy metals such as lead (Pb), cadmium (Cd), and chromium (Cr) were present only at minimal concentrations, remaining far below internationally accepted safety thresholds. Hence, BSFL-derived leachate can be considered environmentally safe for use as an organic liquid fertilizer.

From a theoretical standpoint, this research contributes to a deeper understanding of the relationship between substrate characteristics and nutrient dynamics in BSFL-based bioconversion systems. The application of multivariate statistical approaches provides a robust analytical framework for interpreting inter-element relationships and mineralization mechanisms within complex biological matrices. Nevertheless, this study is limited to a single leachate sampling, which restricts the observation of temporal variations in nutrient and metal content. Future studies involving long-term monitoring, dissolved fraction analysis, and agronomic field evaluations across various crops are recommended to strengthen the practical and agronomic implications of BSFL leachate application.

This study provides both scientific and practical contributions to the development of BSFL-based organic liquid fertilizers. The results offer a theoretical foundation for the formulation of substrate-specific and environmentally sound biofertilizers, supporting the advancement of precision nutrient management and the broader implementation of sustainable circular agricultural systems.

References

1. M. T. Gebremikael, J. V. Campenhout, N. Van Hese, S. Sleutel, Impacts of black soldier fly frass on nitrogen availability, microbial activities, and plant growth. Frontiers in Sustainable Food Systems. 6, 795950 (2022). https://doi.org/10.3389/fsufs.2022.795950
2. N. E. A. Basri, N. F. Ghazali, S. A. Ishak, R. Razali, Potential applications of frass derived from black soldier fly larvae treatment of food waste: A review. Foods. 11, 17, 2664 (2022). https://doi.org/10.3390/foods11172664
3. M. R. Tariq *et al.,* Black soldier fly: A keystone species for the future of organic waste management and fertiliser production. Insects. 16, 8, 750 (2025). https://doi.org/10.3390/insects16080750
4. F. A. Manan *et al.,* Unlocking the potential of black soldier fly frass as organic fertiliser: A review. Science of the Total Environment. 367, 121997 (2024). https://doi.org/10.1016/j.scitotenv.2024.178932
5. T. Green, A biochemical analysis of black soldier fly (Hermetia illucens) larval frass: Plant growth promoting activity. PLoS ONE, 18, 8, e0288913 (2023). https://doi.org/10.1371/journal.pone.0288913
6. G. Lomonaco, D. Caruso, M. Spagnuolo, Larval frass of Hermetia illucens as organic fertilizer: Composition and beneficial effects on different crops. Insects. 15, 110 (2024). https://doi.org/10.3390/insects15010110
7. S. A. Siddiqui *et al.,* Investigating frass recirculation in BSFL treatment as a waste valorisation pathway. Sustainability Science. (2024). https://doi.org/10.1007/s10668-024-04673-8
8. M. J. Salomon, D. Klocke, S. D. Bellingrath-Kimura, Potential of black soldier fly larvae frass (BSFL) as a novel organic fertilizer: Physical and chemical analyses of nutrients under different substrates. Plant and Soil. 1-18 (2025). https://doi.org/10.1007/s11104-024-07187-4
9. M. Y. Chavez, A. Villa Ignacio, J. K. Craver, J. Bousselot, Investigating black soldier fly larval (Hermetia illucens) frass applications as a partial peat replacement and liquid fertilizer in Brassicaceae crop production. Agrochemicals. 4, 2,

