Mycofiltration potential of *Lentinus squarrosulus* (Mont.) Singer on fish pond effluent in Onne, Rivers State, Nigeria

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Abstract

Fish pond effluent discharge into the environment is becoming a nuisance since it contains contaminants that harm the ecology and water quality. In this study, we examined the possibility of using a mushroom *Lentinus squarrosulus* Mont. in removing impurities from fish pond effluents before discharge into the environment. Untreated effluent was obtained from a fish farm in Eleme Local Government Area of Rivers State, Nigeria and tested for contamination levels using physicochemical and microbiological methods within 24 hours of collection. Sterilized sawdust was bagged, inoculated with mushroom spawn and given two weeks to colonization the substrate. The colonized substrate was prepared for mycofiltration after the third week. With a sterilized stainless-steel spoon, holes were drilled into the substrate's centre, and the untreated water sample was then poured into the holes and allowed to filter through into a sterile container. Filtrate samples were taken to a laboratory for evaluation. Statistical analysis of data obtained after 24–48 h of mycofiltration treatment of aquaculture effluents samples showed a substantial ($P \leq 0.05$) decrease in heterotrophic count from 6.7×10^3 to 0 and total fungal/yeast count from 5.3×10^3 to 0. The findings revealed that mycofiltration technique is a useful, efficient and affordable technology as it reduced dissolved oxygen from 7.92 to 2.88 mg L^{-1}, biological oxygen demand from 5.97 to 5.62 mg L^{-1} and chemical oxygen demand from 1.89 to 1.78 mg L^{-1}. Other parameters such as pH, turbidity, electrical conductivity, total dissolved solid, total suspended solid, sulphate, nitrite and phosphate, and algae count were reduced significantly at the end of the filtration process. There were notable variations ($P \leq 0.05$) between values obtained from fish pond effluents and that treated with *L. squarrosulus*, indicating that the mushroom played a promising role in the process of mycofiltration and also showed that mycofiltration is a healthier method for water quality improvement of fish effluent before discharge.

Keywords: Aquaculture effluents, Environmental contamination, *Lentinus squarrosulus*, Mycofiltration.

Introduction

Over the past ten years, aquaculture, often known as fish farming, has made incredible progress. It now accounts for more than one third of all fish consumed by humans (FAO, 2004) despite the fact that it produces a lot of garbage and has a negative impact on the environment (Pillay, 2004).

Environmental contamination arises from incorrect discharge of fish farm or aquaculture effluents into the environment, according to Omofunmi *et al.* (2016). There have been initiatives to investigate the effects of fish farming effluent discharges and utilize mycofiltration to ensure that the water quality released from aquaculture effluents causes no environmental harm. The high concentration of nutrients, organic matter and suspended solids in the water discharged from fish ponds, increases the demand for oxygen, eutrophication and high-water turbidity causing an unneeded algal bloom. Fish and other aquatic life in the river cannot use enough dissolved oxygen as a result of the decomposition and death of the algae. The toxins that algae release pose a threat to humans as well. The environment was poisoned, according to investigations by Omofunmi *et al.* (2016), as a result of wastes from a catfish (*Clarias gariepinus*) farm that were discharged into a river in southwest Nigeria. According to Amirkolaie *et al.* (2008), the buildup of these pollutants (solid and dissolved wastes) lowers water quality and can lead to an increase in fish illness incidence (Liltved and Cripps, 1999).

In several rural communities throughout the world, studies and research have shown that a mycelium-permeated substrate can successfully remove pollutants from contaminated drinking water sources (Stamets, 2005; Akpaja and Olorunfemi, 2014; Mnkandla and Otomo, 2021). Mycofiltration is the original method of employing fungi to filter out contaminants from water, according to Wandle (2014). Additionally, research by Akpaja and Olorunfemi (2014) shows the benefits, effectiveness and affordability of the mycofiltration method as a tool for toxicity reduction in drinking water sources for rural residents in developing countries. One of the few fungi that can digest lignin and allow fungi to break down pollutants is the white-rot fungus (Green, 2019).

An example of edible white rot saprophytic fungus with hard fruiting bodies is known *Lentinus squarrosulus*. It is abundant in minerals, vitamins, B and D, carbohydrates, lipids, amino acids, proteins and sugars (Ayodele *et al.*, 2007). For the effective breakdown of many types of contaminants, mycoremediation depends on the enzymes produced by these mushrooms (Kulshreshtha *et al.*, 2016). Therefore, the objective of the present research was to examine the possibilities of using selected mushroom *Lentinus squarrosulus* in removing pollutants from fish farm effluent before discharging into the environment.

Materials and Methods

Study site and collection of water samples

The fish pond effluent sample was collected in September, 2019 from a fish pond in the International Institute of Tropical Agriculture (IITA), located in Onne (Fig. 1) with GPS location of 4.723816, 7.151618, Rivers State, Nigeria. Water samples were collected using sterile plastic bottles, stored in the refrigerator at 4 °C. Analysis was done within 24 h of collection.

