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Biological Treatment of Agro-Processing Effluents Using Mycofiltration for Improved Maize Root Development in Cross River State, Nigeria

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Abstract. Untreated agro-processing effluents pose a significant and growing threat to soil and water quality in developing economies, underscoring the need for low-cost, environmentally sound biological treatment options. This study evaluated mycofiltration as a biological treatment for effluents from cassava, maize, palm oil mills, and abattoirs. Four fungal species: Shiitake (*Lentinula edodes*), Brick top (*Hypholoma sublateritium*), Phoenix mushroom (*Pleurotus pulmonarius*), and King Stropharia (*Stropharia annulata*) were considered in this study. The experiment used a 5 × 5 factorial design, replicated three times. The study was carried out in 3 sequential phases. The first phase was a 14-day laboratory immersion trial. The second was a screen-house-pot trial. The final phase took place in a rain-fed field over one growing season. Mycofiltration reduced electrical conductivity, total dissolved solids, total suspended solids, and biological and chemical oxygen demands (BOD, COD) in cassava, maize, and abattoir effluents ($p < 0.0001$). Brick top and King Stropharia spawns achieved the greatest reductions. For abattoir effluent, BOD declined from 8,325.46 to 2,001.11 mg/L (76%) and COD from 13,200.12 to 6,019.81 mg/L (54%) under King Stropharia treatment. In the laboratory phase, abattoir effluent filtered with Brick top (E4M2) produced maize root lengths exceeding those of the water control by 178% (7.333 vs. 2.632 cm); this root growth benefit persisted across the screen house and field phases. Palm oil mill effluent inhibited root development by 46-53% across all phases and fungal treatments, reflecting its recalcitrant load (BOD 28,400.56 mg/L; COD 45,760.82 mg/L in raw form). The factorial ANOVA results confirmed that effluent type and mushroom spawn both significantly affect mycofiltration performance. These findings demonstrate that mycofiltration performance is effluent-specific. Findings from this study provide quantitative evidence that mushroom spawn selection must be matched to the target effluent type to achieve both compliant discharge and beneficial agricultural reuse.

Keywords: Basidiomycetous fungi; bioremediation; root development; wastewater treatment; *Zea mays*

1. Introduction

Agro-processing industries contribute significantly to value addition across developing economies. However, agro-processing activities generate large volumes of effluents (Ganesh et al., 2010; Rajinikanth et al., 2013). These effluents contain high levels of biological oxygen demand (BOD) and chemical oxygen demand (COD), posing environmental threats. A critical review of the existing literature reveals the deleterious effects of agro-processing effluents in many countries worldwide. Prominent among such reports are the degraded environment in Malaysia, where palm oil is produced in large quantities for export (Malaysian Palm Oil Council (MPOC), 2013). Ediene et al. (2013) also reported the recalcitrant organic load of POME and its degrading effect on the environment in Biase LGA, Cross River State. Comparable trends have also been documented in Spain and other Mediterranean countries where wine is produced in large quantities (Fia et al., 2012) and Nigeria, where cassava, maize, and abattoir effluents are indiscriminately disposed (Ediene et al., 2016; Ediene & Iren, 2017).

Uncontrolled discharge of effluents with high organic loads into the environment remains widespread. Sustained accumulation of these compounds can alter soil chemistry, promote luxury uptake by plants, and degrade receiving ecosystems (Meksi et al., 2012;

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Ganesh et al., 2010). Low-cost and environmentally sound treatment technologies are urgently needed. Mycofiltration is the use of basidiomycetous fungi (mushrooms) to remediate effluents. This biologic technology consists of a low-cost biological approach (Taylor & Stamets, 2014; Stamets, 2013; Mnkandla & Otomo, 2021). Fungal mycelium networks can assimilate suspended solids from these effluents, resulting in much cleaner filtrates that may be reused sustainably (Stamets, 2013; Singh, 2006). According to Dhouib et al. (2006), Ediene et al., 2026 and Kumar et al. (2022), fungal degradation of complex organic pollutants is driven largely by extracellular ligninolytic enzymes, laccases, manganese peroxidases, and lignin peroxidases. These enzymes oxidize phenolic and aromatic compounds into simpler, less toxic metabolites. Because these same enzymatic pathways can also mineralize organic nitrogen and phosphorus into plant-available forms, mycofiltration is expected not only to reduce pollutant load but also to alter the phytotoxic or phytostimulatory potential of the treated effluent. This mechanistic link is the basis for using maize root elongation as a bioassay endpoint in the present study.

In this study, maize (*Zea mays* L.) was primarily used to evaluate root development, serving as a bio-indicator. This is because root architecture makes a significant contribution to plant growth and productivity. A viable root system promotes nutrient and water absorption. It can provide good anchorage and also act as an indicator of crop health (Bauer et al., 2024). The *Zea mays* root assay is a recognized test method used for environmental toxicity assessment. It is well known as a sensitive and robust index of soil and water quality (Wang et al., 2001; Mahmood et al., 2005; Leme & Marin-Morales, 2009; da Costa et al., 2012; Gvozdenac et al., 2011; Odutayo et al., 2016). Root morphology (length, branching, and coloration) responds quantifiably to phytotoxic and phytostimulatory substances (Fazal et al., 2007; Fageria, 2013).

The primary goal of this study was to examine the ability of mycofiltration to improve wastewater quality. Cassava, maize, palm oil mill (POME), and abattoir effluents were the sources of effluent examined. The objective was to ensure that treated effluents were safe and non-harmful for the environment. Fungal species were selected for three reasons: they are commercially available, contain ligninolytic activity, and yielded favourable outcomes in previous studies. We hypothesized that mycofiltration would significantly reduce key pollutant parameters in agro-processing effluents and that the resulting filtrates would support maize root development comparable to or exceeding water and the effluent raw controls.

To our knowledge, no previous study has simultaneously evaluated four basidiomycetous species across three experimental scales (laboratory, screen house, and field) and four distinct agro-processing effluent types within a single factorial framework. Previous mycofiltration research has generally been restricted to single-effluent systems or laboratory-only assessments (Mnkandla & Otomo, 2021; Taylor & Stamets, 2014), leaving a gap in understanding how treatment efficacy and phytotoxicity outcomes scale from controlled to field conditions. This study addresses that gap, contributing new evidence on both the wastewater-remediation and crop-safety dimensions of mycofiltration, with direct relevance for tropical smallholder and agro-industrial settings where conventional treatment infrastructure is limited.

