

Mass balance, physicochemical characterization and preliminary ecotoxicity assessment of byproducts from *Azadirachta indica*-based biopesticide production: Toward informed biological valorization

Djalalou-Dine MEMOKO

djalmemoko40@gmail.com

University of Lomé <https://orcid.org/0009-0005-7306-1458>

Yao Félicité AMENUTI

University of Lomé

Yawo Amen NENONENE

University of Lomé

Research Article

Keywords: *Azadirachta indica*, biopesticide, byproducts, physicochemical characterization, ecotoxicity, *Eisenia fetida*.

Posted Date: October 13th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10751179/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Mass balance, physicochemical characterization and preliminary ecotoxicity assessment of byproducts from *Azadirachta indica*-based biopesticide production: Toward informed biological valorization

Djalalou-Dine MEMOKO ^{1*}, Yao Félicité AMENUTI ² and Yawo Amen NENONENE ³

¹ University of Lomé, School of Agronomy (ESA); Research Laboratory on Agroresources and Environmental Health (LARASE), P.O. Box 1515; Lomé, Togo; djalmemoko40@gmail.com

² University of Lomé, School of Agronomy (ESA); Research Laboratory on Agroresources and Environmental Health (LARASE), P.O. Box 1515; Lomé, Togo; henocamenuti@gmail.com

³ University of Lomé, School of Agronomy (ESA); Research Laboratory on Agroresources and Environmental Health (LARASE), P.O. Box 1515; Lomé, Togo; amen.nenonene@gmail.com

* Corresponding author: djalmemoko40@gmail.com ; ORCID : 0009-0005-7306-1458

Abstract

Neem (*Azadirachta indica*)-based biopesticide production generates three solid byproducts (pulp, depulping sludge, and husks) that are currently discarded untreated despite their substantial organic matter content and the environmental risks associated with their residual bioactive compounds. This study simultaneously quantified the mass flows and determined the physicochemical characteristics of these three byproduct fractions, and conducted a preliminary ecotoxicity assessment of neem fruit pulps on *Eisenia fetida* to establish a maximum incorporation threshold for vermicomposting. A total of 300 kg of dry neem fruits were processed, yielding byproducts representing 48.79% of the processed fruit dry mass, with pulp as the dominant fraction (39.14%), followed by husks (7.21%) and depulping sludge (2.44%). All three byproducts exhibited high organic matter content (76.87-81.50% DM) and C/N ratios ranging from 24.52 to 37.15, indicative of their potential for anaerobic digestion, composting, and vermicomposting. The ecotoxicity assessment revealed a significant increase in *Eisenia fetida* mortality above a pulp incorporation rate of 30% (w/w), establishing this threshold as the maximum safe incorporation rate for vermicomposting. To the best of our knowledge, this is the first study to simultaneously characterize all three byproduct fractions from neem biopesticide processing, including depulping sludge, which had not been previously described, and to empirically determine an ecotoxicological threshold for earthworm-based valorization of these residues.

Keywords: *Azadirachta indica*, biopesticide, byproducts, physicochemical characterization, ecotoxicity, *Eisenia fetida*.

Highlights

- First simultaneous quantification and characterization of three neem biopesticide byproducts
- Depulping sludge from wet processing characterized for the first time in the literature
- *Eisenia fetida* mortality increases significantly above 30% pulp incorporation rate
- Fraction-specific valorization pathways recommended based on C/N and ecotoxicity data
- Decision framework proposed for biological valorization of neem processing byproducts.

1. Introduction

Recent concerns about the harmful effects of chemical pesticides on human health and the environment [1] have led to a surge in biopesticide use worldwide [2]. Among the various biopesticides available, neem fruit derivatives are among the most popular because of their proven effectiveness and broad activity [3]. The key active component, azadirachtin, targets insects, fungi, and bacteria [4], which explains the increasing demand for neem-based biopesticides. The industrial-scale processing of neem seeds generates substantial volumes of solid residues: for every ton of neem oil produced, approximately four tons of press cake are generated as a solid byproduct [5]. As the neem biopesticide sector expands across Sub-Saharan Africa, where neem trees are abundant and local processing units are being established, the volumes of processing byproducts are expected to increase proportionally, raising urgent questions about their sustainable management.

Extracting neem kernel, which holds the highest concentration of the active compound, involves processing the entire fruit. Initially, fruits are soaked in water to ease depulping, the process where the seed is separated from its pulp, resulting in two byproducts: pulp and depulping sludge. The seeds are then sun-dried and mechanically dehusked to obtain the kernel needed for biopesticide production (either neem powder or neem oil), with husks forming another byproduct. These three byproducts are currently discarded without treatment, despite containing residual bioactive compounds that may pose environmental risks if not properly managed, thereby justifying the need for controlled biological valorization pathways.

Biological recovery processes such as anaerobic digestion, composting, and vermicomposting are well-established strategies for managing organic residues from agro-industrial processing, including those generated in tropical settings [6, 7]. These processes convert organic waste into value-added products such as biogas, compost, and vermicompost, thereby contributing to the principles of the circular economy in biopesticide supply chains. The high organic matter content and high C/N ratios of byproducts from neem fruit processing [6, 8] suggest compatibility with these valorization pathways. However, the presence of concentrated bioactive compounds in the raw byproducts of neem processing, notably azadirachtin, limonoids, and triterpenoids, introduces an ecotoxicological constraint that must be addressed empirically before any attempt at biological valorization, particularly for earthworm-based systems.

Earthworms possess a biochemical defense mechanism that enables them to tolerate dietary bioactive compounds, particularly polyphenols [9]. In fact, *Eisenia fetida* and related species produce gut metabolites termed drilodefensins, which act as biological surfactants to protect them against the inhibition of digestive enzymes. According to [9], a high concentration of bioactive compounds in the worms' feed leads to a 50% increase in drilodefensins secretion, suggesting that this defense system is adaptive but concentration-dependent, though its capacity remains limited. Based on this adaptive capacity of earthworms, several studies have demonstrated the feasibility of vermicomposting with substrates containing high levels of bioactive compounds, including invasive weeds such as *Ageratum conyzoides* [10, 11], *Parthenium hysterophorus* [12, 13], *Chromolaena odorata* [14], and *Lantana camara* [15], as well as agro-industrial residues from the processing of *Jatropha curcas* [16]. However, these studies consistently link earthworm tolerance to the nature of the substrate and, above all, to its concentration of bioactive compounds. These findings indicate that, for any novel bioactive-rich substrate, a preliminary ecotoxicity assessment is a necessary prerequisite before

establishing a vermicomposting process [17]. Specifically regarding byproducts from neem fruit processing, the studies available in the literature relate solely to the effect of concentrations of diluted neem extracts or purified azadirachtin, as recommended for agricultural use, on earthworms [18, 19]. These studies do not provide information on the tolerance of *Eisenia fetida* worms to different incorporation rates of raw neem fruit by-products in an actual vermicomposting process.

To date, three critical knowledge gaps hinder the rational design of biological valorization systems for neem biopesticide processing byproducts. First, no study has simultaneously quantified and physicochemically characterized all three byproduct fractions (pulp, depulping sludge, and husks) generated from a single processing unit operating under real wet-depulping conditions. [6] characterized sun-dried neem pulps exclusively for bioethanol production purposes, while [20] focused solely on the physical properties of the neem nut, leaving the depulping sludge, a byproduct specific to wet mechanical processing, entirely uncharacterized in the available literature. Second, the physicochemical properties of depulping sludge, generated by wet mechanical depulping, have never been reported, representing a complete absence of baseline data for this fraction. Third, the tolerance of *Eisenia fetida* to concentrated raw neem fruit pulps at incorporation rates representative of a real vermicomposting process has never been empirically determined, making it impossible to define safe and effective operating conditions for earthworm-based valorization of these residues. Addressing these three knowledge gaps is a prerequisite for the rational design of biological valorization systems for neem biopesticide processing byproducts.

