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Comparative Study on Nutrient Composition and Biological Activity of Vermiwash Obtained from Different Organic Wastes

Rajwinder Kaur¹, Neeraj Rani², Seema Garcha³, Shilpa⁴, AS Sidhu² and SS Walia²

¹Department of Soil Science, Punjab Agricultural University, Ludhiana, Punjab, India – 141004

²School of Organic and Natural Farming, Punjab Agricultural University, Ludhiana, Punjab, India - 141004

³Department of Microbiology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, Punjab, India - 141004

⁴Department of Biochemistry, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, Punjab, India - 141004

*Correspondence for materials should be addressed to NR (email: neerajsoil@pau.edu)

Abstract

This study optimized vermiwash production using *Eisenia fetida* from organic substrates rice straw + cow dung (1:1), wheat straw + cow dung (1:1), kitchen waste + cow dung (1:1), neem leaves + cow dung (1:1), and pure cow dung in a drip-irrigated earthen pot system at Punjab Agricultural University, Ludhiana. Kitchen waste vermiwash demonstrated superior nutrient enrichment (elevated NPK, micronutrients), microbiological (peak microbial populations, enzyme activities), and biochemical profiles (maximum proteins, pigments, sugars) across treatments ($p < 0.05$). Kitchen waste's labile nutrients enhanced earthworm-microbial synergy over lignocellulosic straws and stabilized cow dung, positioning vermiwash as an optimal organic biostimulant for sustainable agriculture.

Keywords: Vermiwash; *Eisenia fetida*; Kitchen waste; Nutrient enrichment; Biostimulant

Introduction

The increasing demand for sustainable agricultural practices has led to a rise in the search for eco-friendly alternatives to synthetic fertilisers. Among these, vermiwash, a liquid fertilizer derived from the leachate of vermicomposting systems, is increasingly recognized as a good component for agricultural sustainability (Nayak et al., 2019; Mungruaiklang and Iwai, 2021). Produced through the waste decomposition activities of earthworms like *Eisenia fetida*, this organic liquid is rich in plant growth hormones, enzymes, and a full spectrum of nutrients ranging from NPK to trace elements (Bhagat et al., 2022; Sreenivas et al., 2000). Beyond nutrition, vermiwash serves a dual purpose by acting as a natural biopesticide within integrated pest management frameworks. Application is generally conducted through soil drenching or foliar spraying. Both methods improve crop health, though foliar sprays are particularly noted for facilitating quick nutrient uptake and bolstering tolerance against environmental challenges such as drought (Riya et al., 2024). Several earthworm species have been recognized as important contributors to vermicomposting operations. These earthworm species, along with *E. eugeniae*, are considered essential in organic waste processing due to their ability to produce nutrient-rich vermiwash. Earthworms, often referred to as "farmer's friends," improve soil fertility and plant health by enhancing nutrient availability through their bioenzymes and efficient organic matter processing (Sharma et al., 2017). In India, species such as *Eisenia fetida*, *Eudrilus eugeniae*, *Perionyx excavatus*, *Lampito mauritii*, and *Drawida willsi* are all recognised for their potential use in vermicomposting operations (Ranganathan et al., 2020). Specifically, there is limited research on how different organic materials, such as agricultural waste, plant residues, and food scraps, influence the nutrient composition, microbial diversity, and overall quality of vermiwash.



This study aims to investigate the effects of diverse organic residues on the physicochemical, microbiological, and biochemical properties of vermiwash. This study provided valuable insights into the optimal conditions for producing high-quality vermiwash and the different parameters supporting its broader application in sustainable agriculture.

Materials and methods

The research experiment was conducted at the Vermicompost Unit of School of Organic and Natural Farming, PAU, Ludhiana.