8 (2025). https://doi.org/10.3390/agrochemicals4020008
10. D. Gärttling, H. Schulz, Compilation of black soldier fly frass analyses. Journal of Soil Science and Plant Nutrition. 22, 3, 1–15 (2022). https://doi.org/10.1007/s42729-021-00703-w
11. M. J. Salomon, T. R. Cavagnaro, R. A. Burton, Potential of black soldier fly larvae frass (BSFL) as a novel fertilizer: Impacts on tomato growth, nutrient uptake, and mycorrhizal formation. Plant and Soil, 513, 417–434 (2025). https://doi.org/10.1007/s11104-024-07187-4
12. G. A. Sopha *et al.,* Liquid organic fertilizer derived from black soldier fly larvae frass: Nutrient uptake, yield and soil properties. International Journal of Horticultural Science & Technology. 12, 2, 479-490 (2025). https://doi.org/10.22059/ijhst.2024.373373.797
13. D. Beesigamukama, S., Subramanian, C. M. Tanga, Nutrient quality and maturity status of frass fertilizer from nine edible insects. Scientific Reports. 12, 7182 (2022). https://doi.org/10.1038/s41598-022-11336-z
14. J. Poveda, I. Zunzunegui, Ó. Santamaría, J. Martín-García, Insect frass as a novel organic soil fertilizer for the cultivation of spinach (Spinacia oleracea): Effects on soil properties, plant physiological parameters, and nutrient status. Journal of Soil Science and Plant Nutrition. 23, 1121–1135 (2023). https://doi.org/10.1007/s42729-023-01451-9
15. C. L. Solofondranohatra, D. Ramanantenasoa, P. Randriamanantsoa, Black soldier fly frass fertilizer outperforms traditional fertilizers in open-access study. Sustainability. 17, 15, 7152 (2025). https://doi.org/10.3390/su17157152
16. G. Arabzadeh *et al.,* Diet composition influences growth performance, bioconversion of black soldier fly larvae: Agronomic value and in vitro biofungicidal activity of derived frass. Agronomy. 12, 8, 1765 (2022). https://doi.org/10.3390/agronomy12081765
17. D. Mulu, F. Yimer, G. Opande, Characterizing quality of frass produced by black soldier fly (Hermetia illucens) larvae after treating water hyacinth and fruit wastes in various proportions. Biomass Conversion and Biorefinery. 13, 15185–15196 (2023). https://doi.org/10.1007/s13399-023-04552-8
18. J. Beasley, J. A. Schrader, D. Grewell, Black soldier fly frass supports plant growth and reduces nitrogen leaching during Coleus production. HortTechnology. 33, 3, 305–312 (2023). https://doi.org/10.21273/HORTTECH05093-22
19. S. K. Gurung *et al.,* Manure-derived black soldier fly frass enhanced the growth of chilli plants (Capsicum annuum L.) and altered rhizosphere bacterial community. Applied Soil Ecology. 202, 105605 (2024). https://doi.org/10.1016/j.apsoil.2024.105605
20. E. O. Ogello, N. O. Outa, M. Muthoka, F. Juma, J. Hoinkis, Optimizing black soldier fly (Hermetia illucens) production: Effects of substrate variation on biomass, nutritional quality, hatchability, fecundity, and frass quality. Frontiers in Sustainable Food Systems. 9, 1621034 (2025). https://doi.org/10.3389/fsufs.2025.1621034
21. B. Jasso, L. Quinchia, T. M. Waliczek, M. L. Drewery, Black soldier fly larvae (Hermetia illucens) frass and sheddings as a compost ingredient. Frontiers in Sustainable Food Systems. 7, 1297858 (2024). https://doi.org/10.3389/fsufs.2023.1297858
22. N. F. Addeo, R. D'Amato, P. Caputo, Mineral profile and heavy metals bioaccumulation in black soldier fly larvae and frass. Science of the Total Environment. 927, 1828051X (2024). https://doi.org/10.1080/1828051X.2024.2302845
23. K. Böhm, G. A. Hatley, B. H. Robinson, M. J. Gutiérrez-Ginés, Analysis of chemical and phytotoxic properties of frass derived from black soldier fly-based bioconversion of biosolids. Sustainability. 15, 15, 11526 (2023). https://doi.org/10.3390/su151511526
24. D. R. Kidd *et al.*, Black soldier fly frass greatly improves growth of four crops. Journal of Cleaner Production. 520, 146046 (2025). https://doi.org/10.1016/j.jclepro.2025.140592
25. A. De Volder *et al.*, Heat treatment and storage of frass from black soldier fly larvae and yellow mealworm production: Compliance with EU regulation on microbiological quality and safety. MicrobiologyOpen. 14, 3, e70020 (2025). https://doi.org/10.1002/mbo3.70020
26. A. Chepkorir *et al.,* Insect frass fertilizer as a regenerative input for improved bush bean production: Growth, yield, nutrient uptake and biological N fixation. Frontiers in Plant Science. 15, 1460599 (2024). https://doi.org/10.3389/fpls.2024.1460599
27. E. O. Anedo, P. N. Okechukwu, C. S. Obayi, Unpacking the benefits of black soldier fly frass fertilizer: Nematode suppression and potato production. Frontiers in Plant Science.16, 1509643 (2025). https://doi.org/10.3389/fpls.2025.1509643