The objectives of this study were therefore to: (i) quantify the mass flows of the three byproducts generated during neem-based biopesticide production; (ii) determine their physicochemical characteristics; and (iii) conduct a preliminary ecotoxicity assessment of neem fruit pulps on *Eisenia fetida* to establish a maximum incorporation threshold for vermicomposting.

To the best of our knowledge, this is the first study to simultaneously quantify and physicochemically characterize all three byproduct fractions (pulp, depulping sludge, and husks) generated from a neem biopesticide processing unit operating under real wet-depulping conditions. In particular, depulping sludge, a byproduct specific to wet mechanical processing, is characterized here for the first time. Furthermore, this study provides the first empirical determination of an ecotoxicological incorporation threshold for neem fruit pulps in an *Eisenia fetida*-based vermicomposting system, thereby establishing the operational foundation needed for the biological valorization of these residues.

2. Material and Methods

2.1. Study site

The study was conducted at the Lomé Agronomic Experimental Station (SEAL), affiliated with the University of Lomé in Togo (06°17'N, 001°20'E). The station includes an anaerobic digestion unit, a neem fruit processing unit for biopesticide production, and a cattle farm.

2.2. Collection and Processing of Neem Fruits

The neem fruits used in this study were purchased from neem fruit collectors in Dapaong (Savanes Region, Togo). Figure 1 shows a cross-section of a fresh neem fruit, illustrating its various parts. For byproduct quantification, 300 kg of dried neem fruits were soaked in water

for 24 hours (Figure 2) and then mechanically depulped with water. This depulping step yielded pulp and depulping sludge (fine pulp fragments mixed with water). The seeds obtained after depulping were sun-dried for three days and then mechanically dehusked. Dehusking yielded kernels for biopesticide production (neem powder or neem oil) and husks, which constitute the third byproduct.

2.3. Mass quantification of Byproducts

Byproduct mass was quantified by weighing on an electronic balance with a precision of ± 0.01 kg. Husks were weighed directly after mechanical dehulling and collected progressively into basins for weighing.

Mass quantification of pulps and sludge was preceded by a draining step. Pulp was drained through sieves for four hours, whereas sludge, due to its finer particle size, was drained through burlap sacks for 48 hours. The fresh mass of each byproduct was recorded after draining. Figure 3 shows the appearance of pulps and sludge after draining.

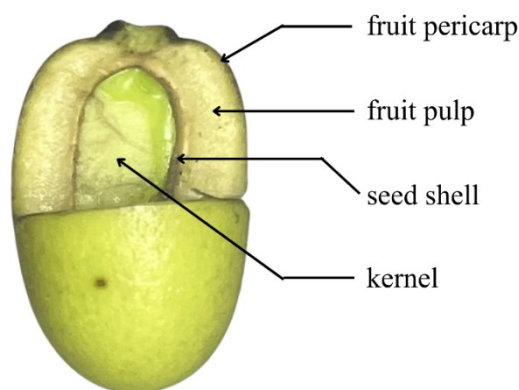


Figure 1. Structure of a fresh, mature neem fruit

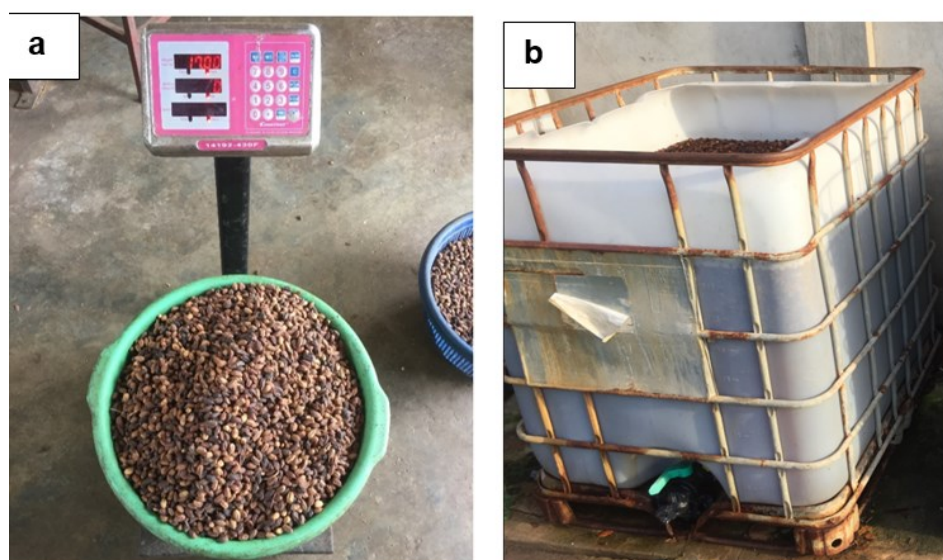


Figure 2. Soaking of dried neem fruits before depulping: (a) weighing of dried neem fruits before filling the soaking tanks; (b) soaked neem fruits

After draining, three subsamples were collected from each byproduct (drained pulps, drained sludge, and husks) to determine dry matter content. The dry matter content for each byproduct was used to calculate the total dry mass generated from the 300 kg of processed neem fruits using the following equation:

$$B_{DM} = B_{FM} \times DM \quad (1)$$

B_{DM} = byproduct dry mass (kg); B_{FM} = Fresh mass of the byproduct after draining (kg); DM = dry matter content determined from 100 g subsamples (%).

Once the dry mass of each byproduct was determined, the byproduct yield relative to the 300 kg of processed fruit was calculated as follows:

$$BP_{yield} = \frac{B_{DM}}{F_{mass}} \times 100 \quad (2)$$

BP_{yield} = byproduct yield relative to the mass of processed fruits (%); B_{DM} = byproduct dry mass (kg); F_{mass} = total mass of processed fruits (300 kg).

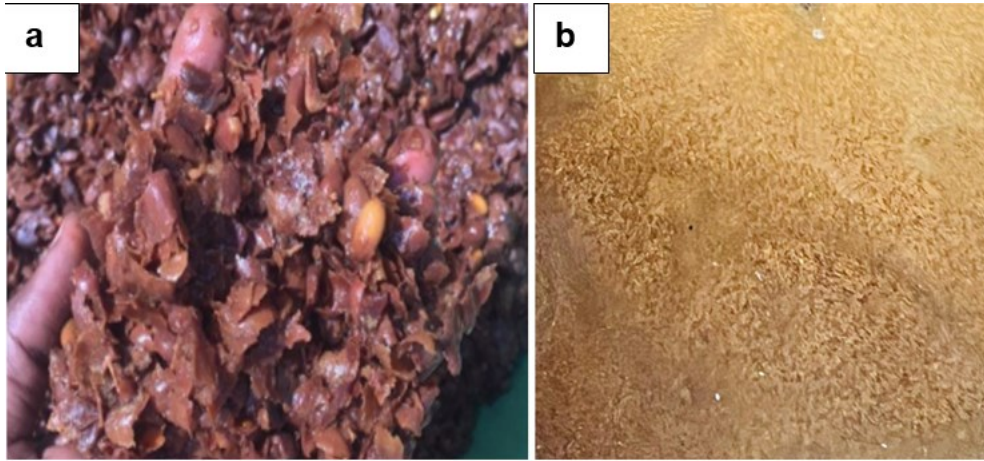


Figure 3. Appearance of pulps (a) and sludge (b) after draining

2.4. Sample collection

Samples for physicochemical analyses were collected immediately after draining for pulps and sludge and after dehulling for husks. Three 1 kg subsamples were collected per byproduct and weighed on a CAMRY electronic balance (precision ± 0.1 g). Samples were sealed in airtight bags and transported primarily to the Soil and Plant Analysis Laboratory of the École Supérieure d'Agronomie (ESA, University of Lomé).

2.5. Chemical Analysis Methods

Physicochemical analyses included dry matter content (DM), pH, ash content (AC), organic matter (OM), total organic carbon (TOC), Kjeldahl total nitrogen (TNK), phosphorus, potassium, and total sugar. Total sugar content was measured only for pulps, given their expected high sugar content. Before analysis, pulps and sludge samples were pre-dried at 40 °C for two hours, then ground and sieved through a 2 mm sieve (all byproducts).