2. Materials and Methods

2.1 Fungal Spawns and Mycofilter Preparation

Pure strain spawns of four mushroom species: Shiitake (*Lentinula edodes*), Brick top (*Hypholoma sublateritium*), Phoenix mushroom (*Pleurotus pulmonarius*), and

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King Stropharia (*Stropharia annulata*), were procured from Fungi Perfecti Laboratory, USA, and stored at –70 °C until use. Maize cobs (substrate) were crushed to less than 2 mm particles to facilitate mycelium colonization. Substrate bags underwent resilience testing (repeated cycles of saturation, drying, heating, and freezing) and sterilization following the protocol of Stamets (2013). Two millilitres of live spawn were inoculated per kilogram of substrate, then incubated in darkness for 30 days until full colonization was achieved. Colonization was scored visually on a 6-point scale. Inter-rater agreement was assessed using Cohen's κ and interpreted using the criteria of McHugh (2012). Twenty-four plastic filtration units were constructed, each comprising four tiers: a filtering chamber, two mycofilter compartments (containing fungi mycelium fully colonized substrate), and a 10-litre receptacle, modified after Stamets (2013). Wire mesh screens (0.2 mm) were installed over all chamber outlets to prevent substrate clogging. Filtration proceeded at ~30 °C. Composite effluent samples were collected on three visits from cassava, maize, POME, and abattoir processing facilities in Cross River State, Nigeria. Ten litres of each effluent was passed through per mycofilter unit, at a flow rate of 0.417 L h^{-1}, and a contact time of 24 hours.

Mycofiltration was carried out weekly to achieve the desired quantity. Effluents were stored below 4 °C and brought to room temperature prior to filtration, following standard preservation protocols (Norweco, 2017). Both raw and mycofiltered effluents were analyzed for their chemical properties, including temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), total suspended solids (TSS), BOD, and COD (APHA, 2017). Abattoir effluent samples were analyzed for microbiological quality using internationally recognized standard methods. Total aerobic bacteria were quantified by heterotrophic plate count per APHA Standard Methods 9215, while *Clostridium perfringens* was enumerated following ISO 14189. Coliforms were detected and counted using the most probable number (MPN) method, following APHA Standard Methods 9221. *Escherichia coli* was confirmed using ISO 4831. *Faecal streptococci* counts were carried out in line with ISO 7899-2. *Pseudomonas spp*. were isolated and counted using membrane filtration per ISO 16266. Mould and yeast counts were determined following ISO 21527-1 (ISO, 2008; APHA, 2017).

2.2 Experimental Design

This study was conducted within a single agro-ecological zone. Three experimental phases were conducted sequentially. Phase 1 involved maize plants grown directly in effluents under laboratory conditions. Phase 2 was a screen house trial using 10-kg soil-filled buckets. Phase 3 was a rain-fed field experiment. All phases used 25 treatment combinations (Table 1) replicated three times, yielding 75 experimental units per phase. Laboratory and screen house experiments followed a 5 × 5 factorial completely randomized design (CRD). For the field experiment, treatments were arranged in a 5 × 5 factorial randomized complete block design (RCBD) with three replicates (blocks). Each block contained all 25 treatment combinations, giving a total of 75 experimental plots.

Within each replicate (laboratory and screen house) or block (field), the 25 treatment combinations were assigned to experimental units using computer-generated random numbers, so that every unit had an equal probability of receiving any treatment. Blocks were separated by alleyways to reduce treatment interference and account for field heterogeneity. Eight border-excluded plants from two centre rows were sampled per plot. Field data were collected during one growing season following the laboratory and screen house pre-trials.

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Table 1. Treatment combinations used in laboratory, screen house, and field experiments

Code	Description	Code	Description
E0M0	Water – no mushroom spawn (absolute control)	E2M3	Maize effluent + Phoenix mushroom spawn
E0M1	Water + Shiitake mushroom spawn (control)	E2M4	Maize effluent + King Stropharia mushroom spawn
E0M2	Water + Brick top mushroom spawn (control)	E3M0	POME – no mushroom spawn
E0M3	Water + Phoenix mushroom spawn (control)	E3M1	POME + Shiitake mushroom spawn
E0M4	Water + King Stropharia mushroom spawn (control)	E3M2	POME + Brick top mushroom spawn
E1M0	Cassava effluent – no mushroom spawn	E3M3	POME + Phoenix mushroom spawn
E1M1	Cassava effluent + Shiitake mushroom spawn	E3M4	POME + King Stropharia mushroom spawn
E1M2	Cassava effluent + Brick top mushroom spawn	E4M0	Abattoir effluent – no mushroom spawn
E1M3	Cassava effluent + Phoenix mushroom spawn	E4M1	Abattoir effluent + Shiitake mushroom spawn
E1M4	Cassava effluent + King Stropharia mushroom spawn	E4M2	Abattoir effluent + Brick top mushroom spawn
E2M0	Maize effluent – no mushroom spawn	E4M3	Abattoir effluent + Phoenix mushroom spawn
E2M1	Maize effluent + Shiitake mushroom spawn	E4M4	Abattoir effluent + King Stropharia mushroom spawn
E2M2	Maize effluent + Brick top mushroom spawn		

E0 = Water (control); E1 = Cassava effluent; E2 = Maize effluent; E3 = POME; E4 = Abattoir effluent. M0 = No spawn; M1 = Shiitake; M2 = Brick top; M3 = Phoenix mushroom; M4 = King Stropharia.

2.3 Treatment Application and Seed Management

In the laboratory, 30 mL of each treatment was applied daily to maintain fresh effluent exposure. In the screen house, 500 mL of each treatment was applied weekly per pot, supplemented with 500 mL of water on alternate days. In the field, 9 L of each treatment was applied weekly per plot, equivalent to 10,000 L ha^{-1}. Normalizing the application rates to mm $week^{-1}$ for comparison across phases, the laboratory rate of 30 mL day^{-1} applied to a 100 mm diameter unit equated to 26.8 mm $week^{-1}$. The screen house rate of 500 mL $week^{-1}$ applied to a 150 mm diameter pot equated to 28.2 mm $week^{-1}$. The field rate of 9 L $week^{-1}$ per unit, at a density of 1,111 units ha^{-1}, equated to 10.0 mm $week^{-1}$, or 10,000 L ha^{-1} $week^{-1}$.

Seed viability was confirmed following AOSA (2005) and ISTA (2005) germination protocols. Seeds were surface-sterilized in 10% sodium hypochlorite solution for five minutes. Two seeds were sown per unit, then thinned to one vigorous seedling. Weeding was carried out manually in the screen house and field trials.

2.4 Data Collection

Maize roots were harvested at 14 days after germination in the laboratory and at cob maturity (118 days) in the screen house and field experiments. Root length was measured for each plant using a calibrated ruler. Data were subjected to analysis of variance (ANOVA), and means were separated using Fisher's least significant difference (LSD) test at $p < 0.05$.

Fisher's LSD was adopted as the mean-separation procedure because the omnibus ANOVA F-tests for all main effects and their interaction were significant at $p < 0.0001$ in every experimental phase, which is the precondition generally recommended before applying LSD. We acknowledge, however, that LSD carries a higher risk of Type I error

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than more conservative procedures such as Tukey's HSD; this is noted as a limitation (Section 4.4). A formal a priori power or sample-size calculation was not performed. The precision achieved (CV of 1.11–7.07% and 50 degrees of freedom across the three phases) is reported as an indication of experimental reliability.

2.5 Statistics

Simple measures of dispersion, factorial analysis of variance (ANOVA), coefficient of variation (CV, %) [(√MSError / Grand Mean) × 100], LSD (5%) [t(0.025, df_error) × √(2·MSError/r)], and LSD (1%) [t(0.005, df_error) × √(2·MSError/r)] were computed using the R statistical package.

