ASSESSMENT OF MYCOFILTRATION EFFICIENCY OF FOUR BASIDIOMYCETOUS FUNGI FOR THE TREATMENT OF AGRO-PROCESSING EFFLUENTS USING *Allium cepa ASSAY*

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Abstract
This study assessed the effectiveness of four types of basidiomycetous fungi (*Lentinula edodes, Hypholoma sublateritium, Pleurotus pulmonarius,* and *Stropharia annulata*) in treating effluents from cassava, maize, palm oil mills, and abattoirs. A 5 × 5 factorial experiment was conducted using a completely randomized design with three replicates. Before treatment, palm oil mill effluent showed the highest pollution load, with biological oxygen demand (BOD) and chemical oxygen demand (COD) values of 28,400.56 and 45,760.82 mg/L, respectively. The Brick Top and King Stropharia spawns achieved the largest reductions in pollutant concentrations. In cassava effluent, BOD decreased from 607.18 to 420.54 mg/L after treatment with King Stropharia. For maize effluent, BOD dropped from 2,067.46 to 1,263.24 mg/L. In palm oil mill effluent, BOD was reduced from 28,400.56 to between 9,112.79 and 9,254.54 mg/L, while COD fell to between 41,005.21 and 42,125.00 mg/L. The BOD in abattoir effluent decreased from 8,325.46 to 2,001.11 mg/L. Untreated cassava and palm oil mill effluents caused significant cytotoxic effects on *Allium cepa*, including mitotic inhibition and chromosome abnormalities along with micronucleus formation. Brick Top and King Stropharia increased the mitotic index in cassava effluent from 6.02% to between 11.03% and 11.48%, while chromosomal abnormalities were reduced from 16.60% to between 2.02% and 2.87%. The two-way ANOVA showed significant impacts of effluent type, fungal species, and their interaction on mitotic index as well as the frequency of abnormalities and micronuclei ($p < 0.001$). The results show that mycofiltration enhanced the physicochemical quality of agro-processing effluents while decreasing their cytotoxicity.

Keywords: Mycofiltration, basidiomycetous fungi, agro-processing effluents, cytotoxicity, *Allium cepa* assay

Introduction

Effluent management is a vital component of environmental stewardship, especially in sectors like agriculture and food processing, which produce significant amounts of effluent as a by-product of their operations (Rajagopal *et al.*, 2013; MPOC, 2013; Ganesh *et al.*, 2010). Waste effluents have an inherent adverse effect on air, water and soil ecosystems due to their discharge quality (Owuli, 2003). Conventional effluents treatment technologies involve chemical or mechanical treatments, and are energy intensive, expensive and pose a negative impact on the environment (Henze, 2001).

Mycofiltration promises a sustainable approach for effluents treatment (Mnkandla & Otomo, 2023; Akpasi *et al*., 2023; Dasgupta *et al.*, 2024). Mycofiltration is the ability of fungi to filter and remove organic pollutants and contaminants from wastewater (Stamets, 2013). Mushrooms exhibit an outstanding capability to decompose organic materials and absorb nutrients from effluent as shown by Mnkandla and Otomo (2026). Typically, mycofiltration systems consist of a substrate, such as wood chips, agricultural waste, or synthetic polymers, that is inoculated with specific fungal species (Stamets, 2005). When effluent passes through mycelial mats, the fungal hyphae colonize the organic pollutant in the effluents, break them down with the aid of extracellular enzymes, and convert the complex compound to simpler ones, ready for adsorption (Singh, 2006; Adenipekun & Isikhuemhen, 2008; Stamets, 2013). Additionally, the mycelial mat serves as a physical barrier for suspended solid matter and microbial pathogens (Isikhuemhen *et al.*, 2003; da Luz *et al.*, 2013; Mnkandla & Otomo, 2024;). Fungi can also be used for breaking down organic contaminants into non-toxic substances without producing any harmful residues (Eskander *et al*., 2012; Tsujiyama *et al*., 2013).

Recent interest in fungal-based effluent treatment has emerged due to filamentous fungi's ability to produce extracellular oxidative enzymes that degrade complex organic pollutants (Jang *et al.*, 2009; Olusola and Anslem, 2010). In particular, white-rot fungi generate laccases, manganese peroxidases, and lignin peroxidases that aid in breaking down resistant organic compounds often impervious to traditional biological treatments (Latif *et al.*, 2023). Beyond enzymatic degradation, the extensive surface area of fungal mycelia enhances biosorption of heavy metals, nutrients, and suspended particles while serving as a physical filtration matrix that captures microorganisms and particulate matter (Taylor and Stamets, 2014; Latif *et al.*, 2023).