2.5.1. Dry matter content and pH

Dry matter content (DM) was determined according to the standard NF EN 13040 (AFNOR, 2007). A 100 g subsample of each byproduct was weighed (m_1) into a pre-tared crucible (m_0) using a balance with 0.1 g precision. Samples were placed in an oven maintained at $103 \pm 2^\circ\text{C}$ and dried to constant mass. Crucibles were then removed, allowed to cool, and reweighed (m_2). Dry matter content was calculated as follows:

$$DM(\%) = \frac{m_2 - m_0}{m_1 - m_0} \times 100 \quad (3)$$

DM (%) = dry matter content of byproduct; m_0 = mass of the empty crucible (g); m_1 = mass of the sample + crucible oven drying (g); m_2 = mass of the sample + crucible after oven drying (g).

The pH of each byproduct was measured in aqueous suspension at a 1:5 (w/v) ratio in accordance with standard NF EN 13037 (AFNOR, 2012). A 20 g subsample of dried, ground, and sieved material was added to 100 mL of distilled water, homogenized by stirring for 15 minutes, and then measured with a VIVOSUN pH meter (precision 0.01).

2.5.2. Organic Matter and Ash Content

Organic matter (OM) and ash content (AC) were determined by combustion in accordance with standard NF EN 13039 (AFNOR, 2011). A 20 g subsample of oven-dried material ($103 \pm 2^\circ\text{C}$) was placed in a pre-calcined crucible (m_0) and then ignited in a muffle furnace at $450 \pm 10^\circ\text{C}$ for three hours. After cooling, the remaining ash mass was weighed (m_2), and ash and organic matter contents were calculated as follows:

$$AC(\%) = \frac{m_2 - m_0}{m_1 - m_0} \quad (4)$$

$$MO(\%) = 100 - MC(\%) \quad (5)$$

AC (%) = ash content; OM (%) = organic matter content; m_0 = mass of the empty crucible (g); m_1 = mass of the dried sample + crucible before combustion (g); m_2 = mass of ash + crucible after combustion (g).

2.5.3. Total Organic Carbon

Total organic carbon (TOC) was determined by wet oxidation using the Walkey & Black (1934) method, with a 0.167 M potassium dichromate solution in concentrated sulfuric acid, followed by back-titration of the unreduced dichromate with ammonium ferrous sulfate.

2.5.4. Total Kjeldahl Nitrogen and C/N ratio

Total Kjeldahl nitrogen content was determined using the modified Kjeldahl method in accordance with standard NF EN 13654-1 (AFNOR, 2002). A 1 g subsample of dried, ground material was placed in a digestion tube, to which 5 g of Kjeldahl catalyst and 12 mL of concentrated sulfuric acid were added. Mineralization was carried out for approximately 75 minutes at 200°C , followed by one hour at 420°C , converting organic nitrogen to ammonium. Distillation was then performed by adding 80 mL of distilled water, 50 mL of sodium hydroxide solution, and 30 mL of boric acid solution. The resulting ammonia was recovered by steam entrainment and collected in a boric acid solution containing a few drops of bromothymol blue indicator. Titration was performed using a 0.1 N hydrochloric acid solution [21]. A blank test

was run in parallel using 2 mL of water, following the same protocol. Total nitrogen content was calculated as follows:

$$TKN(\%) = \frac{(V_1 - V_0) \times C \times 1,401}{m} \quad (6)$$

V_1 = volume of acid used for sample titration (mL); V_0 = volume of acid used for blank titration (mL); C = molar concentration of the acid (mol/L); m = dry mass of the subsample (g).

The C/N ratio of each byproduct was calculated by dividing its TOC content by its TKN content as follows:

$$C/N = \frac{COT(\%)}{NTK(\%)} \quad (7)$$

2.5.5. Total Phosphorus

Total phosphorus content was determined by the ammonium molybdate spectrometric method in accordance with standard NF EN ISO 6878 (AFNOR, 2005). The filtered extract (0.45 μ m) was acidified with an ammonium sulfate/sulfuric acid mixture, then treated with ammonium molybdate and ascorbic acid. A blue phosphomolybdate complex formed, and absorbance was measured at 880 nm after 30 minutes. A linear calibration curve (0.005 to 0.8 mg P) was used to calculate phosphorus content, with a detection limit of 0.005 mg/L. Phosphorus contents, initially expressed as elemental phosphorus (P), were converted to P_2O_5 equivalents and presented as % P_2O_5 .

2.5.6. Potassium

Potassium, expressed in K_2O , was determined by flame atomic absorption spectrometry after extraction. A 5 g subsample was suspended in 200 mL of demineralized water, brought to a gentle boil under reflux for 30 minutes, cooled, filtered through Whatman No. 40 filter paper, and adjusted to 250 mL in a volumetric flask. The extract was diluted 1:100 to bring the concentration within the linear range (0-5 mg K/L), and cesium chloride was added at 0.1 (w/v) as an ionization suppressant. Measurements were performed in an air-acetylene flame at 766.5 nm using a five-point calibration curve.

2.5.7. Total Sugars

Total sugar content was determined using the phenol-sulfuric acid colorimetric method, as described by [22]. A 2 mL aliquot of aqueous pulp extract was introduced into a colorimetric tube, to which 0.05 mL of 80 % (w/w) phenol was added. Five milliliters of concentrated sulfuric acid (95.5 %) were then rapidly poured, directing the stream against the liquid surface to ensure proper mixing. After 10 minutes of rest, the tubes were agitated and placed in a water bath at 30 °C for 20 minutes. The characteristic yellow-orange color was measured at 490 nm for hexoses and at 480 nm for pentoses and uronic acid. Sugar concentration was determined by reference to a standard calibration curve, and results were expressed as a percentage of dry matter.

2.6. Preliminary ecotoxicity assessment of neem fruit pulps

A preliminary ecotoxicity test was conducted to identify the maximum pulp incorporation rate above which *Eisenia fetida* mortality becomes significantly higher than in the control. This step was necessary given that the bioactive compounds concentrated in raw neem fruit pulps,

including azadirachtin, limonoids, and triterpenoids, may be detrimental to earthworm survival during vermicomposting [18, 19].

Seven substrates were prepared in three replicates each: a control consisting of seven days-precomposted cattle manure only, and six substrates combining precomposted cattle manure with neem fruit pulps at incorporation rates of 10%, 20%, 30%, 40%, 50%, and 60% (w/w). Each replicate was inoculated with 10 adult clitellate *Eisenia fetida* earthworms. The test was conducted over two weeks under controlled conditions. Dead earthworms were counted daily and removed to calculate the cumulative mortality rate at the end of the test period. A one-way analysis of variance (ANOVA) was performed to assess the effect of pulp incorporation rate on earthworm mortality, and Tukey's HSD post-hoc test was used for mean comparison. The preliminary ecotoxicity data reported here were used to establish the experimental design of a companion vermicomposting study

3. Results

3.1. Quantities of byproducts generated

The results of the mass quantification of byproducts generated during neem-based biopesticide production are presented in Table 1.

The results show that kernels account for 51.21% and wastes for 48.21% of the total fruit mass processed. Among the wastes, pulps are predominant with 39.14% of the mass of processed fruits, followed by shells (7.21%), while sludges rank last with 2.44%.

Table 1. Mass balance of byproducts generated from the processing of 300 kg of dry neem fruits.

Product/by-product	Fresh mass (kg)	DM (%)	Dry mass (kg)	Rate (%)
Kernel	170.13	90.30 ± 0.15	153.63	51.21
By-products	797.96	-	146.37	48.79
Total soaked seeds	968.09	-	300.00	100.00
<i>Pulps</i>	645.32	18.20 ± 0.36	117.44	39.14
<i>Sludge</i>	125.77	5.82 ± 0.43	7.31	2.44
<i>Husks</i>	23.37	92.50 ± 1.20	21.62	7.21
<i>Handling losses</i>	3.50	-	-	-
Total - by-products	797.96	-	146.36	-

Fresh mass = mass of the byproduct after draining; DM = dry matter content after oven drying; Dry mass = dry mass of the byproduct relative to its total fresh mass; Rate = byproduct rate relative to the mass of processed fruits.