3. Results and Discussion

3.1 Chemical Properties of Raw and Mycofiltered Effluents

The pH of the raw and mycofiltered agro-processing effluents ranged from 6.11 to 8.70 (Table 2a), indicating they were nutrient-rich and conducive to microbial and enzymatic activity. Water controls and POME (E3) were slightly acidic, while cassava (E1), maize (E2), and abattoir (E4) effluents were slightly alkaline. All pH values fell within the WHO and EPA discharge guidelines of 6.0–8.5 for agricultural soils (WHO, 2006; EPA, 2012). The high pH in agro-processing effluents may reflect the presence of phenolic acids and oxidised organic acid compounds. Mycofiltration produced negligible changes in pH across all effluent types.

Electrical conductivity (EC) (Table 2a) in water controls ranged from 546.30 to 551.32 µS/cm, within the WHO/FAO/EPA threshold of 700 µS/cm. All the raw effluent EC were substantially higher: 979.37–2,640.00 µS/cm (cassava, E1), 1,698.76–3,780.00 µS/cm (maize, E2), 5,479.45–6,560.49 µS/cm (POME, E3), and 1,099.76–3,140.49 µS/cm (abattoir, E4). Palm oil mill effluent recorded the highest EC across all treatments. Brick top (M2) and King Stropharia (M4) spawns were most effective at reducing EC, particularly in cassava (E1M2: 979.37 µS/cm), maize (E2M4: 1,700.00 µS/cm), and abattoir (E4M2: 1,007.98 µS/cm) effluents. These results agree with the known capacity of fungal mycelium to adsorb and dissolve ionic compounds (Kumar et al., 2022; Stamets, 2013).

Total Dissolved Solids (TDS) also exceeded the WHO/FAO limit of 450 mg/L in all effluent treatments except E0M2 (375.90 mg/L) and E0M4 (396.21 mg/L). Brick top (M2) reduced TDS in cassava effluent (E1M2: 1,107.41 mg/L), while King Stropharia (M4) was most effective in maize (E2M4: 1,807.52 mg/L), POME (E3M4: 3,195.66 mg/L), and abattoir (E4M4: 997.56 mg/L) effluents. High TDS levels in agro-industrial effluents are linked to dissolved organic matter and inorganic salts (Dhouib et al., 2006).

Total Suspended Solids (TSS) in water controls (E0M0–E0M4) remained below the EPA threshold of 5 mg/L. Raw effluent controls (E1M0, E2M0, E3M0, E4M0) had significantly higher TSS than their mycofiltered counterparts. King Stropharia (M4) produced the highest TSS reductions in cassava (E1M4: 1.65 mg/L) and maize (E2M4: 2.19 mg/L) effluents, while Brick top (M2) performed best for POME (E3M2: 3.79 mg/L) and abattoir (E4M2: 1.25 mg/L) effluents. Fungal mycelium acts as a physical filtration matrix, mechanically retaining suspended particles (Stamets, 2013; Singh, 2006; Ikechi-Nwogu & Akpan, 2022).

High BOD and COD values were recorded across all raw effluent controls. Palm oil mill effluent recorded the highest values (E3M0: BOD 28,400.56 mg/L; COD 45,760.82 mg/L), while cassava effluent had the lowest among agro-processing effluents (E1M0: BOD 607.18 mg/L; COD 986.15 mg/L). The BOD/COD ratios obtained in this study (0.21–0.63) indicate varying degrees of biodegradability among the effluents; ratios of 0.4–0.5 are generally considered indicative of wastewater amenable to biological treatment, while lower ratios suggest more

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recalcitrant organic compounds (Kittikun et al., 2000; Onyia et al., 2001). The moderate BOD/COD ratios suggest that substantial fractions of the organic material in the effluents are biodegradable, supporting the relevance of biological approaches such as mycofiltration; by contrast, the lower ratios recorded for some POME treatments point to a more recalcitrant organic fraction, an interpretation consistent with, but not conclusively proven by, the present dataset.

Table 2a. Mean chemical properties of raw and mycofiltered agro-processing effluents

Treatment	Temp (°C)	pH	EC (µS/cm)	TDS (mg/L)	TSS (mg/L)	BOD (mg/L)	COD (mg/L)	BOD/COD
WHO	-	6-8.5	700	450	-	30	125	1.75
EPA	-	6-8.4	700	-	5	30	120	1.75
FAO	-	6-8.5	700	450	-	-	-	-
E0M0	30.20	6.40	546.30	640.00	4.67	4.91	5.71	0.85
E0M1	30.20	6.50	549.45	549.00	2.56	3.30	5.63	0.57
E0M2	30.10	6.70	551.32	375.90	2.11	3.10	5.60	0.55
E0M3	30.10	6.50	547.44	514.73	2.23	3.11	5.27	0.59
E0M4	30.00	6.90	550.91	396.21	1.14	2.90	5.95	0.48
E1M0	30.50	8.51	2,640.00	1,584.00	20.94	607.18	986.15	0.61
E1M1	30.45	8.39	1,600.91	1,399.09	4.24	493.40	945.78	0.52
E1M2	30.50	8.45	979.37	1,107.41	3.50	453.99	860.18	0.52
E1M3	30.41	8.45	1,620.64	1,498.99	2.30	499.79	935.20	0.53
E1M4	30.46	8.45	995.79	1,295.11	1.65	420.54	845.63	0.49
E2M0	30.70	8.46	3,780.00	2,268.00	20.18	2,067.46	4,600.80	0.44
E2M1	30.67	8.47	2,709.02	2,075.78	3.42	1,654.00	3,600.02	0.45
E2M2	30.62	8.40	1,698.76	2,198.54	2.21	1,564.44	3,676.24	0.42
E2M3	30.70	8.39	1,730.73	2,200.91	4.22	1,345.92	2,960.44	0.45
E2M4	30.70	8.49	1,700.00	1,807.52	2.19	1,263.24	2,936.19	0.43
E3M0	30.30	6.11	6,560.49	3,936.00	30.22	28,400.56	45,760.82	0.62
E3M1	30.30	6.21	6,560.00	3,867.72	7.65	13,840.78	45,575.50	0.30
E3M2	30.31	6.20	5,479.45	3,759.19	3.79	9,112.79	42,125.00	0.21
E3M3	30.28	6.14	5,500.90	3,890.34	7.25	13,563.99	45,500.76	0.29
E3M4	30.24	6.23	5,370.02	3,195.66	4.32	9,254.54	41,005.21	0.22
E4M0	30.27	8.68	3,140.49	1,884.00	18.28	8,325.46	13,200.12	0.63
E4M1	30.25	8.70	1,140.00	1,675.90	3.91	3,543.17	10,500.97	0.33
E4M2	30.18	8.69	1,007.98	1,009.52	1.25	2,113.29	6,025.29	0.25
E4M3	30.23	8.68	1,137.47	1,200.61	2.56	3,432.97	12,415.87	0.27
E4M4	30.27	8.69	1,099.76	997.56	1.43	2,001.11	6,019.81	0.32

EC = Electrical conductivity; TDS = Total dissolved solids; TSS = Total suspended solids; BOD = Biological oxygen demand; COD = Chemical oxygen demand. Rows for WHO, EPA, and FAO show international guideline values.