Method for mycofiltration

The mycofiltration method was done according to the method of Ikechi-Nwogu *et al.* (2020). Bagged sterilized sawdust was injected with mushroom spawn and given 1 to 2 weeks to colonize the substrate. After the third week, the colonized substrate was ready for mycofiltration. They were placed on a funnel and covered with nylon bags to prevent contamination. Using a sterile stainless-steel spoon, holes were drilled into the substrate's center, and the untreated water sample was poured into the holes. The untreated water sample was then given 24 to 48 h to filter through the substrate and into a sterile container. The collected filtrates were brought to a lab for evaluation.

Preparation of media

Powdered agar was appropriately weighed (39 g) and added to1000 mL of sterile distilled water, allowed to dissolve completely and autoclaved at a temperature of 121 ºC for 15 min. After sterilization, it was dispensed into sterile Petri dish and allowed to solidify within 30 min to one hour.

Method for microbial analysis

Surface Drop method as modified by Reshma 2022 was used for the analysis. Sterile dilution blanks (9 mL) were each marked 10^1, 10^2, …, 10^n (desired dilution). One milliliter of the sample using sterile pipette was taken and mixed with the first dilution blank measured. The contents were jiggled gently to obtain uniform distribution of cells. The procedure was repeated until the desired dilution 10^n was obtained. From selected dilutions, 0.02 mL of the suspension was transferred onto the nutrient agar plate. The inoculated agar plates were then Incubated at 35–37 ºC for 18–24 h.

Data analysis

Using SPSS version 15.0 for Windows 2007, quantitative data for pH and other physicochemical parameters were summarized as means ± standard errors, which were then put through Duncan multiple comparison and Dunetts tests in a one-way ANOVA.

Results and Discussion

Mycofiltration is a cutting-edge method that utilizes fungi as biofilters to break down and remove pollutants from soil and water. The waste materials from paper manufacturing plants, pesticides, wood preservatives, synthetic dyes, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and have all been altered by the white-rot fungi (Robles-Hernandez *et al.*, 2008). Based on research findings, it has been determined that mycofilters are capable of removing contaminants. The mycofilters made by the fungus *L. squarrosulus* in this study demonstrated some encouraging outcomes in the bio-remediation of fish pond effluents.

The fact that the fish pond effluent releases substantial loads of organic matter and nutrients into the environment was revealed by the usual values of the physico-chemical parameters of the untreated fish pond effluent. Following 24–48 h of mycofiltration treatment, a change in color and a reduction in pH, turbidity, electrical conductivity, total dissolved solids, total suspended solids, sulphate, nitrite, nitrate, phosphate, dissolved oxygen, biological oxygen demand, chemical oxygen demand, and ammonium-nitrogen were noted as shown in Table 1.

The removal of harmful compounds from wastewater and the remediation of polluted soils has both benefited from fungi's ability to break down a variety of different compounds and materials (Aek and Cajthaml, 2005). Fungi can trap and digest a variety of organisms in addition to their capacity for hyper-accumulating metals. (Stamets, 2005). Robles-Hernandez *et al.* (2008) in their experiment sated that the white-rot fungus has the ability to change a variety of environmental organo-pollutants, including pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, wood preservatives, synthetic dyes, and waste from paper manufacturing companies.

In this work, algae and bacteria from aquaculture effluents were successfully removed using mycofilters made by the fungus *L. Squarrosulus.* In irrigation and aquaculture, pH level is a crucial indicator. It displays the water's acidity and basicity. According to Olubanjo and Alade (2018), irrigation water should have a pH between 5.5 and 7.5. The mycofiltration process lowered the pH of the fish pond effluent from 6.65 to 6.2, allowing it to be used safely for irrigation in agriculture. This means further evaluation on fish pond water will reduce the pH some more, making it very safe for irrigation purposes.

Sulphate as well was significantly reduced in the mycofiltration process making it safe for livestock to ingest. According to Miao *et al.* (2012) the concentrations of sulphate in ingested water has the ability to cause diarrhea in livestock. Jalili *et al*. (2018) recommended that nitrate and nitrite level should be managed as high level of nitrate and nitrite have detrimental consequences to human health. The mycofiltration process likewise reduced the amount of nitrate and nitrite level. Also, mycofiltration has the potentials to reduce the amount of ammonia-nitrogen in fish pond effluent which is brought about by the remains of feed and the waste of fish. When the ammonia level is reduced, it can be used for fish hatchery as too much ammonia in water can lead to convulsion and sometimes, death of fishes.

Phosphorus is an important element in the water bodies that serves as a source of nutrient (Nomosatryo and Lukman, 2011). Discharging water with an excessive amount of phosphorus has a negative impact on the water's quality by generating an algal bloom, which makes it harder for fish to obtain sunlight (Green, 2019) and also depletes the level of oxygen in the water endangering aquatic life. Also, Bernard *et al.,* (2004) stated that pond water from fish farm supplied low amounts of nitrogen and phosphorus for crops, when used for irrigation. From the results, the presence of algae was greatly reduced by mycofiltration procedure (Table 2). Algae in water makes irrigation impossible as they consume nutrients intended for plants and form a barrier that prevents water from reaching the roots zone.