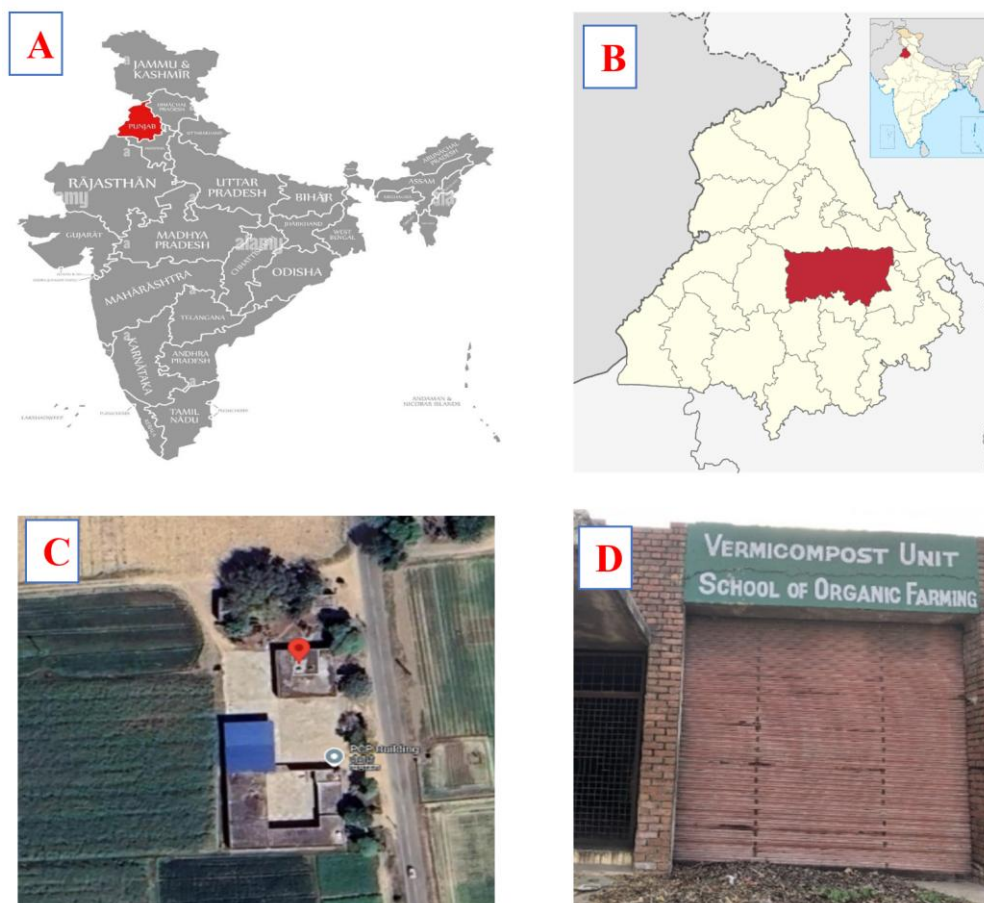


Fig. 1. A- Punjab located on the India map B- Ludhiana located on the Punjab map C- Satellite view of location D- Vermicompost Unit

Treatments details

Raw materials, including chopped paddy and wheat straw, were sourced from the Integrated Farming System Unit at PAU, Ludhiana (Figure 1), while neem leaves (*Azadirachta indica*) were gathered from the areas surrounding the School of Organic and Natural Farming, PAU. Additionally, kitchen waste (free of onion, garlic, and acidic peels) was obtained from PAU hostel messes, and fresh cow dung was procured from the dairy farm unit at GADVASU, Ludhiana. The organic residues were combined with cow dung in a 1:1 ratio on a dry weight basis and allowed to partially decompose for a period of 15 days. The study utilized fifteen earthen pitchers equipped with gate valves; each vessel was filled with the prepared substrate and inoculated with 10-15 *Eisenia fetida* earthworms at a temperature of approximately 25°C. Moisture levels were sustained at 65-70% of the water holding capacity using a drip-wise application method, accompanied by regular turning to ensure proper aeration. The collection of vermiwash commenced 15 days following the inoculation of the earthworms.

Table 1. Details of treatments and material required for preparation of different vermiwash samples.