The overall organic matter degrading capacity was highest in the King Stropharia (M4) treatments. For E4M4, a BOD value of 2,001.11 mg/L and a COD value of 6,019.81 mg/L were obtained against the control (BOD 8,325.46 mg/L; COD 13,200.12 mg/L), consistent with reports for olive mill effluents in which the combination of anaerobic digestion and white-rot fungi decreased the BOD/COD ratio (Dhouib et al., 2006). A remarkable decrease in microbial load was also recorded for abattoir effluent treated with mycofiltration (Table 2b). For all detected microorganisms, counts in the untreated effluent (E4M0) were significantly higher, reflecting the extremely heavy microbial load characteristic of slaughter house effluent.

The aerobic bacterial count decreased gradually from 16 CFU/mL in raw effluent to non-detectable amounts at treatment levels E4M3 and E4M4. The complete removal of coliforms and *Escherichia coli* from treated effluents indicates improved

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safety conditions. Yeast and mould counts were much lower than in the untreated effluent. *Faecal streptococci* decreased from 20 CFU/mL in raw effluent to undetectable levels, and *Pseudomonas spp*. was also not detected in the treated effluents. Overall, mycofiltration significantly improved the microbiological quality of the abattoir effluent, with Phoenix mushroom spawn (E4M3) and King Stropharia mushroom spawn (E4M4) showing the highest efficiency.

Table 2b. Microbial composition and count of raw and mycofiltered abattoir effluent

Test	E4M0	E4M1	E4M2	E4M3	E4M4
Total aerobic bacteria plate count (CFU/mL)	16	2	1	0	0
Mould/yeast (CFU/mL)	10	0	1	1	0
Coliform (MPN/100 mL)	16	0	0	0	0
Escherichia coli (MPN/100 mL)	15	0	0	0	0
Faecal streptococci (CFU/mL)	20	4	2	0	0
Pseudomonas spp. (CFU/mL)	2	0	0	0	0
Clostridium perfringens (CFU/mL)	0	0	0	0	0

3.2 Percentage Reduction in pH, EC, TDS, TSS, BOD, and COD of Mycofiltered Effluents

Total Suspended Solids (TSS) recorded the greatest reductions among all cassava effluent parameters (Figure 1). Treatment E1M4 yielded the highest TSS removal at 92.12%, followed by E1M3, E1M2, and E1M1 (79.75%). Electrical conductivity (EC) was considerably reduced, with E1M2 achieving 62.90% reduction and E1M4 close behind at 62.28%. BOD reductions ranged from 17.68% (E1M3) to 30.74% (E1M4), while COD reductions were comparatively modest (4.10–14.27%). pH reductions were minimal (0.71–1.41%). Brick top (M2) and King Stropharia (M4) were the top-performing mycofilter types across nearly all cassava effluent parameters.

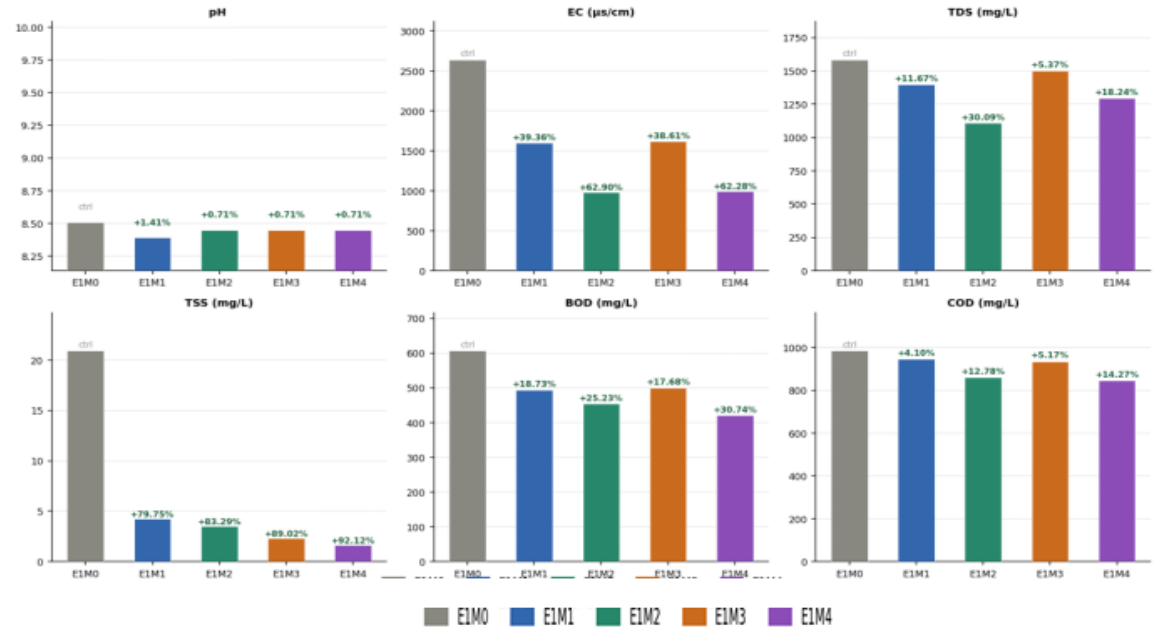

Figure 1. Percentage reduction in pH, EC, TDS, TSS, BOD, and COD for cassava effluent mycofiltered treatments (M1–M4) relative to the raw effluent control (E1M0)

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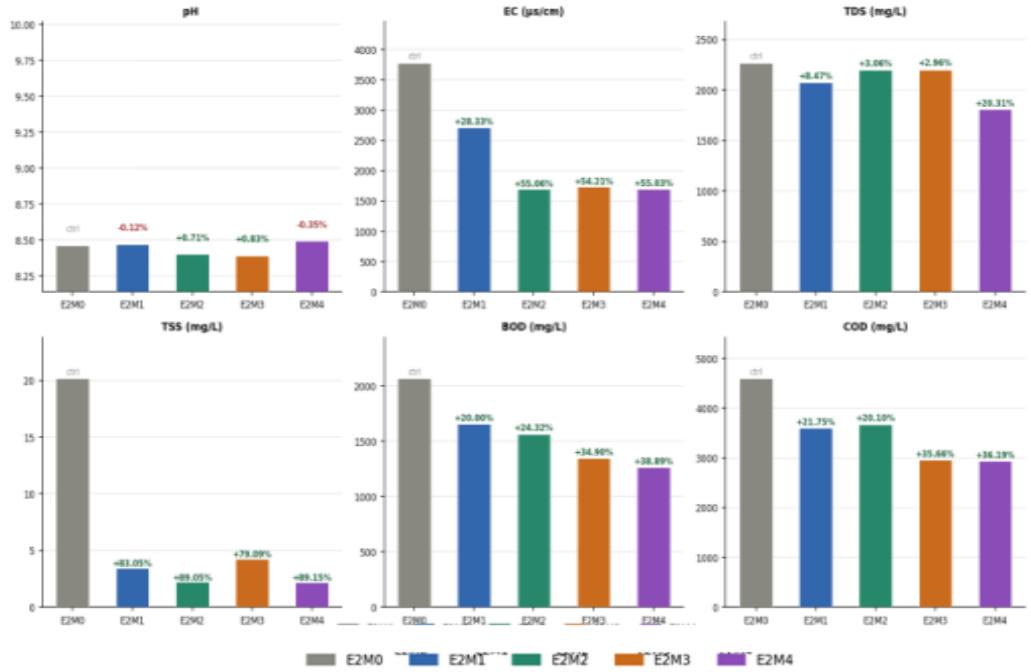