Algal toxins have the power to contaminate soil and groundwater. It can also have an effect on both human and animal health when swallowed, through skin contact (such as while handling irrigation equipment) or when polluted spray mist is inhaled.

A high exposure to algae increases a person's risk of acquiring skin issues as well as other health issues. Moreover, eating a plant that has been consumed after being irrigated with this contaminated water may result in gastroenteritis. Algal toxins may persist on the surface of plant parts for a long time and may potentially cause bodily harm (NSW Food Authority, 2013).

In this study, algal count was completely eliminated. Due to the limited lifespan of algae and the fact that as they die and begin to decompose, dissolved oxygen in the water is consumed, this makes the mycofiltration process effective. The fish could perish if there isn't enough dissolved oxygen in the water. Microbial examination of the effluent was similarly carried out and from the results, as shown in the table 3 below, there was a total elimination of microbes. Data obtained revealed a ($P \leq 0.05$) significant.

Coliform in irrigation water is risky when used in the production of leafy vegetables that will be eaten raw without cooking (Allende and Monaghan, 2015). The results in the table above, showed that the mycelia treatment was able to eliminate coliform/yeast count. *L. squarrosulus* significantly decreased the initial counts over the course of 24–48 h, proving that mycoremediation is a viable and promising method for natural pollutant remediation.

Conclusion

The choice of using fishpond effluent as the test sample, is as a result of the amount of pollution the improper discharge cause to the environment which can be utilized for other uses after mycofiltration. The findings from this study indicated that mycofiltration might be used as a low-cost, self-help method of bioremediating fishpond wastewater employing the fungus *L. squarrosulus* as a valuable tool. The mycofiltered effluent can be used in agriculture for irrigation purposes as it contains an amount of nitrogen and phosphorus which is advantageous to the soil. Advanced work should be done as it helps to reduce the amount of discharge of contaminant in the environment and also helps to enrich the soil.

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Table 1: Physico-chemical and microbial load of water treated through mycofiltration.

Parameters	Before	After
pH	6.65	6.2
Colour (Pt-Co Unit)	77.5	19
Turbidity (NTU)	40.5	13
Electrical conductivity (µS cm^{-1})	536.5	220.5
Total dissolved solid (mg L^{-1})	247.3	148
Total suspended solid (mg L^{-1})	27	7
Sulphate (mg L^{-1})	5.5	3
Nitrite (mg L^{-1})	0.125	0.1
Nitrate (mg L^{-1})	0.133	0.104
Phosphate (mg L^{-1})	1.06	0.68
Dissolved oxygen (mg L^{-1})	7.92	2.88
Biological oxygen demand (mg L^{-1})	7.92	2.88
Chemical oxygen demand (mg L^{-1})	5.62	5.97
Ammonium-nitrogen	1.91	1.79
Algae (cells L^{-1})		
Scenedesmus eichornis	14000	0
Scenedesmus quadricauda	7000	0
Closterium acerosum	7000	0
Cosmarium sp.	7000	0

Table 2: Algal count of fish pond effluents before and after mycofiltration.

Algae	Before (cfu mL^{-1})	After (cfu mL^{-1})
Scenedesmus eichornis	14000	0
Scenedesmus quadricauda	7000	0
Closterium acerosum	7000	0
Cosmarium sp.	7000	0

Table 3: Microbial analysis of aquaculture effluents showing the percentage effect of mycofiltration.

Description	Before mycofiltration (cfu mL^{-1})	After Mycofiltration (cfu mL^{-1})	Percentage change (%)	Significant difference
Heterotrophic count	6.7×10^3	0	100%	S
Total coliform count	8.0×10^3	8.0×10^2	10%	NS
Fecal count	0	0	0%	NS
Total fungal/yeast count	5.3×10^3	0	100%	S

S = significantly different from each other; **NS** = not significantly different from each other.

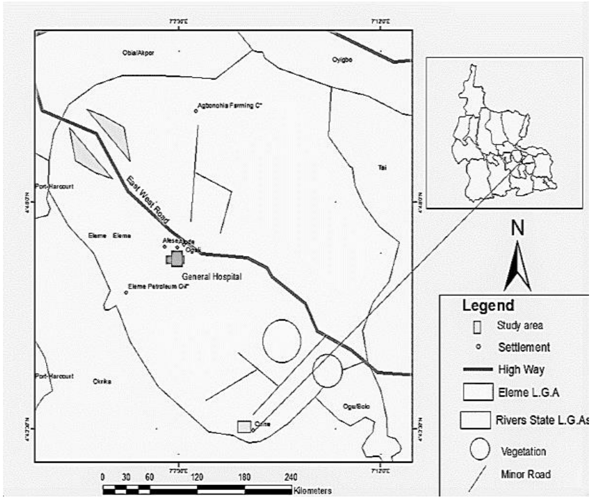

Fig. 1: Map of Eleme Local Government Area, Rivers State Nigeria and Environs showing location of sample collection.

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