Agricultural effluent contains various nutrients and also rich organic and other toxic compounds that cause many environmental pollution problems (Ediene *et al*., 2013; Ediene *et al*., 2016) Effluents are a mixture of numerous components, with documented primary adverse impact on plants ranging from phytotoxicity, to genotoxicity and mutagenicity (Ediene *et al.*, 2017). Therefore, this study is aimed to analyze the effectiveness of mycofiltration using four (4) culturally-relevant basidiomycetes species to treat effluents. The purpose is to reduce biological oxygen demand (BOD), chemical oxygen demand (COD) and overall pollutant load in the effluents. *Allium cepa* (onion) assay was used to assess the effectiveness of the mycofiltration process.

Allium cepa (onion) assay is recognized as one of the most reliable and well-established plant tests for monitoring environmental impact (Grant, 1982; Melo *et al*., 2024). Various studies show that the onion root tip is a very sensitive indicator of cytotoxic effects, genetic damages such as DNA and chromosomal aberrations. Leme and

Marin-Morales (2009), Alias *et al*. (2023), Melo *et al*. (2024) and Alias *et al*. (2024), concluded that *Allium cepa* may be employed in the screening of environmental mutagens and have been frequently used in environmental risk studies. Parameters commonly measured in the onion assay are micronuclei, structural fragmentation, chromosomal abnormalities during cell cycle such as spindle disruption, chromosomal bridges during all phases of mitosis, as well as to verify overall damage of cells (Melo *et al*. 2024; Alias *et al.*, 2024; Nicuță *et al*., 2025). The low cost, simplicity, rapid and good correlation to mutagenicity, make *Allium cepa* the best option for evaluating environmental toxicity (Leme and Marin-Morales, 2009; Alias *et al*., 2023).

This work presents a comparative assessment of four species of basidiomycetous fungi used in the mycofiltration treatment of cassava, maize, palm oil mill and abattoir effluents. We complemented the physicochemical analysis of the treated effluents with a cytogenetic study on *Allium cepa*. This would ensure the establishment of correlation between the improvements made to the effluent's quality and the mitigation of cytotoxicity to plants.

Materials and methods

Fungal spawns and mycofilter preparation

Pure strains of four mushroom species: Shiitake (*Lentinula edodes*), Brick Top (*Hypholoma sublateritium*), Phoenix Mushroom (*Pleurotus pulmonarius*), and King Stropharia (*Stropharia annulata)* were sourced from Fungi Perfecti Laboratory in the USA. The spawns were stored at -70 °C until needed. The maize cobs used as the substrate were crushed into particles less than 2 mm in diameter, to enable mycelial penetration and colonization. The resilience tests for the substrate bags, which comprised of repeated cycles of saturation-drying heating-freezing, and sterilization under sterile conditions were completed according to Stamets' (2013) protocols. Two (2) ml of live spawn was inoculated per kg of substrate, and incubation were carried out in the dark for 30 days to allow full colonization. Full colonization was visually evaluated based on a six-point scale; measured with a Kappa (κ), and statistic interpreted according to McHugh's criteria (2012).

Twenty-four plastic filtration units were constructed and comprised of a filtering chamber, two mycofilter chambers, containing fully colonized substrate, and 10 L collecting bottle. This was modified from Stamets' (2013). All chamber outlets were covered with 2 mm wire mesh to reduce clogging. Filtration was conducted at approximately 30°C. Composite effluent samples were collected three times from cassava, maize, palm oil mill, and abattoir processing facilities in Cross River State, Nigeria. Ten litres of each effluent were filtered through each mycofiltering unit at a flow rate of 0.417 L/h over a contact period of 24 hours. Filtration continued weekly until the desired volume of filtrate was obtained.

Effluents were held at <4°C until required for treatment and was brought to room temperature before use (Norweco, 2017). The parameters for raw and mycofiltered effluents (including the control treatment) included: temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), total suspended solids (TSS), BOD, and COD (APHA, 2023). Additionally, abattoir effluent samples underwent microbiological quality analysis using internationally recognized standard methods: total aerobic bacteria through heterotrophic plate count (APHA Standard Methods 9215). *Clostridium perfringens* was determined by ISO 14189; while coliforms was by the Most Probable Number method (APHA Standard Methods 9221). *Escherichia coli* was determined by ISO 4831; faecal streptococci by ISO 7899-2; *Pseudomonas spp*. by membrane filtration (ISO 16266). Mould and yeast counts targeting both xerophilic and non-xerophilic fungi were determined by ISO 21527-1 (ISO, 2008; APHA, 2017).