Figure 2. Percentage reduction in pH, EC, TDS, TSS, BOD, and COD for maize effluent mycofiltered treatments (M1–M4) relative to the raw effluent control (E2M0)

Removal of TSS was the most notable outcome among all POME parameters, with E3M2 recording the highest reduction at 87.46%, followed by E3M4 and E3M3 at 85.70% and 76.01%, respectively (Figure 3). BOD reductions were substantial, with E3M2 leading at 67.91%, closely followed by E3M4 at 67.41%. COD reductions were modest (0.41–10.39%), reflecting the challenge of degrading the very high COD load (45,760.82 mg/L) recorded in raw POME. EC reductions were low for E3M1 (0.01%) but improved for E3M2 through E3M4 (16.15–18.15%). POME combined with Brick top mushroom spawn (E3M2) and King Stropharia (E3M4) performed best overall for BOD, TSS, EC, and COD removal.

TSS reductions were exceptional for abattoir effluent (Figure 4); E4M2 achieved 93.16%, and E4M4 achieved 92.18%, the highest TSS removals recorded across all four effluent groups. BOD reductions were also the strongest observed, with E4M4 leading at 75.99%, closely followed by E4M2 at 74.61%. COD reductions were very effective for E4M2 and E4M4 (approximately 54.36–54.39%). EC reductions were uniformly robust (63.70–67.90%), and TDS reductions were the best recorded across all effluent groups – King Stropharia (E4M4) achieved 47.04% and E4M2 achieved 46.42%. E4M4 was consistently the most effective treatment for abattoir effluent.

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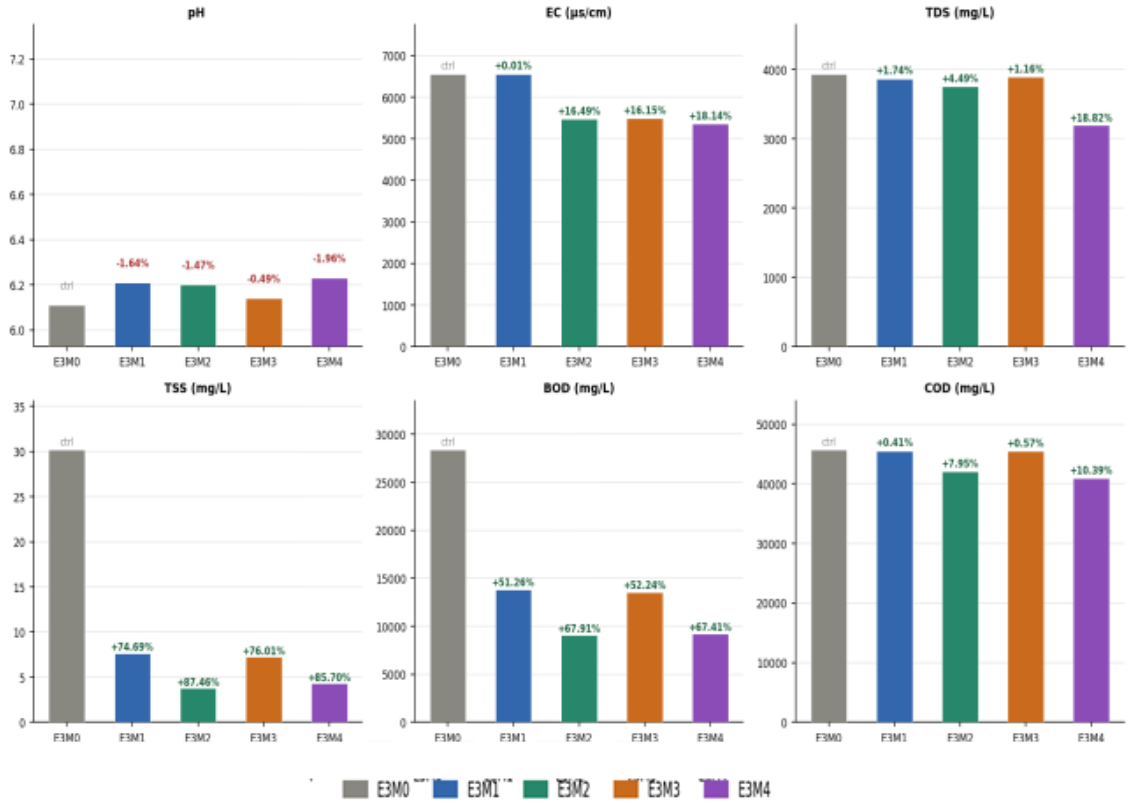

Figure 3. Percentage reduction in pH, EC, TDS, TSS, BOD, and COD for palm oil mill effluent (POME) mycofiltered treatments (M1–M4) relative to the raw effluent control (E3M0)

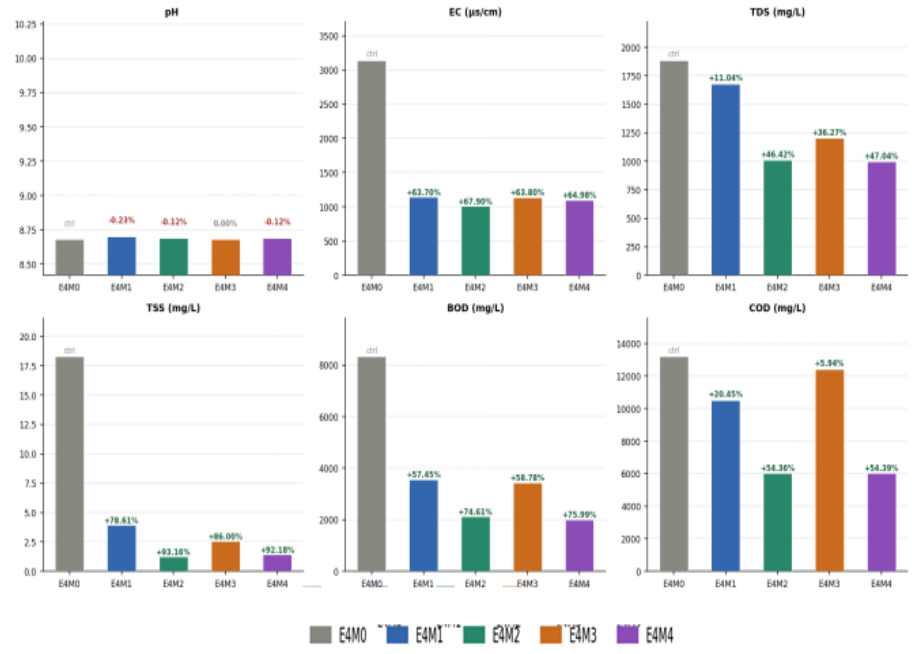

Figure 4. Percentage reduction in pH, EC, TDS, TSS, BOD, and COD for abattoir effluent mycofiltered treatments (M1–M4) relative to the raw effluent control (E4M0)