TRT	Vermiwash	Material used
T ₁	Rice straw	Rice straw chopped (RS) + cow dung (1:1) + earthworms
T ₂	Wheat straw	Wheat straw chopped (WS) + cow dung (1:1) + earthworms
T ₃	Kitchen waste	Kitchen waste (KW) + cow dung (1:1) + earthworms
T ₄	Neem leaves	Neem leaves (NL) + cow dung (1:1) + earthworms
T ₅	Cow dung	100 % Cow dung (CD) + earthworms

Vermiwash (V/W) was produced at the Vermicompost Unit of School of Organic and Natural Farming, Ludhiana, according to the protocol developed by Ismail (1997). The experimental setup consisted of fifteen earthen pots arranged on iron supports. The bottom pots were equipped with plastic gate valves to facilitate the collection of

the liquid extract. The middle pots contained a layer of gravel (1-2 inch size), over which cow dung was placed. Different organic residues were subsequently added and covered with an additional layer of cow dung. To improve aeration and create a favorable environment for earthworm activity, the neck portions of the middle pots were partially broken. Moisture levels were maintained between 60 and 70% throughout the process, and water was supplied continuously in the form of slow dripping through a drip irrigation system using IV (Intravenous) drip pipe. The organic materials were allowed to undergo partial decomposition for 15 days, with regular turning to ensure uniform breakdown. Subsequently, approximately 10-15 adult individuals of *Eisenia fetida*, a surface-dwelling compost earthworm recognized for its high reproductive capacity and efficient utilization of organic wastes, were introduced into each unit. These earthworms accelerated the decomposition and stabilization of the organic substrates. Following an additional 15-day period, vermiwash was harvested daily from the collection pots located at the base of the system. The resulting liquid was generated as water percolated through the decomposing organic matter and earthworm active zones (drilosphere), becoming enriched with soluble nutrients, metabolites, enzymes, and beneficial microbial populations.



Fig. 2. Materials used to prepare different types of vermiwash carrying nutrients from the freshly formed castings

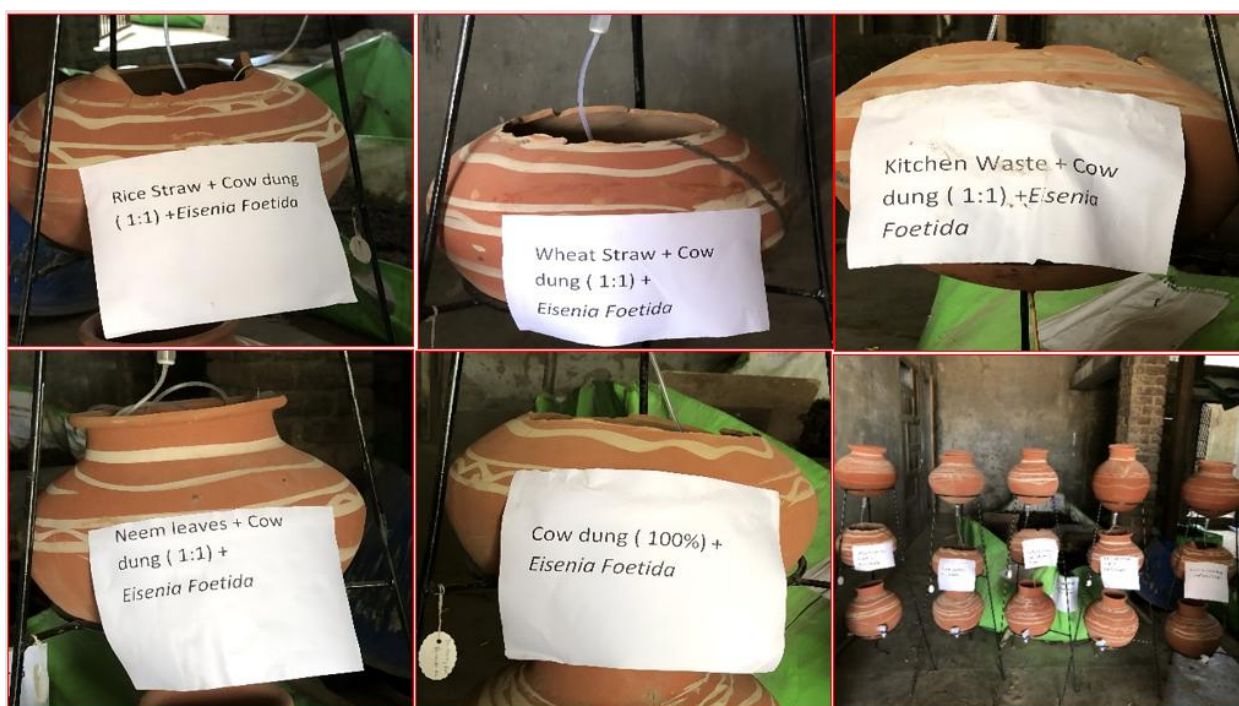


Fig. 3. The collected vermiwash was stored in a deep freezer at -20°C for further analysis of its microbiological and biochemical analysis.