Experimental Design

This laboratory experiment used a 5x5 factorial Completely Randomized Design (CRD) with 25 treatment combinations (Table 1). Each treatment combination had 3 replications, giving a total of 75 experimental units. Treatment combinations were randomly assigned using random allocation procedures to minimize positional bias.

Treatment Application and *Allium cepa* Cytotoxicity Assay

In the laboratory, thirty (30) ml of each treatment were applied to individual experimental plants as treatment daily to ensure continuous supply of freshly prepared effluent. After the exposure period, *Allium cepa* roots were carefully cut using a razor, placed into labeled sample bottles, instantly fixed in chill Carnoy fixative solution (ethanol:acetic acid, 3:1 v/v), and then stored at 4 ^{o}C for 24 hours . The root tips were hydrolyzed using 1 *N* HCl solution in 60 ^{o}C for 5 minutes then, washed with distilled water. The root tips were then flattened between slide and coverslip to obtain a squash. The cells in the squash were carefully stained with acetocarmine solution for 10 minutes. Coverslips were carefully mounted without entrapping any air. The cover slides were sealed with colourless transparent nail varnish to prevent cell evaporation when heated by microscope (Grant, 1982; Sharma, 1983). The roots were then examined under a microscope and photographed to determine all the indices as described in Table 1.

Table 1: Treatments used in the laboratory study

Treats	Description	Treats	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	Palm oil mill effluent (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		

Effluent Analysis

Effluents pH were measured on-site with a pH meter. All effluents were analyzed according to the standard procedures set out in the Central Pollution Control Board's guide manual. Total dissolved solids were determined by Winkler method, BOD by titrimetric method, and COD was calculated using the open reflux method (CPCB, 2012). The total solids (suspended and dissolved) were calculated from the residue left after evaporating 100 mL of the sample at a temperature between 103 and 105 °C, using the formula:

$$\text{Total solids (mg/L)} = \frac{(W1-W2)}{'(Volume\ of\ samples(ml)} \text{ x1000}$$

Where

W_1 represents the weight of the sample and W_2 is the weight of the dry sample.

Cytogenetic Indices

The formulas used for calculating cytogenetic indices are as follows:

$$\text{Mitotic Index (MI, \%)} = \frac{Dividing\ cells}{Cells\ scored} x100$$

Relative Division Rate (RDR, %) = $\frac{\text{MI of treatment}}{\text{MI of corresponding untreated effluent control}} x100$

In each effluent block, the untreated effluent (M0) served as the reference. For the water series (E0), E0M0 was used as the absolute control reference.

Mitotic Inhibition (%) = $100 - RDR$

Abnormality Frequency (%) = $\frac{\text{Abnormal dividing cells}}{\text{Total dividing cells}} x100$

Micronucleus Frequency (%) = $\frac{\text{Micronucleated cells ls}}{\text{Cells scored}} x100$

All indices were calculated per replicate (n = 3 per treatment with 2000 cells scored per replicate) and then averaged.

Statistical Analysis

A one-way ANOVA was conducted for 25 treatments, while a two-way ANOVA was used for the five effluent levels combined with five mushroom-spawn levels, including interaction effects. The analyses were based on replicate-level data for each index. This was followed by Tukey's HSD and Fisher's LSD post-hoc comparisons at $\alpha = 0.05$.

Results and Discussion

Physicochemical Characteristics of Raw and Mycofiltered Effluents

Temperatures of the effluent were generally, from 30.0 to 30.7 °C, which is generally favourable for plant production. The value of pH ranged from 6.11 to 8.70, and indicated slightly acidic to moderately alkaline condition that could influence the nutrient status in the effluents. There were substantial variations in pollution load among the four agro-industrial effluents studied (Table 2).

Palm oil mill effluent (POME) had the highest concentration of pollutants among the raw effluents with electrical conductivity of 6560.49 µS/cm, TDS of 3936.00 mg/L, TSS of 30.22 mg/L, BOD of 28,400.56 mg/L, and COD of 45,760.82 mg/L. These values exceed those of the other effluents used in this experiment but align with values from previous studies (Ediene *et al*., 2016). However, in the treated POME samples, BOD decreased. Brick top (E3M2 = 9112.79 mg/L) and King Strophoria (E3M4 = 9254.54 mg/L) showed greater reduction efficiencies. A similar trend was observed for COD values (45760.82 mg/l E3M0) with the two best performers being Brick Top, (E3M2, 9112.79 mg/l) and King Strophoria (E3M4, 9254.54 mg/l). Although the COD values were still higher than normal level, the trend indicates reduction in pollution degree. The reduction in COD and BOD levels could be due to fungal ability to degrade complex organic materials commonly found in palm oil mill effluent. This could be mainly due to the activities of laccase and peroxidases enzymes. This supports findings that white-rot fungi produce a diverse array of lignocellulolytic enzymes which enable them to degrade xenobiotic substances including those found in effluent (Latif *et al*., 2023).

