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Bioremediation Potential of *Lentinus squarrosulus* (Mont.) Singer in Runoff Wastewater from Rivers State, Nigeria

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ABSTRACT

Background and Objective: Industrial and agricultural activities contribute to water pollution, necessitating eco-friendly remediation methods. *Lentinus squarrosulus* (Mont.) Singer, a white-rot fungus, has shown potential for biodegradation of pollutants. This study evaluates its efficiency in treating runoff wastewater from selected areas in Rivers State, Nigeria. **Materials and Methods:** Runoff wastewater samples were collected from multiple sites and analyzed for physicochemical properties before and after fungal treatment. *Lentinus squarrosulus* was cultured and applied under controlled conditions. Standard analytical techniques were used to measure reductions in contaminants such as heavy metals and organic matter. The study was analyzed using SPSS version 15.0 and underwent Duncan multiple comparisons and Dunnett's tests in a One-way ANOVA $p<0.05$. **Results:** The fungus demonstrated significant pollutant reduction, improving water quality parameters. Notable decreases were observed in biochemical oxygen demand (BOD), chemical oxygen demand (COD), and heavy metal concentrations, indicating effective bioremediation potential. **Conclusion:** The findings highlight *Lentinus squarrosulus* as a promising candidate for wastewater treatment. Its application in bioremediation could contribute to sustainable water management strategies in polluted environments.

KEYWORDS

Surface runoff water, mycofiltration, environmental pollution, *Lentinus squarrosulus*, bioremediation, runoff wastewater, heavy metal removal, water pollution, fungal treatment

INTRODUCTION

The water that runs off after rain or snowfall is known as stormwater. Runoff gathers up fertilizer, oil, and gas from automobiles, pesticides, dirt, bacteria, and other contaminants as it flows unfiltered via drainage channels and ditches and into our streams, rivers, lakes, and oceans. One of the main causes of water contamination is runoff or stormwater[1]. These pollutants are then transported to our ponds, streams, and wetlands[2].

There is a high increase in the amount of toxic waste coming into these bodies[3] which not only affects the biological productivity of the aquatic organisms found in them[4] but also destroys their aesthetic value[5].

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Our water bodies have become dumpsites for all kinds of pollutants because of the continuous flow of surface runoff wastewater into them. This surface runoff waste may be the reason why the microbial activities and physiochemical parameters of some water bodies increase affecting the growth, reproduction, and development of some organisms.

According to Kadiri *et al.*[6] surface runoff water that runs directly into the aquatic environment has both acute and chronic environmental effects that can be very severe, reduce biodiversity, and significantly reduce populations of sensitive species.

The effects of untreated surface runoff water on nearby rivers and streams are evident, and proper wastewater treatment is also essential to preserving public health, safeguarding the quality of drinking water, and ultimately fostering economic development. This study therefore explored the possibilities of using *Lentinus squarrosulus* to treat surface runoff wastewater through the process of mycofiltration.

MATERIALS AND METHODS

Study site and collection of water samples: The surface runoff water sample was collected in September, 2019 from Choba Park, University of Port Harcourt, Rivers State, Nigeria. Using sterile plastic bottles, water samples were taken and kept at 4°C in the refrigerator. Within a day of collection, the analysis was completed.

Method for mycofiltration: The mycofiltration procedure was carried out following the methodology of GloriaIkechi-Nwogu *et al.*[7]. Mushroom spawn was injected into bags of sterilized sawdust, and the substrate was allowed to colonize for one to two weeks. The colonized substrate was prepared for mycofiltration after the 3rd week. To stop contamination, they were put on a funnel and covered with nylon bags. The surface runoff water sample was poured into holes drilled in the center of the substrate using a sterile stainless steel spoon. After that, the water sample was allowed to pass through the substrate and into a sterile container for a whole day or two. The gathered filtrates were taken to a laboratory for analysis.

Preparation of media: Agar powder was precisely weighed (39 g), added to 1000 mL of sterile distilled water, dissolved entirely, and autoclaved for 15 min at 121°C. It was injected into a sterile Petri dish after sterilization, and it was left to harden for 30-60 min.

Method for microbial analysis: Blanks for sterile dilution (9 mL) were labeled 101, 102, ..., and 10 n (the desired dilution). Using a sterile pipette, 1 mL of the sample was obtained and combined with the first measured dilution blank. To ensure that the cells were distributed evenly, the contents were gently shaken. Until the required dilution of 10 n was achieved, the process was repeated. The 0.02 mL of the suspension was put onto the nutrient agar plate from specific dilutions. After that, the inoculated agar plates were incubated for 18 to 24 hrs at 35-37°C.