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Mycofiltration was observed to decrease EC, TDS, TSS, BOD, and COD levels for cassava (22.3% mean reduction), maize (28.0%), and abattoir effluents (8.6%), as shown in Table 2a and Figures 1-4. All raw effluents had BOD/COD ratios below 1.75, indicating general biodegradability and the applicability of biological treatments such as mycofiltration (Kittikun et al., 2000; Onyia et al., 2001). Brick top (M2) and King Stropharia (M4) consistently outperformed Shiitake (M1) and Phoenix mushroom (M3), indicating that basidiomycetous species vary in their enzymatic capacity for degradation. The superior performance of Brick top and King Stropharia may be attributable to increased extracellular oxidoreductase (laccase/peroxidase) activity relative to the other species; this remains a proposed explanation, as enzymatic assays were not performed directly in this study (see Section 4.4). Nonetheless, similar results have been reported for species from the *Pleurotus*, *Stropharia*, and *Trametes* genera, as achieving the most consistent contaminant removal and are largely attributed to their strong extracellular laccase and peroxidase activity (Mnkandla & Otomo, 2026; Hultberg et al., 2020; Sen et al., 2023).

Most of the treated effluents also failed to meet WHO, EPA, or FAO guidelines for direct discharge, indicating that single-pass mycofiltration functions as a preconditioning step rather than a complete treatment method. This finding is consistent with reported removal efficiencies across previous mycofiltration studies, which range widely from below 30% to above 90% depending on contaminant, fungal species, and contact time, and rarely reach complete removal in a single pass (Mnkandla & Otomo, 2026; Mnkandla & Otomo, 2021). Titilawo et al. (2023), reported that a *Macrolepiota procera* mycofilter on corncob reduced turbidity, heavy metals, and microbial load in surface water, with the slowest flow rate (0.2 mL/min) giving the greatest contaminant reduction. Beltrán-Flores et al. (2022), demonstrated that *Trametes versicolor* immobilized on wood chips in a rotating drum bioreactor, operated continuously for 225 days, achieved a stable fungal consortium and sustained pesticide removal (diuron up to 54%, bentazon up to 48%). Obayagbona et al. (2024) similarly found that *Lentinus squarrosulus* mycofilters reduced TDS, EC, turbidity, and heterotrophic bacterial counts in raw urban stormwater, though trace-metal reductions were not statistically significant at their sample size. Kim et al. (2023) introduced a continuously operated fungal wheel reactor using solid-state-fermented *Trametes versicolor* for pharmaceutical and personal-care-product removal, showing that periodic replacement of spent fungal substrate restores removal capacity after enzyme depletion.

3.3 Effects of Mycofiltered Effluents on Maize Root Length

3.3.1 Laboratory Experiment

Table 3a summarizes the laboratory assay root length data. Among the 25 treatments, E4M2 and E4M4 yielded the longest roots. The average root length for E4M2 (abattoir effluent treated with Brick top mushroom spawn) was 7.333 cm, approximately 178 % higher than the absolute control (E0M0: 2.632 cm). E4M4 (treated with King Stropharia) followed closely with 7.200 cm, and E4M1 recorded 6.167 cm. Treated abattoir effluent generally recorded the greatest root elongation at this stage.

All POME treatments significantly inhibited root growth compared with E0M0 (absolute control). The E3M0 (untreated POME) treatment showed the shortest roots of the entire experiment, averaging 1.300 cm, 50.6% shorter than the absolute control. Factorial ANOVA results (Table 3b) showed that effluent type, mushroom spawn type, and their interaction all significantly influenced maize root length ($p < 0.0001$).

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Table 3a. Mean maize root length (cm) per treatment for the Laboratory Incubation Experiment (CRD)

Treatment	Mean (cm)	Treatment	Mean (cm)
E0M0	2.632	E2M3	3.333
E0M1	3.967	E2M4	5.867
E0M2	4.400	E3M0	1.300
E0M3	3.997	E3M1	2.767
E0M4	3.867	E3M2	3.607
E1M0	2.835	E3M3	2.510
E1M1	3.332	E3M4	4.997
E1M2	6.000	E4M0	3.567
E1M3	2.833	E4M1	6.167
E1M4	5.993	E4M2	7.333
E2M0	3.232	E4M3	4.500
E2M1	4.567	E4M4	7.200
E2M2	5.783		

E0 = Water; E1 = Cassava effluent; E2 = Maize effluent; E3 = POME; E4 = Abattoir effluent. M0 = No spawn; M1 = Shiitake; M2 = Brick top; M3 = Phoenix; M4 = King Stropharia.

Table 3b. Factorial ANOVA output for the Laboratory Incubation Experiment (CRD)

Source of variation	Sum sq.	df	F	Pr(>F)
Effluent	60.8614	4	161.31	<0.0001
Mushroom spawn	92.9172	4	246.27	<0.0001
Effluent × Mushroom interaction	20.6174	16	13.66	<0.0001
Residual	4.7163	50		

Grand mean = 4.2635; √MSE = 0.3013; CV = 7.07%; LSD (5%) = 0.4942; LSD (1%) = 0.6588.

Effluent type significantly influenced root development (F = 161.31), and mushroom species exerted a significant influence (F = 246.27). The interaction effect indicates that fungal performance was dependent on effluent type.

3.3.2 Screen House Experiment

Root length data from the screen house experiment are presented in Table 4a. Abattoir effluent mycofiltered with King Stropharia mushroom spawn (E4M4) had the longest roots (37.800 cm), followed by E1M4 (37.300 cm), E2M4 (36.900 cm), and E4M3 (36.867 cm).

All treatments involving cassava, maize, and abattoir effluent had root lengths greater than or equal to the absolute control (E0M0: 29.867 cm). This phase showed the greatest root inhibition for POME: the mean root length of untreated POME (E3M0) was only 14.167 cm, 52.6% lower than the absolute control. Factorial ANOVA (Table 4b) indicated that effluent type (F = 5,077.71), mushroom species (F = 535.56), and their interaction (F = 102.07) significantly influenced root length ($p < 0.0001$).

3.3.3 Field Experiment

Field root length results are given in Table 5. E2M3 (maize effluent treated with Phoenix mushroom) had a root length of 28.750 cm. E3M3 and E3M4, which performed poorly in the earlier phases, showed notably improved root lengths in the field (28.200 cm and 28.670 cm, respectively), possibly due to soil buffering, microbial interactions, and environmental dilution. Factorial ANOVA for the field experiment indicated significant effects of

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effluent type (F = 717.84, p < 0.0001), mushroom spawn type (F = 352.24, p < 0.0001), and their interaction (F = 207.66, p < 0.0001). POME generated the shortest root lengths of all treatments; E3M0 recorded 12.847 cm, 46.2% lower than the absolute control (E0M0: 23.873 cm). Cassava and abattoir effluent treatments maintained root lengths similar to or greater than those of the control treatment. An experimental precision of 1.43% CV was obtained.