Maize effluent (E2M0) ranked second in organic pollution load (BOD at 2067.46 mg/L; COD at 4600.80 mg/L). The

reduction of BOD from 2067.46 mg/l to 1263.24 mg/l in maize effluent (E2M4), as well as decrease of COD from 4600.80 mg/l to 2936.19 mg/l in E2M4 demonstrates effectiveness of mycofiltration system (King Strophoria, E2M4).

Table 2: Mean physicochemical properties of raw and mycofiltered agro-processing effluents

Treat.	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
Mean	30.12	6.60	549.08	495.17	2.54	3.66	5.63	0.61
E1M0	30.50	8.51	2640.00	1584.00	20.94	607.18	986.15	0.61
E1M1	30.45	8.39	1600.91	1399.09	4.24	493.40	945.78	0.52
E1M2	30.50	8.45	979.37	1107.41	3.50	453.99	860.18	0.52
E1M3	30.41	8.45	1620.64	1498.99	2.30	499.79	935.20	0.53
E1M4	30.46	8.45	995.79	1295.11	1.65	420.54	845.63	0.49
Mean	30.46	8.45	1567.34	1376.02	6.52	494.98	914.58	0.53
E2M0	30.70	8.46	3780.00	2268.00	20.18	2067.46	4600.80	0.44
E2M1	30.67	8.47	2709.02	2075.78	3.42	1654.00	3600.02	0.45
E2M2	30.62	8.40	1698.76	2198.54	2.21	1564.44	3676.24	0.42
E2M3	30.70	8.39	1730.73	2200.91	4.22	1345.92	2960.44	0.45
E2M4	30.70	8.49	1700.00	1807.52	2.19	1263.24	2936.19	0.43
Mean	30.67	8.44	2323.70	2110.15	6.44	1579.01	3554.73	0.43
E3M0	30.30	6.11	6560.49	3936.00	30.22	28400.56	45760.82	0.62
E3M1	30.30	6.21	6560.00	3867.72	7.65	13840.78	45575.50	0.30
E3M2	30.31	6.20	5479.45	3759.19	3.79	9112.79	42125.00	0.21
E3M3	30.28	6.14	5500.90	3890.34	7.25	13563.99	45500.76	0.29
E3M4	30.24	6.23	5370.02	3195.66	4.32	9254.54	41005.21	0.22
Mean	30.28	6.17	5894.17	3729.78	10.64	14834.53	43993.45	0.32
E4M0	30.27	8.68	3140.49	1884.00	18.28	8325.46	13200.12	0.63

E4M1	30.25	8.70	1140.00	1675.90	3.91	3543.17	10500.97	0.33
E4M2	30.18	8.69	1007.98	1009.52	1.25	2113.29	6025.29	0.25
E4M3	30.23	8.68	1137.47	1200.61	2.56	3432.97	12415.87	0.27
E4M4	30.27	8.69	1099.76	997.56	1.43	2001.11	6019.81	0.32
Mean	30.24	8.68	1505.14	1353.51	5.48	3883.20	9632.41	0.36

Legend: E0 = water; E1 = cassava effluent; E2 = maize effluent; E3 = palm oil mill effluent; E4 = abattoir effluent; M0 = no mushroom spawn; M1 = Shiitake spawn; M2 = Brick top spawn; M3 = Phoenix spawn; M4 = King Stropharia spawn.

Cassava (E1M0) effluent showed moderate pollution levels. The concentration of EC in cassava effluent decreased from 2640.00 µS/cm (E1M0) to 979.37 µS/cm (E1M2) and 995.79 µS/cm (E1M4), respectively. Similarly, the concentration of BOD decreased from 607.18 to 453.99 mg/l (E1M2) and 420.54 mg/l (E1M4), with the latter giving the highest reduction value (King Strophoria, +abattoir effluent E1M4). The reductions in BOD in cassava effluent (607.18 mg/l to 453.99 mg/l and 420.54 mg/l E1M2 and E1M4, respectively) are environmentally significant because cassava effluent is widely associated with soil acidity and toxicity. Therefore, mycofiltered cassava effluent offers potential for safe utilization of effluents in irrigation systems. It also supports the previous reports of decreased toxic components of cassava effluent as a result of treatment by means of some plant beneficial microorganisms and fungi (Aslantürk, 2024).

Abattoir raw (E4M0) effluent equally showed moderate BOD levels (BOD of 8325.46 mg/L). in the treated effluents. The BOD was reduced from 8325.46 mg/l (E4M0) to 2001.11 mg/l (E4M4) and 2113.29 mg/l (E4M2), while COD was reduced to 6019.81mg/l and 6025.29 mg/l, respectively.