3.2. Physicochemical Characteristics of Byproducts

The results of laboratory analyses of each byproduct are presented in Table 2. Neem husks were naturally dry (DM = 92.50 %), while pulps (DM = 18.20 %) and sludge (DM = 5.82 %) were moist. Regarding pH, all three byproducts fell within the acidic range, with values of 5.80, 5.20, and 5.50 for pulps, sludge, and husks, respectively.

All three byproducts exhibited high organic matter content (76.87-81.50 %DM), with a similar trend observed for total organic carbon, ranging from 44.59 to 47.27% DM. C/N ratios ranged from 24.52 for sludge to 37.15 for husks, with pulps displaying an intermediate value of 33.52.

Major nutrient contents (N, P, K) were modest across all the byproducts. Nitrogen ranged from 1.20 % DM (husks) to 1.85 %DM (sludge); phosphorus from 0.22 DM (sludge) to 0.27 % DM (husks); and potassium from 1.70 DM (sludge) to 1.94 % DM (husks). Total sugar content, measured exclusively in pulps, was 69.77 ± 2.31 %DM.

Table 2. Physicochemical characteristics of the three byproducts

Parameter	Pulps	Sludge	Husks
DM (%)	18.20 ± 0.36	5.82 ± 0.43	92.50 ± 1.20
pH	5.80 ± 0.04	5.20 ± 0.10	5.50 ± 0.15
OM (% DM)	81.50 ± 1.20	78.20 ± 2.10	76.87 ± 1.50
TOC (% DM)	47.27 ± 0.70	45.36 ± 1.22	44.59 ± 0.80
TKN (% DM)	1.41 ± 0.03	1.85 ± 0.10	1.20 ± 0.12
P ₂ O ₅ (% DM)	0.25 ± 0.02	0.22 ± 0.03	0.27 ± 0.04
K ₂ O (% DM)	1.91 ± 0.12	1.70 ± 0.15	1.94 ± 0.20
C/N	33.52	24.52	37.15
Total sugars (% DM)	69.77 ± 2.31	-	-

DM = dry matter content; OM = organic matter content; TOC = total organic carbon; TKN = total Kjeldahl nitrogen.

3.3. Preliminary ecotoxicity assessment of neem fruit pulp

The results of the preliminary ecotoxicity test are presented in Table 3. Analysis of variance revealed a highly significant effect of pulp incorporation rate on *Eisenia fetida* mortality ($p < 0.001$). At incorporation rates of 10% and 20% (T10 and T20), mortality remained low (6.7% and 13.3%, respectively) and did not differ significantly from the control (T0: 3.3%; $p > 0.05$). A significant increase in mortality was first observed at T30 (23.3%; $p < 0.05$), marking the threshold above which pulp incorporation rate had a statistically significant detrimental effect on earthworm survival. Beyond this threshold, mortality rose sharply and progressively, reaching 46.7% at T40, 76.7% at T50, and 100% at T60, with each treatment forming a statistically distinct group. Based on these results, a pulp incorporation rate of 30% (w/w) was identified as the maximum safe threshold for *Eisenia fetida*-based vermicomposting of neem fruit pulps.

Table 3. Effect of neem pulp incorporation rate on *Eisenia fetida* mortality during the preliminary ecotoxicity test

Treatments	Mortality (%)
T0: control (cattle manure)	3.3 a
T10: 10 % pulp incorporation	6.7 ab
T20: 20 % pulp incorporation	13.3 ab
T30: 30 % pulp incorporation	23.3 b
T40: 40 % pulp incorporation	46.7 c
T50: 50 % pulp incorporation	76.7 d
T60: 60 % pulp incorporation	100.0 e

Means followed by the same letter are not significantly different at 5% threshold (Tukey's HSD test).

4. Discussion

4.1. Mass balance of products generated during neem fruit processing

Kernels accounted for 51.21% of the total mass of processed dry neem fruits. The kernel fraction obtained in this study is close to the value of 51.35% reported by [6] for fruits collected at the Mora site in Cameroon, whereas [20] reported a higher kernel fraction of 55.83%. This discrepancy is mainly attributable to the basis used for calculating the kernel fraction: [20] expressed kernel yield relative to dry seeds, while in the present study, kernel yield was calculated relative to the total mass of processed dry neem fruits.

Wastes generated from neem fruit processing represented 48.79% of the total mass of dry neem fruits, with pulps being the dominant waste fraction at 39.14%. [6] reported a pulp fraction of 48.65%, which differs from the value obtained in the present work. This difference may be explained by the fact that they did not account explicitly for shells and depulping sludge fractions; instead, they partitioned neem fruits into only kernels and pulps, whose combined proportion was set to 100%. The pulp obtained here, therefore, represents a potentially valuable resource for energy and agronomic valorization, in line with the recommendations of [7], which are consistent with the principles of the circular economy.

Shells represented 7.21% of the neem fruit processing wastes. This proportion is lower than the 44.17% shell fraction reported by [20]. The difference between these values is again related to the calculation basis: their shell fraction was expressed relative to seed mass, whereas in the present study it is expressed relative to the total mass of dry neem fruits.

Depulping sludges (2.44%) constituted the smallest waste fraction and were highly liquid, as they consisted of a mixture of pulp fragments and water. This liquid nature can hinder the valorization of this waste stream, since it requires substantial reduction of its moisture content. To increase the concentration of solid residues in neem fruit sludges and reduce water consumption, [7] recommends using a depulper equipped with a water recirculation system and filtration units that capture pulp-laden water and recycle it within the process.

Beyond the agronomic implications of individual byproduct fractions, the overall mass balance reveals that byproducts collectively represent 48.79% of the dry mass of processed neem fruits, nearly equivalent to the kernel fraction itself (51.21%). At industrial processing scale, where hundreds of tons of neem fruits are processed annually to meet growing biopesticide demand [5], this proportion translates into substantial biomass flows that cannot be sustainably managed through simple discarding. This finding reinforces the urgency of developing structured biological valorization pathways for these residues, as advocated by [7].

4.2. Dry Matter Content and pH

The dry matter results show that husks are naturally dry (DM = 92.50 %), consistent with their rigid lignocellulosic structure, comparable to *Jatropha curcas* husks for which [23] report a moisture content of only 5-6%. Pulps (DM = 18.20 %) and sludge (DM = 5.82%) were moist due to the water-based depulping process. These results differ from those reported by [6], who obtained dry matter contents ranging from 87 to 90% for neem pulps. This discrepancy is explained by the fact that those authors worked with sun-dried pulps, whereas our depulping process involves water soaking followed by mechanical depulping.

All three byproducts displayed acidic pH values (5.20-5.80), attributable to the presence of fermentable sugars and naturally occurring organic acids in fruit tissues, further accentuated by

the preliminary soaking of the fruits. These results are consistent with those of [6], who reported a natural must pH of 5.4 for neem pulps. Sludge displayed the lowest pH, likely due to the concentration of organic acids during the aqueous depulping process.

The acidic pH values recorded across all three byproducts (5.20-5.80) have direct implications for biological valorization. In anaerobic digestion, optimal methanogenesis occurs within a pH range of 6.8-7.2 [24], meaning that direct digestion of these byproducts without pH adjustment or co-digestion with a buffering substrate, such as cattle manure, would risk inhibiting methanogenic activity. For composting and vermicomposting, mildly acidic substrates are generally tolerated, as microbial activity and earthworm metabolism tend to progressively neutralize pH during the process [15].

4.3. Organic matter (OM), total organic carbon (TOC) and C/N ratio

All three byproducts exhibited high organic matter contents (76.87-81.50% DM), with pulps showing the highest values. These results confirm the predominantly organic nature of these residues, consistent with data reported by [25] on neem byproducts, who highlighted their high labile organic matter content, favorable to biological processes. TOC values (44.59-47.27% DM) followed the same trend and are representative of substrates rich in both structural and soluble carbohydrates.