Table 4a. Mean maize root length (cm) per treatment for the Screen House Experiment (CRD)

Treatment	Mean (cm)	Treatment	Mean (cm)
E0M0	29.867	E2M3	35.967
E0M1	32.833	E2M4	36.900
E0M2	32.800	E3M0	14.167
E0M3	33.133	E3M1	21.867
E0M4	30.700	E3M2	21.800
E1M0	32.537	E3M3	17.400
E1M1	32.200	E3M4	25.530
E1M2	36.400	E4M0	33.335
E1M3	33.930	E4M1	35.933
E1M4	37.300	E4M2	34.700
E2M0	30.467	E4M3	36.867
E2M1	32.500	E4M4	37.800
E2M2	36.637		

E0 = Water; E1 = Cassava; E2 = Maize; E3 = POME; E4 = Abattoir. M0 = No spawn; M1 = Shiitake; M2 = Brick top; M3 = Phoenix; M4 = King Stropharia.

Table 4b. Factorial ANOVA output for the Screen House Experiment (CRD)

Source of variation	Sum sq.	df	F	Pr(>F)
Effluent	2,466.6316	4	5,077.71	<0.0001
Mushroom spawn	260.1628	4	535.56	<0.0001
Effluent × Mushroom interaction	198.3298	16	102.07	<0.0001
Residual	6.0722	50		

Grand mean = 31.3427; √MSE = 0.3485; CV = 1.11%; LSD (5%) = 0.5715; LSD (1%) = 0.7619.

From the results, abattoir effluent mycofiltered with Brick top (E4M2) produced the longest roots in the laboratory phase (mean: 7.333 cm), exceeding the absolute control by 178%; E4M4 (King Stropharia) followed at 7.200 cm. In the screen house and field phases, E4M4 again ranked among the top treatments. These findings suggest that mycofiltration of abattoir effluent may convert high-concentration organic nitrogen and phosphorus into plant-accessible forms, consistent with the work of Darch et al. (2019), who linked nutrient-enriched amendments to improved root architecture in maize. Abattoir effluent is rich in organic nitrogen (blood proteins, urinary urea) and phosphorus (bone fragments); fungal enzymatic action (proteases, phosphatases) may mineralize these into plant-available ammonium-N and orthophosphate-P, functioning as a biostimulant rather than solely a remediation agent (Darch et al., 2019; Schimel & Bennett, 2004). This mechanism is supported by evidence that fungal mycofiltration removes organic

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pollutants mainly through extracellular enzyme activity and biosorption onto the mycelial surface, processes that also mobilize bound nutrients (Mnkandla & Otomo, 2026; Arregui et al., 2019). Cassava and maize effluents also produced root lengths comparable to or above the water control at the tested application rates; partial remediation may convert low-to-moderate organic loading from phytotoxic to mildly phytostimulatory (Kumar et al., 2022). The consistency of these results across three experimental phases strengthens confidence in the observation, though multi-season validation would be needed before firm conclusions are drawn.

Table 5. Mean maize root length (cm) per treatment for the Field Experiment (RCBD)

Treatment	Mean (cm)	Treatment	Mean (cm)
E0M0	23.873	E2M3	28.750
E0M1	26.420	E2M4	28.420
E0M2	27.043	E3M0	12.847
E0M3	25.417	E3M1	19.837
E0M4	26.147	E3M2	17.083
E1M0	26.170	E3M3	28.200
E1M1	25.917	E3M4	28.670
E1M2	26.627	E4M0	26.670
E1M3	26.500	E4M1	28.293
E1M4	28.043	E4M2	26.543
E2M0	27.873	E4M3	25.793
E2M1	26.210	E4M4	28.753
E2M2	26.670		

E0 = Water; E1 = Cassava; E2 = Maize; E3 = POME; E4 = Abattoir. M0 = No spawn; M1 = Shiitake; M2 = Brick top; M3 = Phoenix; M4 = King Stropharia.

Table 5b. Factorial ANOVA output for the Field Experiment (RCBD)

Source of variation	Sum sq.	df	F	Pr(>F)
Effluent	387.9772	4	717.84	<0.0001
Mushroom spawn	190.3773	4	352.24	<0.0001
Effluent × Mushroom interaction	448.9454	16	207.66	<0.0001
Residual	6.756	50		

Grand mean = 25.7108; √MSE = 0.3676; CV = 1.43%; LSD (5%) = 0.6028; LSD (1%) = 0.8037.

3.4 Phytotoxic Effects of Palm Oil Mill Effluent

Palm oil mill effluent (E3) inhibited maize root development in all experimental phases, regardless of fungal treatment. E3M0 reduced root length by approximately 50% in the laboratory, 52.6% in the screen house, and 46.2% in the field, relative to the water control. Similar phytotoxic effects were reported by Ediene et al. (2026) for palm oil mill effluent in their *Allium cepa assay*. The most plausible mechanisms for the partial field-phase reversal of POME inhibition observed in E3M3 and E3M4 are proposed, rather than experimentally confirmed, and include: (a) dilution by rain events; (b) soil microbial buffering of phenolic toxicity; and (c) adsorption of phenolics onto clay-organic complexes in the field soil. Fungal degradation of pollutants depends on active extracellular enzymes, such as laccases, which lose activity at high concentrations of

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phenolic and solvent-type compounds. This offers a further explanation for the limited effect of single-pass mycofiltration on phenol-rich POME (Rodakiewicz-Nowak et al., 2000).

Considered together, the three experimental phases show that all main and interaction effects were significant at $p < 0.001$, confirming the robustness and reproducibility of the mycofiltration system. In the laboratory, mushroom spawn type was the primary driver of variation; in the screen house, effluent type became more influential; and in the field, the interaction between the two factors was dominant. This shift suggests that as environmental complexity increased, the relationship between substrate chemistry and fungal biology became increasingly important. The F-value for the effluent × mushroom interaction increased from 13.66 in the laboratory to 102.07 in the screen house and 207.66 in the field, indicating that optimal spawn selection should be matched to the specific effluent type and deployment setting. Despite increasing environmental complexity across settings, the residual sums of squares (4.72, 6.07, and 6.76, respectively) indicate reliable measurements throughout.

3.4.1 Mechanistic and plant–microbe interpretation

Brick top and King Stropharia outperformed Shiitake and Phoenix mushroom, this could be linked to two established mechanisms outlined in the mycofiltration literature: (a) physical biosorption of particulates onto the mycelial network; (b). enzymatic transformation via extracellular ligninolytic enzymes (laccase, manganese peroxidase, lignin peroxidase). Sen et al. (2023), directly demonstrated bacterial DNA capture within *Stropharia rugosoannulata* mycelium. Mnkandla and Otomo (2026) review and attribute organic-contaminant removal primarily to adsorption via hydroxyl/carboxyl/amino functional groups in fungal chitin and glucan, plus non-specific enzymatic biodegradation. The plant–microbe dimension is more explicitly connected by the root-promoting effect of mycofiltered abattoir effluent to the mineralization of organic nitrogen and phosphorus by fungal proteases and phosphatases into ammonium-N and orthophosphate-P. This presents the mycofilter–effluent–root system as a three-way interaction in which the fungus conditions the effluent chemistry that the root subsequently responds to, rather than treating mycofiltration and root growth as independent outcomes.