Sampling of vermiwash

The prepared vermiwash was collected from each set of different materials. The samples from each pitcher were collected and properly labelled. The collected samples were carried to the laboratory for analysis. The samples were

filtered and then stored in a deep freezer at -20 °C in lab and stored for physico-chemical, microbiological and biochemical analysis. To evaluate the changes in physico-chemical, microbiological and biochemical characteristics of vermiwash, the following parameters were analyzed.

a) Chemical parameters	
Parameters	Method used
pH	The pH was measured using a pH meter (Jackson, 1967).
EC (dS m ⁻¹)	The EC was determined using a conductivity bridge in a 1:2 ratio (vermiwash sample: water suspension) (Jackson, 1967).
Total organic carbon	The total organic carbon was evaluated using a Muffle furnace. (Nelson and Sommers, 1996).
Total nitrogen	The total nitrogen was determined using the Kjeldahl digestion process described by Bremner and Mulvaney (1982).
Total phosphorus	The diacid (HNO ₃ : HClO ₄ 3:1) procedure was followed for determining the phosphorus content (Jackson, 1967).
Total Potassium	The same digested material used for phosphorus analysis was used for potassium (Jackson, 1967)
Micronutrients (Fe, Cu, Mn, Zn)	The Lindsey and Norvell (1978) approach was used to determine the micronutrient content.
Heavy metals	The heavy metals were analyzed on ICAP-MS.
C:N ratio	Total organic carbon divided by total nitrogen content
b) Microbiological parameters	
Parameters	Method used
Microbial (Bacteria, Fungi and Actinomycetes)	The serial dilution plate technique was used to measure the microbial population (Dingra and Sinclair, 1993).
Dehydrogenase activity	The method of Casida et al. (1964) was applied to measure the activity of dehydrogenase.
Alkaline phosphatase activity	The approach of Tabatabai and Bremner (1969) was used to measure phosphatase activity.
Urease activity	Determined by the Kandeler and Gerber (1988) colorimetric ammonium release method.
Cellulase activity	Determined by the Ghose (1987) cellulase assay (DNS method)
Amylase activity	Determined by the Bernfeld (1955) DNS reducing sugar method
c) Biochemical parameters	

Parameters	Method used
Total proteins (Unit)	Lowry's Method (1951)
Chlorophyll a and b, Lycopene and Carotenoids (Unit)	Barnes et al. (1992)
Total phenols (Unit)	Folin-Ciocalteu Method (Swain and Hillis, 1959)
Total soluble sugars (Unit)	Phenol-Sulfuric Acid Method (Dubois et al., 1956)
Reducing sugars (Unit)	Dinitrosalicylic Acid Method (Miller, 1972)
Non-reducing sugars (Unit)	Total soluble sugars - Reducing sugars

Statistical Analysis

The different parameters were statistically analyzed using the analysis of variance (ANOVA) approach (Gomez and Gomez, 1984) for a randomized block design with the aid of CPCS-1 software developed by the Department of Mathematics and Statistics, PAU, Ludhiana (Cheema and Singh, 1991).

Results and Discussion

Physiochemical Characteristics

The analysis revealed that the source of organic waste significantly influenced the physicochemical properties of the prepared vermiwash (Figure 4). Among the treatments, Kitchen waste emerged as the most nutrient-rich substrate, recording the highest concentrations of macronutrients (N, P, K) and micronutrients (Fe, Mn, Zn, Cu), followed closely by Neem leaves. Organic waste exhibits higher nutrient content due to the diverse essential nutrient compounds present in different substrates, as reported by Gopal et al. (2010); Jadhav et al. (2024); Kumar et al. (2019). Yassen et al. (2020) and Tadayyon et al. (2018) showed that vermiwash contributed to higher micronutrients in plants, enhancing their growth.