The contrasting C/N ratios observed across the three byproduct fractions reflect their distinct biochemical compositions. Depulping sludge, with the lowest C/N ratio (24.52), is enriched in soluble nitrogen-containing compounds leached from pulp tissue during the aqueous depulping process, a phenomenon well documented for fruit-derived processing byproducts subjected to wet transformation [26, 27], which explains both its elevated nitrogen content (1.85% DM) and its lowest C/N ratio among the three fractions. Pulps displayed an intermediate C/N ratio (33.52), consistent with their high total sugar content (69.77% DM) diluting the relative nitrogen proportion. Husks exhibited the highest C/N ratio (37.15), reflecting their predominantly lignocellulosic structure, which is inherently carbon-rich and nitrogen-poor, as similarly reported for *Jatropha curcas* husks [23].

Regarding anaerobic digestion, sludge showed the most favorable C/N ratio (24.52), falling within the optimal range of 20-30 reported for this process [28, 29]. For pulps, the C/N ratio of 33.52 slightly exceeds this optimal range, requiring co-digestion with a nitrogen-rich substrate to restore a favorable C/N balance. Cattle manure, with typical C/N ratios of 15-25 [30], represents a readily available co-substrate in Sub-Saharan African contexts and could effectively compensate for the nitrogen deficit of neem fruit pulps. Additionally, the high total sugar content of pulps (69.77% DM) is favorable for rapid hydrolysis and acidogenesis, but may trigger volatile fatty acid accumulation and process acidification if the organic loading rate is not carefully controlled [31].

Regarding composting, pulps displayed a C/N ratio (33.52) within the optimal range [32, 33], comparable to that of banana peels (30–35; [34]) and pineapple waste (30–45; [29]). Husks exhibited the highest C/N ratio (37.15), exceeding the commonly recommended upper threshold for efficient composting [33], which would slow nitrogen cycling and delay compost maturation. Co-amendment with a nitrogen-rich material, such as depulping sludge (C/N: 24.52) or cattle manure, could therefore serve a dual purpose: correcting the C/N ratio of husks while simultaneously valorizing the sludge fraction, which is otherwise difficult to manage owing to its high moisture content (DM: 5.82%). This co-composting strategy is supported by

recent studies demonstrating that co-amendment of lignocellulosic materials with nitrogen-rich organic wastes consistently improves compost quality and accelerates maturation [35–37].

4.4. Nitrogen, Phosphorus, and Potassium Contents

Nitrogen (1.20-1.85% DM), phosphorus (0.22-0.27% DM), and potassium (1.70-1.94% DM) contents were modest but consistently present across all three byproducts. Sludge showed the highest nitrogen content (1.85% DM), consistent with its lowest C/N ratio among the three fractions. These values are comparable to those reported for neem seed cake from West African processing units, which contains approximately 31.4% protein alongside modest mineral fractions [38], and are consistent with the NPK ranges documented for other agro-industrial fruit processing byproducts (0.61-2.29% N, 0.07-0.55% P, 0.19-3.72% K; [39]) and for *Jatropha curcas* husks, which similarly display low protein and mineral contents [40].

Potassium was the dominant mineral element across all three byproduct fractions (1.70-1.94% DM), a characteristic of particular agronomic relevance in the Togolese context. The soils of southern Togo are dominated by tropical ferruginous soils (Lixisols; [41]), characterized by low cation exchange capacity, high leaching potential, and inherent mineral nutrient deficiencies [42]. Field studies conducted on these soils have demonstrated that nitrogen is the primary limiting nutrient for maize production, followed by phosphorus, with potassium ranking third in order of priority (N > P > K; [43]). While potassium deficiency is therefore a less immediate constraint than nitrogen or phosphorus in these systems, the consistent potassium content of neem byproducts (1.70-1.94% DM) nonetheless represents a complementary agronomic contribution when these residues are valorized through composting or vermicomposting. Organic amendments have been shown to improve overall nutrient availability, including potassium, in southern Togolese soils while simultaneously increasing soil organic carbon stocks and crop yields [44].

Although the nutrient concentrations of these byproducts in their raw state are insufficient for direct use as mineral fertilizers, their valorization through biological processes is expected to enhance nutrient availability and organic matter stability, thereby improving their agronomic value as soil amendments [45]. This potential is further supported by the high organic matter content of all three fractions (76.87-81.50% DM), which constitutes the primary substrate for biological transformation and humus formation.

4.5. Total Sugar Content

Pulps displayed a total sugar content of $69.77 \pm 2.31\%$ DM, confirming the high sugar richness of this byproduct. [6] reported values of 73-74% DM for sun-dried pulps, showing a slight discrepancy with our results. This difference is attributable to partial leaching of soluble sugars during the wet mechanical depulping process used in our study, a phenomenon consistent with the documented transfer of soluble organic compounds from fruit-derived processing byproducts to the aqueous phase during wet transformation [26, 27]. This leaching mechanism also accounts for the acidic pH values recorded in pulps and sludge (5.20-5.80), as partial fermentation of soluble sugars during soaking and wet processing generates organic acids that lower the substrate pH. Total sugar content was determined exclusively for pulps, as this byproduct was identified as the primary candidate for fermentation-based valorization based on preliminary literature data [6], a conclusion confirmed by the characterization results obtained in this study.

The high total sugar content of neem fruit pulps has differentiated implications for each valorization pathway considered. For anaerobic digestion, soluble sugars are rapidly hydrolyzed and converted to volatile fatty acids during acidogenesis, making pulps a highly reactive substrate. Substrates with an acidic initial pH and high soluble sugar content are particularly prone to rapid acidification, as demonstrated for food waste with similar pH profiles (5.2-5.5) by [46], which may inhibit methanogenic activity if the organic loading rate is not carefully controlled [31]. For composting, soluble sugars constitute a highly labile carbon fraction that is rapidly consumed by microorganisms during the thermophilic phase, stimulating microbial activity and accelerating organic matter decomposition [47, 48]. This behavior is analogous to that reported for olive oil processing residues, another sugar-rich, phenolic-bearing agro-industrial byproduct, for which composting has been proposed as the primary stabilization pathway [49]. For vermicomposting, soluble sugars constitute a readily available energy source for *Eisenia fetida*. Studies on sugar-rich agro-industrial residues such as sugar cane press mud have shown that *Eisenia fetida* can tolerate and actively process these substrates at moderate incorporation rates [37, 50], which may partially explain the earthworms' capacity to tolerate pulp incorporation rates up to 30% (w/w) as observed in the preliminary ecotoxicity assessment of the present study.

4.6. Bioactive compounds and preliminary ecotoxicity assessment

The physicochemical characterization carried out in this study does not include quantitative determination of the bioactive compounds present in neem byproducts, particularly azadirachtin and limonoids, as the required analytical equipment (HPLC or LC-MS/MS) was not available within the constraints of this study. However, the absence of quantitative bioactive compound data does not preclude a meaningful ecotoxicological assessment. Following an approach widely adopted in ecotoxicological studies on complex organic matrices [17, 51], we substituted analytical quantification with a functional bioassay using *Eisenia fetida* as a sentinel organism. This approach is justified by two considerations: first, *Eisenia fetida* is an internationally recognized test organism for soil ecotoxicology [52], whose mortality response integrates the cumulative toxic effect of all bioactive compounds present in a substrate rather than targeting a single molecule; and second, the quantitative determination of azadirachtin and limonoids in neem fruit processing byproducts constitutes a methodological priority for future work, particularly given the contrasting ecotoxicological profiles reported for different neem-derived products and exposure formats in the literature.