3.4.2 Scale-up feasibility

On the practical constraints of moving from laboratory/screen-house/field trial scale to routine on-farm use. Drawing on Beltrán-Flores et al. (2022) and Kim et al. (2023), both of which needed periodic substrate/biomass renewal to sustain removal performance over months of continuous operation, we note that: (i) mycofilter substrate has a finite working life and will need scheduled replacement or regeneration. This represents a recurring material and labour cost. (ii) maintaining colonization quality under non-sterile conditions requires protection from competing molds and from substrate washout, both of which we encountered operationally in preparing the filtration units. (iii) flow-through versus batch-retention configurations trade off treatment thoroughness against footprint and hydraulic capacity. A facility discharging effluent continuously (e.g., an abattoir) would need either a larger filter bank or a shorter retention time than the 24-hour contact time used here, with a corresponding effect on removal efficiency. (iv) spawn procurement, substrate sourcing (maize cobs are already an abundant local by-product in Cross River State), and labour for filter assembly and monitoring are the main recurring cost drivers, while capital costs are modest relative to conventional physicochemical treatment. We note that this remains a preliminary economic picture, since a formal cost or life-cycle analysis was outside the scope of the present study, and we explicitly flag it as a priority for future work.

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4. Limitations and Future Directions

Only four basidiomycete species were tested, all sourced as commercial pure-culture spawn from a single supplier. Wild or regionally adapted strains, or other genera reported elsewhere to perform well in mycofiltration (e.g., *Pleurotus ostreatus*, *Trametes versicolor*), were not included, so the relative species ranking reported here cannot be assumed to hold for other fungal taxa or for wild isolates that may be more locally competitive under non-sterile field conditions. The experiments were conducted within a single agro-ecological zone in Cross River State, Nigeria; generalization to other tropical regions requires multi-site validation. The field trial covered a single rain-fed cropping season, and nutrient speciation analysis was beyond the scope of this study. Effluent composition, ambient temperature, humidity, and background soil/water microbial communities are all agro-ecology-specific, so the absolute removal percentages and root-length responses reported here may not transfer directly to other climatic zones or effluent sources. Only the relative ranking of fungal species and the qualitative effluent-specific pattern (POME being consistently more recalcitrant, abattoir effluent being consistently more amenable) are likely to generalize. Direct enzymatic characterization (e.g., laccase, manganese peroxidase, and protease activity assays) was not performed, so the biochemical mechanisms proposed to explain differences in fungal performance (Section 4.1) remain inferential rather than confirmed. Long-term data on the environmental fate of pollutants retained within the mycofilter substrate, and on its safe disposal or reuse, were also not collected and represent an important gap. The use of Fisher's LSD, while justified by the significant omnibus F-tests obtained in all three phases, carries a greater risk of Type I error than more conservative post hoc procedures, and a formal a priori power analysis was not conducted. Multi-season and multi-site studies, coupled with direct enzymatic assays, are needed to confirm and extend these findings. These gaps are not peculiar to this study. A systematic review of the mycofiltration literature found no standardized methods for mycofilter design or substrate preparation, and almost no assessment of mycofilter saturation, across 40 appraised articles (Mnkandla & Otomo, 2026; Osarenotor et al., 2021). Addressing these gaps would support more reliable comparison and upscaling of mycofiltration systems such as the one tested here.

Future research should focus on multi-site and multi-season validation. Efforts could also be directed at enzymatic profiling of the fungal species tested and evaluation of treated abattoir effluent as a biofertilizer. Exploring multi-stage treatment systems for palm oil mill effluent, as well as an economic and life-cycle comparison of mycofiltration with conventional treatment methods, is important. Direct quantification to test whether Brick top and King Stropharia genuinely show higher oxidoreductase output than Shiitake and Phoenix mushroom, and whether phosphatase/protease activity tracks the nutrient-mineralization hypothesis proposed for abattoir effluent.

Molecular/community profiling of the mycofilter itself: DGGE or amplicon sequencing of the fungal ITS region, to track whether the inoculated species remains dominant in the substrate over repeated use or is displaced by co-occurring environmental fungi and bacteria. qPCR-based tracking of bacterial DNA retained within the mycelium, to test whether physical capture/biosorption or biodegradation is the dominant removal route for the abattoir-effluent microbial load.

Metabolomics/transformation-product analysis: LC-MS or GC-MS profiling of raw versus mycofiltered effluent to identify whether organic nitrogen and phosphorus compounds are actually being converted to ammonium-N and orthophosphate-P as hypothesized, and to check for the accumulation of any toxic intermediate breakdown products, particularly for the phenolic fraction of POME. Multi-site and multi-season replication and formal techno-

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economic/life-cycle assessment to test the identified generalizability limitations and to place mycofiltration on a comparable cost basis with conventional physicochemical effluent treatment.

5. Conclusion

This study demonstrated that mycofiltration can improve the chemical and microbiological quality of agro-processing effluents and stimulate maize root growth under both controlled and field conditions. Brick top and King Stropharia were consistently the most effective fungal agents for chemical remediation and root growth outcomes. In practical terms, these findings indicate that mycofiltered abattoir, cassava, and maize effluents, particularly when treated with Brick top or King Stropharia spawn, can be reused for irrigation without harming maize root development. Mycofiltered effluents may even provide a mild biostimulant benefit, offering smallholder and agro-industrial operators in tropical settings a low-cost option for safer effluent reuse. Palm oil mill effluent, in contrast, remains unsuitable for direct reuse after single-pass mycofiltration and would require multi-stage treatment due to its high phenolic content and high physical oil load. The factorial ANOVA results provide quantitative evidence that effluent type and mushroom spawn type jointly determine mycofiltration performance, particularly under field conditions, underscoring the need for context-specific optimization of mycofiltration systems that match mushroom spawn to the target effluent and deployment environment.

These findings are based on a single location and growing season and should be regarded as preliminary. They, however, suggest that mycofiltration could be a viable component of integrated effluent management in tropical agro-industrial settings where conventional treatment infrastructure is lacking. These findings can provide a foundation for future work on enzymatic characterization, multi-site and multi-season validation, and economic and life-cycle assessment of mycofiltration relative to conventional treatment.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Authorship Contribution Statement

Victoria Francis Ediene: Conceptualization, Methodology, Investigation, Data curation, Writing of the original draft. Peggy O. Willie: Conceptualization, Methodology & review. Ekama Derrick Elemi: Validation, review & editing. Mary-Ibenreh O. Agba: Validation, Writing & review. Ene Emmanuel Aki: Methodology, Investigation, Data, Writing. Benjamin O. Unuigbe: Validation, review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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