In contrast, the Cow dung and Rice straw treatments generally exhibited lower nutrient profiles and higher pH values. A study by Kumar et al. (2025) also highlighted that different organic materials, including kitchen waste,

yield vermiwash with varied pH and EC levels, impacting plant growth. Furthermore, while Kitchen waste contained the highest Total Organic Carbon (TOC), it demonstrated a significantly lower C:N ratio compared to the Cow dung. This narrower C:N ratio indicates a higher degree of mineralization and stability, likely driven by the rapid decomposition of the succulent, nitrogen-rich organic matter in kitchen waste and neem leaves compared to the slower degradation of lignocellulosic cereal straws. This result is consistent with the findings of Mukhopadhyay (2025); Chauhan and Singh (2015); Nahar et al. (2024) reported that carbon-rich organic materials such as kitchen waste typically lead to higher TOC levels in vermiwash. A lower C:N ratio is typically preferred for enhanced microbial activity and indicated more efficient nutrient cycling in vermiwash (Hatti et al., 2010). Similarly, Heavy metal analysis of vermiwash from different organic substrates (Table 3) showed significant variation across treatments, with cow dung consistently exhibited the highest concentrations of As, Cr, Ni, and Pb due to bioaccumulation from livestock feed. Kitchen waste yielded the lowest levels, while Cd was undetectable in all samples. All values remained well below maximum permissible limits, confirming the environmental safety of these vermiwash preparations for agricultural use. Elbanna et al. (2021) reported that no detectable levels of Cd, Ni, and Co were found in the vermiwash, while low Cr concentrations were observed across vermiwash produced by the three earthworm species.

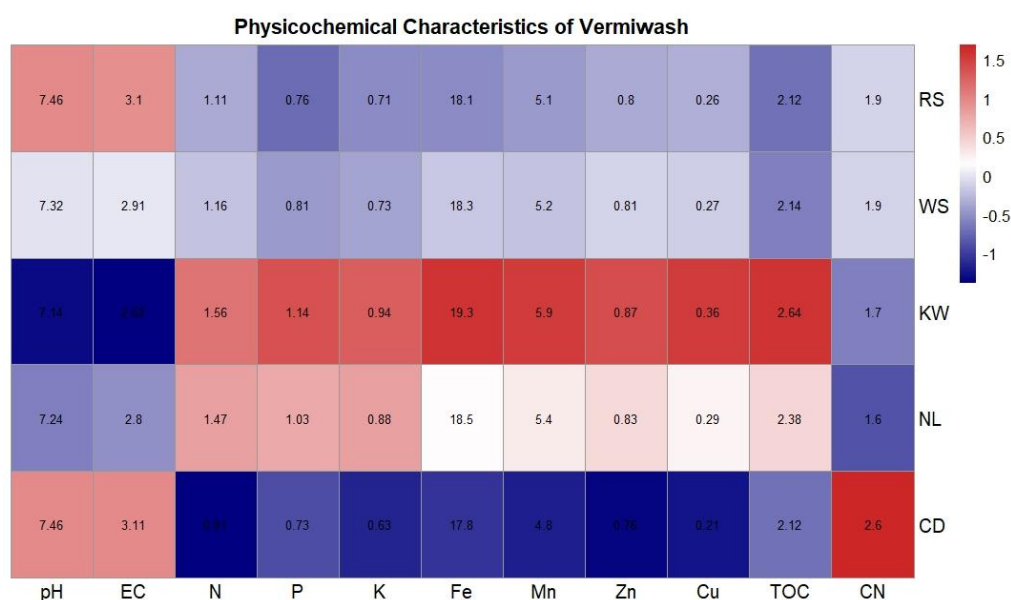


Fig. 4. Physicochemical parameters of vermiwash prepared from different organic substances