The preliminary ecotoxicity assessment revealed a clear dose-dependent relationship between pulp incorporation rate and *Eisenia fetida* mortality ($p < 0.001$). At low incorporation rates (T10: 6.7%; T20: 13.3%), mortality did not differ significantly from the control (T0: 3.3%), indicating that earthworms tolerated these concentrations through their biochemical defense mechanisms, notably the production of drilodefensins, gut surfactant metabolites that increase by approximately 50% in response to bioactive-rich diets [53]. The significant increase in mortality first observed at T30 (23.3%; $p < 0.05$) marks the threshold above which the cumulative ecotoxicological effect of neem pulp bioactive compounds exceeds the adaptive capacity of *Eisenia fetida*. Beyond this threshold, mortality rose sharply and progressively, 46.7% at T40, 76.7% at T50, and 100% at T60, with each treatment forming a statistically distinct group. This pattern is consistent with the dose-response relationships reported for plant-derived extracts and essential oils on *Eisenia fetida*, where mortality increases progressively with concentration and the response is highly dependent on the nature and concentration of the

bioactive compounds involved [51, 54]. These results establish a pulp incorporation rate of 30% (w/w) as the maximum safe operational threshold for *Eisenia fetida*-based vermicomposting of neem fruit pulps.

The dose-response pattern observed contrasts with previous studies that reported no significant toxicity of neem-derived products on earthworms. [55], working with neem cake incorporated into tropical soil at agronomic doses, found no significant mortality in earthworms, while [18] reported an absence of negative effects at field-recommended concentrations of commercial neem extract. These apparent contradictions are reconciled by considering the exposure format: diluted neem derivatives applied to soil differ fundamentally from concentrated raw byproducts incorporated at high rates in a vermicomposting substrate, where earthworms are in direct and prolonged contact with the material. This distinction between diluted field applications and concentrated processing byproducts represents a critical gap that the present study addresses for the first time. The results are consistent with the broader ecotoxicological literature on bioactive-rich substrates, where *Eisenia fetida* tolerance has been demonstrated for invasive plant species, including *Parthenium hysterophorus* [13, 56], *Lantana camara* [15], and *Chromolaena odorata* [14], but always within substrate- and concentration-specific limits. The 30% threshold identified in this study is also consistent with the tolerance range reported for sugar-rich agro-industrial residues such as sugar cane press mud, where *Eisenia fetida* survival and growth remained compatible up to 30-50% incorporation [50], suggesting that the energy substrate availability from neem pulp sugars (69.77% DM) may partially offset the ecotoxicological stress at moderate incorporation rates. The specific interactions between neem bioactive compounds, azadirachtin, limonoids, and triterpenoids, and the drilodefensin defense system of *Eisenia fetida* remain unknown and constitute a priority research question for the mechanistic understanding of neem byproduct vermicomposting.

4.7. Integrated decision framework for biological valorization

The physicochemical characterization and preliminary ecotoxicity assessment reported in this study collectively provide a multi-parameter evidence base for guiding the selection of biological valorization pathways for neem biopesticide processing byproducts. Rather than treating valorization pathway selection as a binary choice, the data presented here support a fraction-specific, criteria-based decision framework that integrates C/N ratio, organic matter content, moisture content, sugar content, nutrient profile, and earthworm ecotoxicity into a coherent set of operational recommendations. Such decision frameworks have been proposed for other complex agro-industrial byproduct streams [7, 45] and are increasingly recognized as essential tools for translating laboratory characterization data into actionable valorization strategies, particularly in Sub-Saharan African contexts where processing infrastructure and input availability constrain pathway selection.

4.7.1. Pulps: anaerobic digestion or composting recommended

Pulps combine the highest organic matter content (81.50% DM), the highest total sugar content (69.77% DM), and an optimal C/N ratio for composting (33.52), making them the fraction with the highest biological valorization potential. Their elevated fermentable sugar content makes anaerobic digestion the primary recommended pathway, as soluble sugars are rapidly hydrolyzed and converted to volatile fatty acids during acidogenesis, supporting high biogas yields. Co-digestion with cattle manure, widely available in Sub-Saharan African contexts, is recommended to correct the slightly elevated C/N ratio (33.52) and buffer the risk of process

acidification associated with rapid volatile fatty acid accumulation [31, 46]. Composting represents a viable alternative, taking advantage of the high labile carbon content to stimulate thermophilic microbial activity [47]. Vermicomposting is possible but not prioritized for this fraction: the preliminary ecotoxicity assessment demonstrated significant *Eisenia fetida* mortality above a pulp incorporation rate of 30% (w/w), making this pathway the most constrained of the three options and better suited to the less toxic fractions, depulping sludge and husks.

4.7.2. Depulping sludge : anaerobic digestion or co-composting with husks recommended

Depulping sludge presents the most favorable C/N ratio for anaerobic digestion (24.52) and the highest nitrogen content (1.85% DM) among the three fractions, making it the preferred candidate for methane production. Its very high moisture content (DM: 5.82%), however, constitutes a major management constraint, direct handling and transport are difficult, and pre-dewatering would be required before composting or vermicomposting. An alternative strategy consists of using sludge as a nitrogen-rich co-amendment for husk composting, thereby simultaneously addressing the high C/N of husks and valorizing the sludge fraction without the need for dewatering.

4.7.3. Husks: co-composting with nitrogen-rich co-amendment recommended

Husks exhibited the highest C/N ratio (37.15) and the lowest moisture content (DM: 92.50%), reflecting their predominantly lignocellulosic structure. This profile is incompatible with anaerobic digestion and requires C/N correction for efficient composting. Co-amendment with depulping sludge (C/N: 24.52) or cattle manure represents a dual valorization strategy that corrects the C/N ratio of husks while simultaneously managing the sludge fraction [35, 36]. The high dry matter content of husks is an operational advantage, they are easy to store, transport, and mix, making them suitable as a bulking agent in composting systems.

Table 4. Decision framework for biological valorization selection for neem biopesticide processing byproducts

Parameter	Pulps	Depulping sludge	Husks
DM (%FM)	18.20	5.82	92.50
pH	5.80	5.20	5.50
OM (%DM)	81.50	78.20	76.87
TOC (%DM)	47.27	45.36	44.59
TKN (%DM)	1.41	1.85	1.20
P ₂ O ₅ (%DM)	0.25	0.22	0.27
K ₂ O (%DM)	1.91	1.70	1.94
C/N	33.52	24.52	37.15
Total sugars (%DM)	69.77	ND	ND
Anaerobic digestion	Preferred (high fermentable sugars + co-digestion with cattle manure for C/N correction)	Recommended (optimal C/N, highest TKN)	Not recommended (C/N too high + lignocellulosic structure)
Composting	Recommended (optimal C/N, high labile C)	Possible as N-rich co-amendment for husks	Recommended with N-rich co-amendment
Vermicomposting	Possible but not prioritized, ecotoxicity threshold $\leq 30\%$ (w/w) limits feasibility	Feasible	Feasible
Priority constraint	High sugar content = rapid acidification risk (OLR control required) + ecotoxicity limits vermicomposting	Very high moisture (5.82% DM), dewatering required	High C/N (37.15), co-amended required
Recommended pathway	Anaerobic digestion (co-digestion with cattle manure) or composting	Anaerobic digestion or co-composting with husks	Co-composting with sludge or cattle manure

ND = not determined; OLR = organic loading rate; FM = fresh matter; DM = dry matter

5. Conclusion

This study simultaneously quantified the mass flows and determined the physicochemical characteristics of three solid byproducts generated during neem (*Azadirachta indica*) biopesticide production (pulps, depulping sludge, and husks) and conducted a preliminary ecotoxicity assessment of neem fruit pulps on *Eisenia fetida* to establish a maximum incorporation threshold for vermicomposting. The three byproduct fractions collectively represent 48.79% of the dry mass of processed neem fruits, with pulps as the dominant fraction (39.14%), followed by husks (7.21%) and depulping sludge (2.44%). All three fractions exhibited high organic matter content (76.87-81.50% DM) and C/N ratios ranging from 24.52 to 37.15, confirming their suitability as substrates for biological valorization. The preliminary ecotoxicity assessment revealed a significant dose-dependent increase in *Eisenia fetida* mortality above a pulp incorporation rate of 30% (w/w), establishing this value as the maximum safe operational threshold for earthworm-based processing of neem fruit pulps.

To the best of our knowledge, this study provides three original contributions to the literature. First, it is the first simultaneous quantification and physicochemical characterization of all three byproduct fractions from a neem biopesticide processing unit operating under real wet-depulping conditions. Second, depulping sludge, a byproduct specific to wet mechanical processing, is characterized here for the first time, filling a complete gap in the available literature. Third, this study provides the first empirical determination of an ecotoxicological incorporation threshold for neem fruit pulps in an *Eisenia fetida*-based vermicomposting system, resolving a critical operational unknown that previously prevented the rational design of earthworm-based valorization systems for these residues.