Generally, these results showed significant variation in effluent composition across the different agro-processing industries (Table 2). It suggests the need for specific treatment strategies for each type of effluent. The mycofiltered treatment in general, resulted in decreases in the concentration of pollutants in all the effluents but the degree of treatment effectiveness differed among fungal species. All treated and untreated effluents recorded BOD/COD ratios of 0.21-0.85. Usually, higher BOD/COD ratios indicate easily biodegradable organic matter in waste. The reduction in the oxygen demand (BOD) for mycofiltration shows utilization and metabolism of the labile portion of organic components and not just separation through filtration (Latif et al., 2023). These improvements have positive environmental implications since effluents usually contaminate the receiving waters with oxygen- depleting biodegradable organic matter and high levels of bacterial count, which could severely harm aquatic organisms and increase health hazards.

Cytotoxic Effects of Raw and Mycofiltered Effluents on *Allium cepa*

Mitotic and Chromosomal Indices in *Allium cepa* root tips exposed to raw and mycofiltered agro-processing effluents are presented on Table 3 and Plates 1-5. Mitotic and Chromosomal Indices for water controls (E0M0–E0M4): MI ranged from 12.7-13.9% while abnormality frequency was <1.6%. The micronucleus frequency for the water controls were ≤0.10% with active normal mitotic (prophase, prometaphase, and telophase) activity. There were no visible chromosomal abnormalities (Table 3) (Plate 1). The water control values were statistically indistinguishable from one another (Table 3), confirming a healthy, unstressed baseline experimental condition that supported normal cell division and validating the assay system.

Untreated cassava effluent (E1M0) induced resting (interphase) cells and chromosome clumping with clumping with MI of 6.02% and 16.60% abnormal cells, indicating that the cells were not actively dividing. Clumping of this kind is generally attributed to environmental stressors, and in this study, was most likely induced by residual toxic constituents of the raw effluent. Meanwhile, cassava effluent treated with Shiitake spawn (E1M1) resulted in chromosome fragmentation and disorientation at prometaphase. This suggests that cytotoxic compounds partially persisted and could have disrupted spindle function, possibly through mutations in genes responsible for spindle activity. Similar disruptions have been associated with pesticide exposure in other studies (Saxena et al., 2007). The Phoenix-treated cassava effluent (E1M3) showed lagging chromosomes and clumping at metaphase, a phenomenon that Bakale and Hadke (1981) associated with hardening and swelling of chromosomes. In contrast, treatments with Brick top (E1M2) and King stropharia (E1M4) did not show any mito-depressive abnormalities. This indicates significant detoxification of cassava effluent by these two species. Statistically, Brick top (E1M2) and King Stropharia (E1M4) increased the MI to between 11.0% and 11.5% while reducing abnormalities to between 2.0% and 2.9%. This implies significant differences compared to E1M0 as well as Shiitake/Phoenix treatments as shown by Tukey's HSD test, though still remaining significantly lower than those from the water/maize/abattoir-treated group (Table 3).

All maize effluent treatments (E2M0-E2M4) showed normal active cell division during the prophase, metaphase, and prometaphase stages, with no significant chromosomal abnormalities (Plate 3). This indicates that maize effluent has lower inherent cytotoxicity compared to cassava, palm oil mill, or abattoir effluent, and that mycofiltration further enhances its already positive biological safety profile. The five maize effluent treatments (E2M0 - E2M4) had mitotic index ranging from 12.0-12.9 % and an abnormality frequency of ≤2.2%. The results were statistically similar to the water-control and mycofiltered abattoir treatments (Table 3).

The palm oil mill effluent caused the most severe cytological responses in the cells

across the different treatments. Mitotic arrest and inhibition of the cell cycle were noted in E3M0 and E3M1 while mostly inactive cells were seen in E3M2, E3M3, and E3M4. These responses correspond closely with the extremely high pollutant load (elevated concentrations of organic matter, suspended solids, and dissolved compounds) in the effluents which can interfere with cellular metabolism and inhibit DNA replication, ultimately suppressing cell division. The severity of cytological damage thus appears directly related to the pollutant burden of the effluent, consistent with previous *Allium cepa* based toxicity studies **(**Macar *et al*., 2025, da Silva *et al*., 2025; Animasaun *et al*., 2024). Palm oil mill effluent had the most statistically different cytological results (MI 1.5-2.9%, abnormality frequency 25-56%, micronucleus frequency 0.48–0.83%) (Table 3). The numbers for cell division and abnormalities were quite high and stood out from every other treatment, even after fungal treatment.