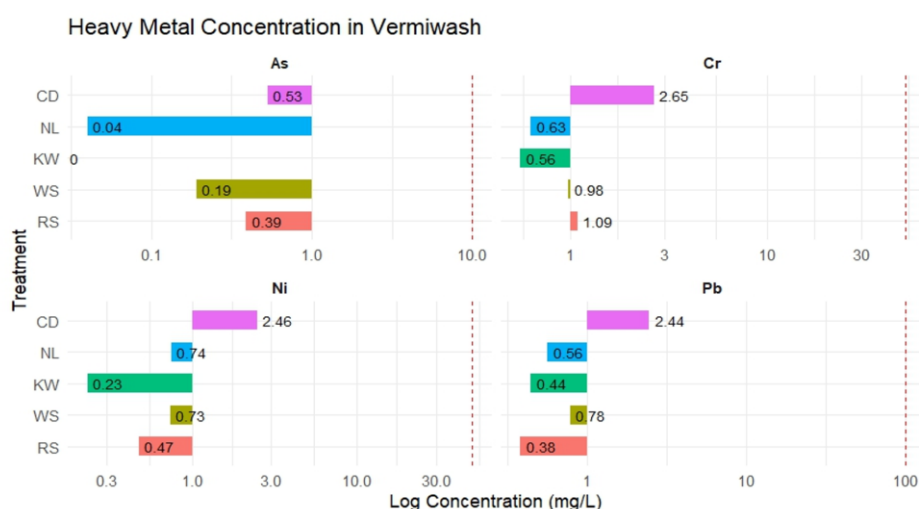


Fig. 5. Heavy metal content in vermiwash prepared from different organic substrates

Microbiological parameters

The organic substrate significantly influenced the microbial populations and enzymatic activities in vermiwash (Figure 6 & Table 2). Kitchen waste consistently supported the highest counts of bacteria, fungi, and actinomycetes, followed by neem leaves and straw substrates, with only cow dung showing the lowest densities. This trend reflects kitchen waste's superior nutritional quality, featuring easily degradable carbohydrates and proteins with a narrow C:N ratio that rapidly fuel microbial growth. Singh et al. (2020) reported that vermiwash contains abundant plant-beneficial bacteria, including *Azotobacter chroococcum* and *Azospirillum brasilense*, which support nitrogen fixation. Rajan and Murugesan (2012) observed that vermiwash improves seed germination and plant growth, likely due to

heightened microbial activity. Josephine et al. (2024) further showed that bacterial interactions in vermiwash enhance plant growth by increasing nutrient availability, underscoring vermiwash's promise as a bioinoculant for sustainable agriculture by enriching soils with beneficial microorganisms. In contrast, cow dung's pre-digested, stabilized organic matter limits further proliferation, while straw's lignocellulosic complexity slows decomposition. Kumar et al. (2024) have shown that fungal diversity in vermiwash is essential for improving soil health and promoting plant growth.

Table 2. Microbiological parameters of vermiwash prepared from different organic substrates

Parameters	Rice straw	Wheat straw	Kitchen waste	Neem leaves	Cow dung (100%)	CD ($p<0.05$)
Bacterial Count ($\log_{10}$ CFU ml ⁻¹)	9.38	9.39	9.73	9.48	9.27	0.06
Fungal count ($\log_{10}$ CFU ml ⁻¹)	8.08	8.06	8.38	8.14	7.94	0.27
Actinomycetes count ($\log_{10}$ CFU ml ⁻¹)	7.05	7.07	7.28	7.14	6.97	0.43
Dehydrogenase activity ($\mu\text{g TPF g}^{-1} \text{h}^{-1}$)	13.0	15.3	20.5	15.7	11.1	2.61
Alkaline phosphatase ($\mu\text{g PNP g}^{-1} \text{h}^{-1}$)	8.10	8.70	11.6	9.1	7.3	1.7
Urease activity ($\text{mg NH}_3/\text{g/h}$)	0.173	0.185	0.236	0.209	0.153	0.03
Cellulase activity ($\mu\text{g glucose g}^{-1} \text{h}^{-1}$)	3.1	3.4	5.3	4.2	2.4	0.94
Amylase activity ($\mu\text{g glucose g}^{-1} \text{h}^{-1}$)	19.0	21.5	35.7	24.3	15.2	3.65