Based on the integrated physicochemical and ecotoxicological evidence generated in this study, fraction-specific valorization pathways are recommended. Pulps, are best suited for anaerobic digestion, preferably in co-digestion with cattle manure to correct the C/N ratio and buffer the risk of process. Depulping sludge is recommended for anaerobic digestion or as a nitrogen-rich co-amendment in husk composting. Husks are best valorized through co-composting with a nitrogen-rich co-amendment such as depulping sludge or cattle manure.

Authors Contributions

Conceptualization, D-D.M. and Y.A.N.; methodology, D-D.M. and Y.A.N.; software, D-D.M. and Y.F.A; validation, D-D.M. and Y.F.A; formal analysis, D-D.M. and Y.F.A; investigation, D-D.M. and Y.F.A; resources, D-D.M. and Y.F.A; data curation, D-D.M. and Y.F.A; writing-original draft preparation, D-D.M.; writing-review and editing, D-D.M.; visualization, Y.A.N.; supervision, Y.A.N.; project administration, Y.A.N.; All authors have read and agreed to the published version of the manuscript.

Data Availability

The datasets generated during this study are not publicly available, as they form part of an ongoing doctoral research program, but are available from the corresponding author upon reasonable request.

Ethics approval

Non applicable

Informed consent

Non applicable

Funding

No funding was received for conducting this study.

Acknowledgments

The authors thank the Head and the entire staff of the LARASE laboratory for their continuous support and contributions to this study.

Conflicts of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

References

1. Wan N-F, Fu L, Dainese M, et al (2025) Pesticides have negative effects on non-target organisms. *Nat Commun* 16:1360. <https://doi.org/10.1038/s41467-025-56732-x>
2. Wylie MR, Merrell DS (2022) The Antimicrobial Potential of the Neem Tree *Azadirachta indica*. *Front Pharmacol* 13:891535. <https://doi.org/10.3389/fphar.2022.891535>
3. Ayilara MS, Adeleke BS, Akinola SA, et al (2023) Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides. *Front Microbiol* 14:1040901. <https://doi.org/10.3389/fmicb.2023.1040901>
4. Kilani-Morakchi S, Morakchi-Goudjil H, Sifi K (2021) Azadirachtin-Based Insecticide: Overview, Risk Assessments, and Future Directions. *Front Agron* 3:676208. <https://doi.org/10.3389/fagro.2021.676208>
5. Saleem S, Muhammad G, Hussain MA, Bukhari SNA (2018) A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of *Azadirachta indica*. *Phytother Res* 32:1241–1272. <https://doi.org/10.1002/ptr.6076>
6. Sabba G, Aboubakar A, Njintang Y n, Mbofung C m f (2018) Production du bioéthanol à base de pulpe des fruits de neem (*Azadirachta indica*). *Revue Energ Renouvelables* 21:1–10
7. Kishore SG, Rajkumar P, Sudha P, et al (2024) Optimization of process conditions of the neem fruit depulping machine for the production of premium quality seeds and oil. *Biomass Convers Biorefinery* 14:22539–22551. <https://doi.org/10.1007/s13399-023-04156-2>
8. Diedhiou D (2017) Fractionnement analytique de la graine de neem (*Azadirachta indica* A. Juss.) et de la graine de dattier du désert (*Balanites aegyptiaca* L.) - Valorisation des constituants de la graine de neem par bioraffinage. Phd
9. Liebeke M, Strittmatter N, Fearn S, et al (2015) Unique metabolites protect earthworms against plant polyphenols. *Nat Commun* 6:7869. <https://doi.org/10.1038/ncomms8869>

10. Devi C, Khwairakpam M (2020) Feasibility of vermicomposting for the management of terrestrial weed *Ageratum conyzoides* using earthworm species *Eisenia fetida*. Environ Technol Innov 18:100696. <https://doi.org/10.1016/j.eti.2020.100696>
11. Gusain R, Suthar S (2020) Vermicomposting of invasive weed *Ageratum conyzoids*: Assessment of nutrient mineralization, enzymatic activities, and microbial properties. Bioresour Technol 312:123537. <https://doi.org/10.1016/j.biortech.2020.123537>
12. Devi C, Khwairakpam M (2021) Management of invasive weed *Parthenium hysterophorus* through vermicomposting using a polyculture of *Eisenia fetida* and *Eudrilus eugeniae*. Environ Sci Pollut Res 28:29710–29719. <https://doi.org/10.1007/s11356-021-12720-4>
13. Rajiv P, Rajeshwari S, Rajendran V (2014) Impact of Parthenium weeds on earthworms (*Eudrilus eugeniae*) during vermicomposting. Environ Sci Pollut Res 21:12364–12371. <https://doi.org/10.1007/s11356-014-3149-4>
14. Ganguly RK, Al-Helal MdA, Chakraborty SK (2022) Management of invasive weed *Chromolaena odorata* (Siam weed) through vermicomposting: An eco-approach utilizing organic biomass valorization. Environ Technol Innov 28:102952. <https://doi.org/10.1016/j.eti.2022.102952>
15. Suthar S, Sharma P (2013) Vermicomposting of toxic weed — *Lantana camara* biomass: Chemical and microbial properties changes and assessment of toxicity of end product using seed bioassay. Ecotoxicol Environ Saf 95:179–187. <https://doi.org/10.1016/j.ecoenv.2013.05.034>
16. Das M, Uppal HS, Singh R, et al (2011) Co-composting of physic nut (*Jatropha curcas*) deoiled cake with rice straw and different animal dung. Bioresour Technol 102:6541–6546. <https://doi.org/10.1016/j.biortech.2011.03.058>
17. Gudeta K, Kumar V, Bhagat A, et al (2023) Ecological adaptation of earthworms for coping with plant polyphenols, heavy metals, and microplastics in the soil: A review. Heliyon 9:e14572. <https://doi.org/10.1016/j.heliyon.2023.e14572>
18. Muangphra P, Gooneratne R (2011) Toxicity of Commercial Neem Extract to Earthworms (*Pheretima peguana*). Appl Environ Soil Sci 2011:1–8. <https://doi.org/10.1155/2011/925950>
19. Sarawaneeyaruk S, Krajangsang S, Pringsulaka O (2015) The effects of neem extract and azadirachtin on soil microorganisms. J Soil Sci Plant Nutr 0–0. <https://doi.org/10.4067/S0718-95162015005000075>
20. Visvanathan R, Palanisamy PT, Gothandapani L, Sreenarayanan VV (1996) Physical Properties of Neem Nut. J Agric Eng Res 63:19–25. <https://doi.org/10.1006/jaer.1996.0003>
21. Yassine K (2022) Détermination de la teneur en azote et en protéines par la méthode KJELDAHL. Natl Inst Appl Sci Technol-INSAT2022