Data analysis: Quantitative data for pH and other physicochemical parameters were summarized as Means±Standard Errors using SPSS version 15.0 for Windows 2007. These were then subjected to Duncan's multiple range test and Dunnett's test in a One-way ANOVA at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

According to the observation, *Lentinus squarrosulus* significantly reduced pollutants, increasing the indices of water quality. Significant reductions in heavy metal concentrations, chemical oxygen demand (COD), and biochemical oxygen demand (BOD) were noted, suggesting promising bioremediation potential.

The whole system of water bodies and land that makes up our watershed benefits from clean stormwater, not just our neighborhood and community.

Table 1: Effect of mycofiltration on the microbial analysis of surface runoff waste water

Plate sample	Before THC	Before TCC	Before TF and YC	After THC	After TCC	After TF and YC
P1	4	6	1	40	0	0
P2	1	14	3	0	40	0
P3	1	6	5	0	0	0
DF	3	1	1	2	1	1
AV	80	347	120	13	13	0
Total	8×10^4	3.47×10^3	1.2×10^3	1.3×10^3	1.3×10^2	0

P: Plate, THC: Total heterotrophic count, TCC: Total coliform count and TF and YC: Total fungi and yeast count

Table 2: Microbial analysis of surface runoff wastewater showing the percentage effect of mycofiltration

Description	Before mycofiltration (CFU/mL)	After mycofiltration (CFU/mL)	Change (%)	Significant difference
Heterotrophic count	8×10^4	0	100	S
Total coliform count	3.47×10^3	1.3×10^2	10	NS
Fecal count	0	0	0	NS
Total fungal/yeast count	1.2×10^3	0	100	S

S: Significantly different from each other and NS: Not significantly different from each other

A trailblazing technique called mycofiltration assesses the potential of fungi for bioremediation as biofilters that degrade and eliminate contaminants from soil and water.

Table 1 presents microbial plate counts before and after a treatment, categorized under total heterotrophic count (THC), total coliform count (TCC), and total fungi and yeast count (TF and YC) across different plate samples (P1, P2, P3), dilution factor (DF), and average values (AV). Before treatment, THC values ranged from 1 to 4, with an average of 80 and a total count of 8×10^4 , while TCC varied between 1 and 14, averaging 347 with a total of 3.47×10^3. The TF and YC ranged from 1 to 5, averaging 120 with a total of 1.2×10^3. After treatment, THC values ranged from 0 to 40, with an average of 13 and a total count of 1.3×10^3, whereas TCC ranged from 0 to 40, also averaging 13 with a total of 1.3×10^2. Notably, TF and YC was eliminated across all samples post-treatment. This indicates a significant reduction in microbial counts, particularly in fungal and yeast populations.

Table 2 shows microbial reductions before and after mycofiltration. Heterotrophic and fungal/yeast counts were completely eliminated (100% reduction, significant), while total coliforms decreased by 10% (not significant). The fecal count remained unchanged at 0 CFU/mL. These results suggest that mycofiltration effectively removes heterotrophic bacteria and fungi but has a limited impact on coliforms.

Table 3 presents water quality parameters before and after treatment. The pH decreased from 8.5 to 6.7, while color and turbidity were reduced from 107.5 to 56 Pt-Co units and 94 to 36.5 NTU, respectively. Electrical conductivity and total dissolved solids decreased significantly from 379 to 158.5 μS/cm and 179-78.5 mg/L, respectively. Suspended solids, sulfate, and phosphate levels also declined, whereas nitrite slightly increased from 0.13 to 0.15 mg/L. Dissolved oxygen dropped from 12.16-3.75 mg/L, and both biological and chemical oxygen demands showed reductions, indicating improved organic matter breakdown.

Both natural environmental processes and man-made pollutants have the potential to pollute surface waterways[8]. The quality of the rainfall in a region mostly determines the quality of the surface water. Elevated rainfall causes the soil to become more wet, which raises the amount of surface runoff. In order to preserve the health of surface waterways, it is imperative that these problems be addressed.

Shirasuna *et al.*[9] argue that seasonality, traffic, catchment, and street paving factors influence rainfall quality. Rainwater must be treated before it enters water bodies, as it contains fuel combustion products, industrial emissions, and ground surface mineral particles that can contaminate them[10].

Table 3: Physico-chemical and microbial load of surface runoff wastewater sample treated through mycofiltration

Parameters	Before	After
pH	8.5	6.7
Colour (Pt-Co unit)	107.5	56
Turbidity (NTU)	94	36.5
Electrical conductivity (µS/cm)	379	158.5
Total dissolved solids (mg/L)	179	78.5
Total suspended solids (mg/L)	73	42
Sulphate (mg/L)	16	6.5
Nitrite (mg/L)	0.13	0.15
Phosphate (mg/L)	1.4	1.1
Dissolved oxygen (mg/L)	12.16	3.75
Biological oxygen demand (mg/L)	11.28	3.36
Chemical oxygen demand (mg/L)	21.42	19.62

This study showed that mycofiltration, in addition to the well-known filtration method, may be used to filter pollutants and alter the organic and inorganic components of surface runoff wastewater. It indicates that fungus on a colonized substrate can capture pollutants and lower the waste water's degree of contamination. The shift in microbial growth and physiochemical parameter levels demonstrated that mycofiltration is an effective filtration technique.