Actinomycetes present in vermiwash facilitate the synthesis of plant growth regulators, such as indole acetic acid (IAA), which are essential for root development and overall plant health. These observations are supported by Dakshayini et al. (2025), who emphasized the critical role of actinomycetes in organic farming. Furthermore, specific strains isolated from vermicompost-amended soil, such as *Streptomyces sampsonii* and *Streptomyces flavovariabilis*, have demonstrated strong antagonistic activity against phytopathogenic fungi. Enzyme activities mirrored these patterns, with kitchen waste exhibiting peak levels across dehydrogenase (microbial respiration indicator), nutrient cycling enzymes (urease, alkaline phosphatase), and hydrolytic enzymes (amylase, cellulase). Elevated alkaline phosphatase and dehydrogenase activity in kitchen waste vermiwash indicate strong potential for phosphorus mobilization and overall microbial respiration. Zeng et al. (2025) and Sharma et al. (2025) found that kitchen waste substrates stimulate microbial enzyme synthesis essential for nutrient cycling. Mishra et al. (2025) further attribute this enhanced activity to the buffered pH and high nitrogen content in vermi-casting materials. Nahar et al. (2024) reported that vermiwash contains vitamins, plant growth-promoting hormones including auxin and gibberellins, enzymes including protease, amylase, urease, and phosphatase that act as antimicrobials, symbiotic microbes including nitrogen-fixing bacteria such as *Azotobacter* sp., *Agrobacterium* sp., and *Rhizobium* and phosphate-solubilizing bacteria (PSB).

The starch-rich components in kitchen waste specifically induce high amylase, while its diverse organics drive overall metabolic activity, superior to the recalcitrant substrates in straw or stabilized cow dung. These findings highlight kitchen waste vermiwash as optimal for soil bio-stimulation due to its enhanced biological potency.

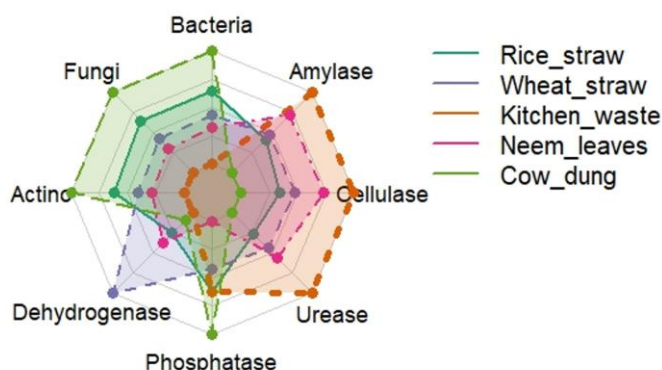


Fig. 6. Microbiological parameters of vermiwash prepared from different organic substrates

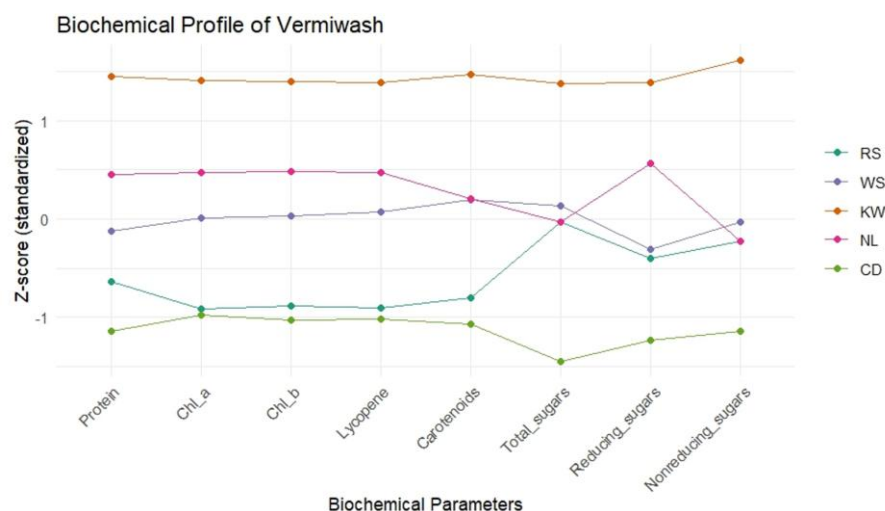


Fig. 7. Biochemical parameters of different vermiwash prepared from different organic substrates

Table 3. Biochemical parameters of different vermiwash prepared from different organic substrates