22. DuBois Michel, Gilles KA, Hamilton JK, et al (1956) Colorimetric Method for Determination of Sugars and Related Substances. *Anal Chem* 28:350–356. <https://doi.org/10.1021/ac60111a017>
23. Wever D-AZ, Heeres HJ, Broekhuis AA (2012) Characterization of Physic nut (*Jatropha curcas* L.) shells. *Biomass Bioenergy* 37:177–187. <https://doi.org/10.1016/j.biombioe.2011.12.014>
24. Sarker S, Lamb JJ, Hjelme DR, Lien KM (2019) A Review of the Role of Critical Parameters in the Design and Operation of Biogas Production Plants. *Appl Sci* 9:1915. <https://doi.org/10.3390/app9091915>
25. Smith BAM, Eudoxie G, Stein R, et al (2020) Effect of neem leaf inclusion rates on compost physico-chemical, thermal and spectroscopic stability. *Waste Manag* 114:136–147. <https://doi.org/10.1016/j.wasman.2020.06.026>
26. Seikova I, Simeonov E, Ivanova E (2004) Protein leaching from tomato seed—experimental kinetics and prediction of effective diffusivity. *J Food Eng* 61:165–171. [https://doi.org/10.1016/S0260-8774\(03\)00083-9](https://doi.org/10.1016/S0260-8774(03)00083-9)
27. Rojas-Sossa JP, Murillo-Roos M, Uribe L, et al (2017) Effects of coffee processing residues on anaerobic microorganisms and corresponding digestion performance. *Bioresour Technol* 245:714–723. <https://doi.org/10.1016/j.biortech.2017.08.098>
28. Santos LAD, Valença RB, Silva LCSD, et al (2020) Methane generation potential through anaerobic digestion of fruit waste. *J Clean Prod* 256:120389. <https://doi.org/10.1016/j.jclepro.2020.120389>
29. Otieno EO, Kiplimo R, Mutwiwa U (2023) Optimization of anaerobic digestion parameters for biogas production from pineapple wastes co-digested with livestock wastes. *Heliyon* 9:e14041. <https://doi.org/10.1016/j.heliyon.2023.e14041>
30. Li X, Li L, Zheng M, et al (2009) Anaerobic Co-Digestion of Cattle Manure with Corn Stover Pretreated by Sodium Hydroxide for Efficient Biogas Production. *Energy Fuels* 23:4635–4639. <https://doi.org/10.1021/ef900384p>
31. Mohammed KI, Ramayya Ancha V, Beyene Lemma D (2024) Biogas Production Optimization in the Anaerobic Codigestion Process: A Critical Review on Process Parameters Modeling and Simulation Tools. *J Chem* 2024:1–25. <https://doi.org/10.1155/2024/4599371>
32. Topal EIA, Ünlü A, Topal M (2016) Determination of the effect of C/N ratio on composting of vegetable-fruit wastes. *Int J Environ Waste Manag* 18:181. <https://doi.org/10.1504/IJEW.2016.080405>
33. Guo R, Li G, Jiang T, et al (2012) Effect of aeration rate, C/N ratio and moisture content on the stability and maturity of compost. *Bioresour Technol* 112:171–178. <https://doi.org/10.1016/j.biortech.2012.02.099>
34. Kalemelawa F, Nishihara E, Endo T, et al (2012) An evaluation of aerobic and anaerobic composting of banana peels treated with different inoculums for soil nutrient

35. Aydın Temel F (2023) Evaluation of the influence of rice husk amendment on compost quality in the composting of sewage sludge. *Bioresour Technol* 373:128748. <https://doi.org/10.1016/j.biortech.2023.128748>
36. Wang S-P, Li X-X, Sun Z-Y, et al (2023) Evaluation of physicochemical properties, bacterial community, and product fertility during rice straw composting supplemented with different nitrogen-rich wastes. *Bioresour Technol* 369:128462. <https://doi.org/10.1016/j.biortech.2022.128462>
37. Kumar R, Verma D, Singh BL, et al (2010) Composting of sugar-cane waste by-products through treatment with microorganisms and subsequent vermicomposting. *Bioresour Technol* 101:6707–6711. <https://doi.org/10.1016/j.biortech.2010.03.111>
38. Djenontin TS, Wotto VD, Avlessi F, et al (2012) Composition of *Azadirachta indica* and *Carapa procera* (Meliaceae) seed oils and cakes obtained after oil extraction. *Ind Crops Prod* 38:39–45. <https://doi.org/10.1016/j.indcrop.2012.01.005>
39. Krasowska M, Kowczyk-Sadowy M, Obidziński S (2023) Composting of stabilized municipal sewage sludge with residues from agri-food processing in Poland. *Ekon Śr - Econ Environ* 83:103–116. <https://doi.org/10.34659/eis.2022.83.4.509>
40. Makkar HPS, Becker K, Sporer F, Wink M (1997) Studies on Nutritive Potential and Toxic Constituents of Different Provenances of *Jatropha curcas*. *J Agric Food Chem* 45:3152–3157. <https://doi.org/10.1021/jf970036j>
41. IUSS Working Group WRB (2022) World Reference Base for Soil Resources, 4th edition. Vienna
42. Lamouroux, Maurice (1969) Carte pédologique du Togo au 1/1 000 000: notice explicative n° 34. ORSTOM, Paris
43. Mawussi, G., Adden, A.K, Sogbedji, J.M., et al (2015) Identification et hiérarchisation d'éléments nutritifs déterminants pour la production du maïs (*Zea mays* L.) sur sols ferrugineux tropicaux au Sud du Togo. *Rev. CAMES* 3:45–51
44. Aziandeke DK, Dos Santos Vianna M, Bouka EC, et al (2025) The influence of rice husk biochar, compost, and their mixture on maize yields and soil organic carbon content in southern Togo. *Geoderma Reg* 42:e00996. <https://doi.org/10.1016/j.geodrs.2025.e00996>
45. Boafo DK, Kraisornpornson B, Panphon S, et al (2020) Effect of organic soil amendments on soil quality in oil palm production. *Appl Soil Ecol* 147:103358. <https://doi.org/10.1016/j.apsoil.2019.09.008>
46. Kuruti K, Begum S, Ahuja S, et al (2017) Exploitation of rapid acidification phenomena of food waste in reducing the hydraulic retention time (HRT) of high rate anaerobic digester without conceding on biogas yield. *Bioresour Technol* 226:65–72. <https://doi.org/10.1016/j.biortech.2016.12.005>

47. Sanchez-Monedero MA, Cayuela ML, Roig A, et al (2018) Role of biochar as an additive in organic waste composting. *Bioresour Technol*. <https://doi.org/10.1016/j.biortech.2017.09.193>
48. Said-Pullicino D, Erriquens FG, Gigliotti G (2007) Changes in the chemical characteristics of water-extractable organic matter during composting and their influence on compost stability and maturity. *Bioresour Technol* 98:1822–1831. <https://doi.org/10.1016/j.biortech.2006.06.018>
49. Alburquerque J (2004) Agrochemical characterisation of “alperujo”, a solid by-product of the two-phase centrifugation method for olive oil extraction. *Bioresour Technol* 91:195–200. [https://doi.org/10.1016/S0960-8524\(03\)00177-9](https://doi.org/10.1016/S0960-8524(03)00177-9)
50. Sangwan P, Kaushik CP, Garg VK (2008) Vermiconversion of industrial sludge for recycling the nutrients. *Bioresour Technol* 99:8699–8704. <https://doi.org/10.1016/j.biortech.2008.04.022>
51. Ferraz CA, Pastorinho MR, Palmeira-de-Oliveira A, Sousa ACA (2022) Ecotoxicity of plant extracts and essential oils: A review. *Environ Pollut* 292:118319. <https://doi.org/10.1016/j.envpol.2021.118319>
52. OECD (1984) Test No. 207: Earthworm, Acute Toxicity Tests. OECD
53. Liebeke M, Strittmatter N, Fearn S, et al (2015) Unique metabolites protect earthworms against plant polyphenols. *Nat Commun* 6:7869. <https://doi.org/10.1038/ncomms8869>
54. Vasantha-Srinivasan P, Senthil-Nathan S, Ponsankar A, et al (2018) Acute toxicity of chemical pesticides and plant-derived essential oil on the behavior and development of earthworms, *Eudrilus eugeniae* (Kinberg) and *Eisenia fetida* (Savigny). *Environ Sci Pollut Res* 25:10371–10382. <https://doi.org/10.1007/s11356-017-9236-6>
55. Zortéa T, Da Silva AS, Dos Reis TR, et al (2018) Ecotoxicological effects of fipronil, neem cake and neem extract in edaphic organisms from tropical soil. *Ecotoxicol Environ Saf* 166:207–214. <https://doi.org/10.1016/j.ecoenv.2018.09.061>
56. Devi C, Khwairakpam M (2021) Management of invasive weed *Parthenium hysterophorus* through vermicomposting using a polyculture of *Eisenia fetida* and *Eudrilus eugeniae*. *Environ Sci Pollut Res*. <https://doi.org/10.1007/s11356-021-12720-4>