For the abattoir effluent, the untreated sample showed some resting cells and early stages that pointed to mild stress. The mycofiltered abattoir effluents exhibited more normal and regular prophase stages with no detectable chromosomal abnormalities (Plate 5). This points to the treatments effectiveness in reducing the toxic effects of the effluent. Abattoir effluents, untreated (E4M0) MI of 7.52% rose to 13.0-14.0% (Table 3) after the four fungal treatments. The results were statistically equivalent to the water controls and not significantly different from each other.

Put together, the chromosome clumping seen in E1M0 and E1M3 is consistent with chromatin stickiness, which is generally viewed as basically an irreversible toxic outcome from abnormal chromosome compaction. The fragmentation plus disorientation visible in E1M1 suggests that the spindle apparatus is getting disrupted and that DNA damage is possible. Leme and Marin-Morales (2009) mentioned that chromosomal irregularities such as stickiness, laggards, bridges, and spindle disruptions are dependable readouts for environmental mutagens and cytotoxic contaminants. These same kinds of abnormalities are commonly described in plants after they are exposed to industrial and agricultural pollutants, and they are usually tied to genotoxic stress. Their presence in the untreated and partially treated cassava effluent treatments indicates that some toxic constituents remained biologically active and can disrupt normal cell-cycle progression and chromosome segregation. In contrast, the lack of mito-depressive abnormalities in E1M2, E1M4, all maize effluent treatments (E2M1-E2M4), and E4M1-E4M4 indicate that mycofiltration significantly reduced the concentration of cytotoxic compounds in the raw effluents.

This restoration of normal prophase, metaphase, and telophase stages reflects an improvement in biological safety. Conversely, their absence of chromosomal aberrations after treatment with Brick top and King Stropharia mycofilters supports previous findings that fungal treatment systems do not only decrease pollutant

concentrations, but also lower wastewater toxicity through biodegradation and detoxification (Akpasi et al., 2023; Mnkandla, & Otomo 2023, Mnkandla, & Otomo 2023).

Table 3: Mitotic and Chromosomal Indices in *Allium cepa* Root Tips Exposed to Raw and Mycofiltered Agro-Processing Effluents

Treatment	Effluent	Mushroom Spawn	MI (%) Mean ± SD	RDR (%)	Mitotic Inhibition (%)	Abnormality Freq. (%)	Micronucleus Freq. (%)
E0M0	Water (control)	No spawn	13.38 ± 0.28 ab	100.00	0.00	0.99 ± 0.20 h	0.05 ± 0.00 e
E0M1	Water (control)	Shiitake	13.02 ± 0.30 bc	97.26	2.74	1.41 ± 0.20 h	0.07 ± 0.03 e
E0M2	Water (control)	Brick top	13.20 ± 0.30 b	98.63	1.37	1.01 ± 0.20 h	0.05 ± 0.00 e
E0M3	Water (control)	Phoenix	12.72 ± 0.30 bc	95.02	4.98	1.44 ± 0.20 h	0.07 ± 0.03 e
E0M4	Water (control)	King Stropharia	13.90 ± 0.30 ab	103.86	-3.86	0.96 ± 0.19 h	0.05 ± 0.00 e
E1M0	Cassava	No spawn	6.02 ± 0.20 f	100.00	0.00	16.60 ± 0.96 f	0.52 ± 0.03 cd
E1M1	Cassava	Shiitake	6.53 ± 0.28 f	108.59	-8.59	13.00 ± 0.22 g	0.47 ± 0.08 cd
E1M2	Cassava	Brick top	11.03 ± 0.23 d	183.38	-83.38	2.87 ± 0.65 h	0.12 ± 0.03 e
E1M3	Cassava	Phoenix	7.03 ± 0.28 ef	116.90	-16.90	12.80 ± 0.66 g	0.42 ± 0.03 d
E1M4	Cassava	King Stropharia	11.48 ± 0.26 d	190.86	-90.86	2.02 ± 0.62 h	0.10 ± 0.00 e
E2M0	Maize	No spawn	12.05 ± 0.25 cd	100.00	0.00	2.21 ± 0.20 h	0.10 ± 0.00 e
E2M1	Maize	Shiitake	12.38 ± 0.28 c	102.77	-2.77	1.75 ± 0.20 h	0.10 ± 0.00 e
E2M2	Maize	Brick top	12.87 ± 0.28 bc	106.78	-6.78	1.42 ± 0.20 h	0.07 ± 0.03 e
E2M3	Maize	Phoenix	12.18 ± 0.28 cd	101.11	-1.11	2.19 ± 0.19 h	0.10 ± 0.00 e
E2M4	Maize	King Stropharia	12.52 ± 0.25 bc	103.87	-3.87	1.59 ± 0.37 h	0.07 ± 0.03 e