The typical values of the surface runoff wastewater showed that it discharges significant loads of nutrients and organic matter into the environment.

Not only can fungi hyper-accumulate metals, but they can also capture and consume a wide range of species[11].

An experiment by Robles-Hernández *et al.*[12] shows that the white-rot fungus may change a variety of ambient organo-pollutants, including pesticides, polychlorinated biphenyls, polycyclic, aromatic hydrocarbons, wood preservatives, synthetic dyes, and waste from paper manufacture firms.

Human activities, particularly in rural regions, rely heavily on water bodies, as do aquatic species for development, eating, and reproduction. However, polluted surface water might have a deleterious influence on these activities. Clean water has a pH of 7 at ambient temperature, but it rises to 8.5 when exposed to air carbon dioxide. Following filtering, the pH drops to 6.7. The pH of water impacts pipelines used for piping and plumbing, as well as metals in aquifers and fixtures. Filtered water minimizes metal ion leaching, precipitate formation, and pipe corrosion. It also decreases hazardous metals in the water, making it safe for aquatic species to ingest and perform their natural functions.

The pH level is also an important indication in aquaculture and irrigation. It shows how basic and acidic the water is. Olubanjo and Alade[13] state that the ideal pH range for irrigation water is 5.5-7.5. The mycofiltration technique safely reduced the pH of runoff wastewater from 8.5-6.7, indicating that further examination could further decrease the pH for irrigation applications.

Conductivity is one of the main parameters used to determine the suitability of water for irrigation and firefighting[14]. It is used to monitor water quality and potential pollution. The electrical conductivity of surface runoff waste water decreased to less than half its initial value, from 379-158.5 µS/cm. The conductivity value before treatment suggested a significant level of contamination. This might be due to agricultural runoff or a sewage leak, which causes an increase in chloride, phosphate, and nitrate ions. These extra dissolved solids have a deleterious effect on water quality. When wastewater enters water bodies and alters their conductivity, it has a detrimental influence on aquatic life and water quality. The wastewater was treated by mycofiltration to lower the ions and conductivity of the metals, making it safe for aquatic life to ingest. It also helps them endure floods and weather-related stressors.

According to the current study, *Lentinus squarrosulus* is a powerful bioremediation agent due to its capacity to filter a wide range of contaminants from water, hence decreasing their harmfulness. Numerous studies have concluded that mycofilters are capable of eliminating contaminants based on their findings. Mycofilters made by *Lentinus squarrosulus* were successfully employed to filter impurities out of drinking water[14]. *Lentinus squarrosulus* exhibited the power to lower parameters like pH, conductivity, turbidity, TDS, and TSS to be below the recommended limits of the World Health Organization (WHO). As well-known bioremediation agents, white-rot fungi (WRF) can break down a wide range of contaminants.

The study of Ikechi-Nwogu and Akpan[15], in which the authors employed white-rot fungus species to reduce toxicity in dumpsite leachate supports this.

Mycofilters are effective in eliminating pollutants based on the findings of this study. The research was limited to the use of *Lentinus squarrosulus* in bioremediation runoff wastewater. To decrease microbiological and physiochemical contaminants, mycofiltration should be used to filter waste fluids entering water bodies, particularly those connected to them. Further research into the filtering properties of *Lentinus squarrosulus*, as well as public education about its use, should be conducted.

CONCLUSION

It is evident that *Lentinus squarrosulus* (Mont.) Singer has a high potential for utilization as a mycelium-permeated substrate in removing pollutants from surface runoff wastewater through the process of microfiltration. Wastewater obtained from surface runoff should not be allowed to run into the water bodies without proper filtration to remove the contaminants in them. Microfiltration can be used to filter these contaminants which are very detrimental to man, aquatic life, and the environment as a whole. The fungal-based filtration process, microfiltration, is new but has experienced a wide range of acceptance around the world and has similar filtering potential as any other filtration process. Therefore, microfiltration proves to be a highly efficient method of reducing the harm caused by contaminated surface runoff wastewater as well as improving the biological activities in water bodies.

SIGNIFICANCE STATEMENT

Surface runoff pollution threatens aquatic ecosystems, but this study shows that mycofiltration using *Lentinus squarrosulus* can effectively remove pollutants, providing a practical and affordable solution. This research contributes to environmental sustainability, improved public health, and conservation of aquatic ecosystems, highlighting the potential of mycofiltration as a valuable tool for mitigating surface runoff pollution.

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