Treatments	Rice straw	Wheat straw	Kitchen waste	Neem leaves	Cow dung (100%)	CD (p<0.05)
Total proteins (mg/L)	0.5286	0.5914	0.7801	0.6604	0.4686	0.029
Chlorophyll a (Unit)	0.0189	0.0315	0.0504	0.0378	0.0181	0.010
Chlorophyll b (Unit)	0.03028	0.05047	0.08075	0.06056	0.02707	0.002
Lycopene (Unit)	0.0102	0.0177	0.0279	0.0208	0.0093	0.006
Carotenoids (Unit)	0.1397	0.2003	0.278	0.2009	0.1232	0.015
Total phenols (Unit)	0.0028	0.0031	0.0037	0.0035	0.0026	NS
Total soluble sugars (Unit)	0.2638	0.2794	0.40125	0.26375	0.1256	0.093
Reducing sugars (Unit)	0.2231	0.2363	0.4788	0.3613	0.1044	0.096
Non-reducing sugars	0.0275	0.0288	0.04	0.0275	0.0213	0.009

Biochemical Parameters of Vermiwash

The biochemical composition of vermiwash varied significantly with substrate type (Figure 7 & Table 3). Kitchen waste produced vermiwash richest in total proteins, chlorophylls (a and b), carotenoids, lycopene, total soluble sugars, reducing sugars, and non-reducing sugars, outperforming neem leaves, straw substrates, and the cow dung control. This superiority stems from kitchen waste's diverse, labile organic pool-rich in nitrogenous compounds and simple carbohydrates that earthworms and microbes efficiently convert into bioavailable proteins and photosynthetic pigments. Proteolytic enzymes originating from the earthworm gut likely facilitate the rise in protein levels. This result supports previous studies by Zambare et al. (2008), which confirmed the existence of both proteins and carbohydrates in vermiwash. Straw substrates showed moderate levels due to partial lignocellulose breakdown yielding secondary metabolites like carotenoids, while cow dung's pre-digested nature yielded the lowest values, limited by depleted soluble fractions. Total phenols remained statistically similar across treatments (NS), reflecting consistent polyphenolic synthesis regardless of substrate recalcitrance. Several researchers, Senthilmurugan (2018), Luján-Hidalgo (2017), Ravindrakumar (2023) and Iqbal (2025) have investigated the biochemical characteristics of vermiwash. Their studies reported the presence of proteins, soluble sugars, phenolic compounds, photosynthetic pigments, and various enzymes, highlighting the potential of vermiwash as a nutrient-rich biofertilizer and plant growth-promoting substance. These patterns confirm kitchen waste vermiwash as nutritionally superior for plant biostimulation, enhancing pigment synthesis and carbohydrate mobilization over lignocellulosic or stabilized alternatives.

Conclusion

The findings of the present study demonstrate that the quality and biological efficacy of vermiwash are strongly influenced by the type of organic substrate used during its preparation. Among the evaluated substrates, kitchen waste combined with cow dung (1:1) consistently produced the most superior vermiwash, exhibiting significantly higher concentrations of essential macronutrients (N, P, and K), micronutrients (Fe, Mn, Zn, and Cu), beneficial microbial populations, enzymatic activities, and biochemical constituents including proteins, chlorophylls, carotenoids, lycopene, and soluble sugars. The enhanced performance of kitchen waste-based vermiwash can be attributed to its nutrient-rich, readily degradable organic matter, which promoted greater earthworm activity, microbial proliferation, and efficient nutrient mineralization. Neem leaf-based vermiwash also exhibited promising

characteristics, whereas vermiwash prepared from rice straw, wheat straw, and cow dung showed comparatively lower nutrient enrichment and biological activity. Importantly, heavy metal concentrations in all treatments remained well below the permissible limits, confirming the environmental safety of the prepared vermiwash for agricultural applications. These findings highlight that substrate selection plays a crucial role in determining the physicochemical, microbiological, and biochemical quality of vermiwash. The study establishes kitchen waste as an efficient and sustainable feedstock for producing high-quality vermiwash with enhanced potential as an eco-friendly biofertilizer and biostimulant. Its application can contribute to improved nutrient availability, enhanced soil microbial activity, and sustainable crop production while simultaneously promoting the recycling of biodegradable organic wastes. Further field-based investigations across different crops and agro-climatic conditions are recommended to validate its agronomic performance and optimize its large-scale utilization in sustainable agricultural systems.

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Author Contributions

RK, NR, SG, S, ASS and SSW conceived the concept, wrote and approved the manuscript.

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