Treatment	Effluent	Mushroom Spawn	MI (%) Mean ± SD	RDR (%)	Mitotic Inhibition (%)	Abnormality Freq. (%)	Micronucleus Freq. (%)
E3M0	POME	No spawn	2.03 ± 0.10 h	100.00	0.00	49.19 ± 0.71 c	0.83 ± 0.06 a
E3M1	POME	Shiitake	2.50 ± 0.05 gh	122.95	-22.95	43.32 ± 1.60 d	0.70 ± 0.05 b
E3M2	POME	Brick top	1.67 ± 0.08 h	81.97	18.03	53.03 ± 1.56 b	0.62 ± 0.03 bc
E3M3	POME	Phoenix	1.48 ± 0.08 h	72.95	27.05	56.22 ± 1.22 a	0.57 ± 0.03 c
E3M4	POME	King Stropharia	2.90 ± 0.15 g	142.62	-42.62	25.30 ± 0.65 e	0.48 ± 0.03 cd
E4M0	Abattoir	No spawn	7.52 ± 0.30 e	100.00	0.00	11.75 ± 0.20 g	0.42 ± 0.08 d
E4M1	Abattoir	Shiitake	13.05 ± 0.25 bc	173.61	-73.61	1.53 ± 0.35 h	0.07 ± 0.03 e
E4M2	Abattoir	Brick top	13.40 ± 0.25 ab	178.27	-78.27	1.37 ± 0.20 h	0.07 ± 0.03 e
E4M3	Abattoir	Phoenix	13.18 ± 0.28 b	175.39	-75.39	1.39 ± 0.20 h	0.07 ± 0.03 e
E4M4	Abattoir	King Stropharia	14.02 ± 0.30 a	186.47	-86.47	1.07 ± 0.33 h	0.05 ± 0.00 e

Legend: E0 = water; E1 = cassava effluent; E2 = maize effluent; E3 = palm oil mill effluent (POME); E4 = abattoir effluent. M0 = no mushroom spawn; M1 = Shiitake; M2 = Brick top; M3 = Phoenix; M4 = King Stropharia. MI = mitotic index; RDR = relative division rate; SD = standard deviation (n = 3 replicates, 2000 cells scored per replicate). Values within a column followed by the same letter are not significantly different by Tukey's HSD test (p > 0.05). Shaded rows mark each effluent's untreated reference (M0) used as the RDR/Mitotic Inhibition baseline for that block.

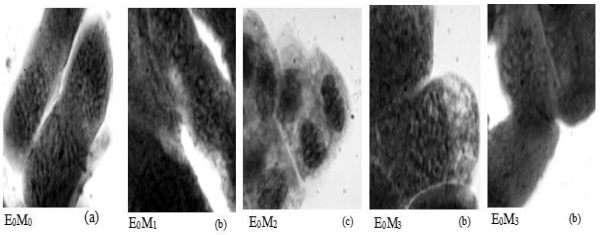

Plate 1: Effects of water and mycofiltered distilled water on *Allium cepa* root development showing (a) normal prometaphase (b) normal prophase (c) normal telophase.
Legend: Distilled water - no mushroom spawn (E_0M_0), Distilled water + Shiitake mushroom spawn (E_0M_1), Distilled water + Brick top mushroom spawn (E_0M_2), Distilled water + Phoenix mushroom spawn (E_0M_3), Distilled water + King Stropharia mushroom spawn (E_0M_4).

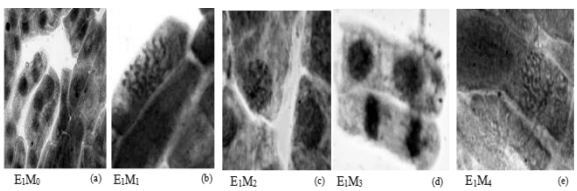

Plate 2 : Effects of raw and mycofiltered cassava effluents on *Allium cepa* root development showing (a) resting cells and clumping chromosomes (b) fragmentation and disorientation of chromosomes at prometaphase (c) early prophase (d) laggards and chromosome clumping at metaphase (e) normal prophase stages.
Legend: Cassava effluent - no mushroom spawn (E_1M_0), Cassava effluent + Shiitake mushroom spawn (E_1M_1), Cassava effluent + Brick top mushroom spawn (E_1M_2), Cassava effluent + Phoenix mushroom spawn (E_1M_3), Cassava effluent + King Stropharia mushroom spawn (E_1M_4).

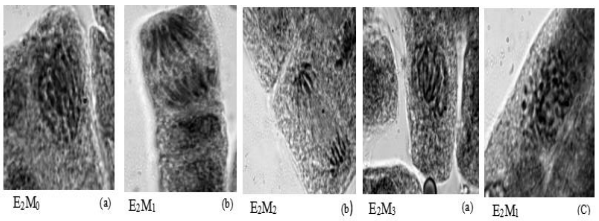

Plate 3: Effects of raw and mycofiltered maize effluents on *Allium cepea* root development showing (a) prophase (b) metaphase (c) Prometaphase stages in normal and active division phases.
Legend : Maize effluent - no mushroom spawn (E_2M_0), Maize effluent + Shiitake mushroom spawn (E_2M_1), Maize effluent + Brick top mushroom spawn (E_2M_2), Maize effluent + Phoenix mushroom spawn (E_2M_3), Maize effluent + King Stropharia mushroom spawn (E_2M_4)

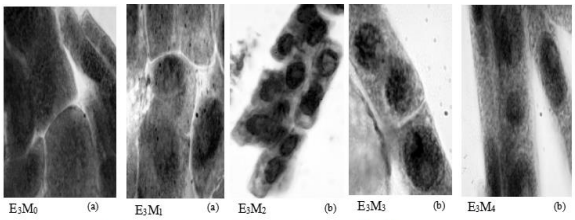

Plate 4: Effects of raw and mycofiltered palm oil mill effluents on *Allium cepa* root development showing (a) cell cycle inhibition and mitotic arrest (b) inactive cells stages.
Legend: Palm oil mill effluent - no mushroom spawn (E_3M_0), Palm oil mill effluent + Shiitake mushroom spawn (E_3M_1), Palm oil mill effluent + Brick top mushroom spawn (E_3M_2), Palm oil mill effluent + Phoenix mushroom spawn (E_3M_3), Palm oil mill effluent+ King Stropharia mushroom spawn (E_3M_4)

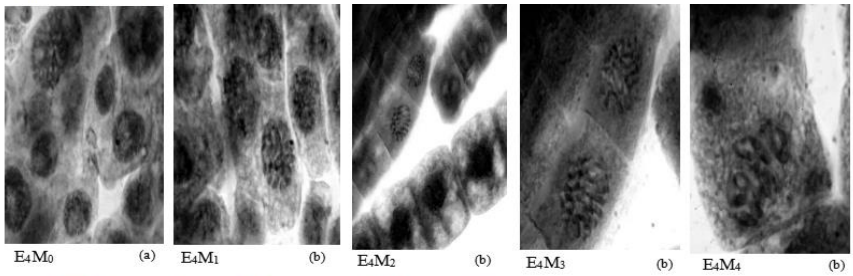

Plate 5: Effects of raw and mycofiltered abattoir effluents on *Allium cepa* root development showing (a) early prophase and resting cells (b) normal prophase.

Legend: Abattoir effluent - no mushroom spawn (E_3M_0), Abattoir effluent + Shiitake mushroom spawn (E_3M_1), Abattoir effluent + Brick top mushroom spawn (E_3M_2), Abattoir effluent + Phoenix mushroom spawn (E_3M_3), Abattoir effluent+ King Stropharia mushroom spawn (E_3M_4).

Environmental Implications

This study showed that a diverse range of physicochemical indicators in all untreated agro-industrial effluents were significantly decreased by the application of fungi to mycofilters. Most importantly, however, the four types of effluents, even at high concentration, produced varied degree of cytological aberrations. The present study revealed that Mycofiltration technology can address this environmental issue by effectively reducing cytogenetic damages and by making effluents bio safe for their re-use as irrigation water. The Brick top mushroom and the king stropha are two potential candidates that could be highly useful for treating agro industrial wastewater due to their abilities to improve quality of treated effluent and their effective reduction in levels of toxicity in both treatments of this study.

Limitations

This study was carried out under controlled laboratory conditions.

Conclusion

This research confirmed the use of fungal mycofiltration technique in the improvement of quality of the agro-industrial effluents. The decrease in conductivity, BOD, COD, TSS and other parameters due to the use of the fungal- colonized materials in treating these different effluents showed remarkable performance. The level of improvements depends on fungal species and effluent type as Bricktop and king stroparia showed significant effective results compared to other tested fungal isolates in most parameters. Cytotoxicity on *Allium cepa* root cells were analyzed and most of the treated effluents were significantly less toxic compared to the untreated effluent samples. The reduction in abnormalities in cassava

effluents (607.18 to 1.4%, E1M2 ;420.54 to0.8%, E1M4) as well as palm oil mill effluent was observed to be very promising and encouraging to develop Mycofiltration technology for industrial wastewater treatment.

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