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Mycoremediation Potential of Edible Mushroom (*Lentinus squarrosulus* Mont) for Reduction of Physicochemical Properties and Microbial Load of Landfill Leachate obtained from Choba Dumpsite, Rivers State, Nigeria

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ABSTRACT: Dumpsite leachate samples are characterized by high concentration of pollutants that pose several environmental health risks in both short and long term. This study was therefore undertaken to investigate the efficacy of mycoremediation capacity of edible mushroom (Lentinus squarrosulus Mont.) for reduction of physicochemical properties and microbial load of landfill leachate obtained from Choba dumpsite, Rivers State, Nigeria using mycofiltration technique and standard methods. Data obtained after a 24-48 hours mycofiltration treatment, revealed a significant ($p<0.05$) reduction in the concentration of pH from 7.4 to 7.7, Colour 227 to 108, Turbidity 152 to 50 (NTU), Electrical conductivity 415 to 201(µS/cm), Total Dissolved Solid 560 to 218 (mg L^{-1}), Total Suspended Solid 22 to 20 (mg L^{-1}), Nitrite 0.124 to 0.120, Nitrate 0.130 to 0.125, Phosphate 1.4 to 1.1, Dissolved Oxygen 8.64to 1.52 (mg/L^{-1}), Biological Oxygen Demand 7.2 to 1.52 (mg/L^{-1}), Chemical Oxygen Demand 4.96 to 1.32 (mg/L^{-1}) and microbes at the end of the filtration process. The findings from this study showed that mycofiltration technique could be a useful, efficient and affordable technology for toxicity reduction in dumpsite leachate.

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In the past decades, there has been a rapid increase in population due to development of countries. The increase in population, has put tremendous pressure on the environment by generating huge amount of solid waste (Khapre, 2015). In developing countries across the world, disposing of these solid wastes has been a global concern (Aderemi *et al.,* 2011) as managing them has been a little difficult owing to high urbanization rates, poverty, population growth, lack of funds from the governments (Doan, 1998), lack of waste management policies and poor urban planning. According to Aljaradin and Persson (2012), land filling is the simplest, cheapest and most cost-effective method of disposing of waste in developed and developing countries. Although these wastes disposed in landfills can pose several public and environmental health risks including groundwater pollution (Oyelami, 2013; Yan, 2015). When it rains the rain water penetrates through landfills producing leachate (Fluid from the landfill that percolates through the solid waste dumpsite). According to Salam and Nilza (2020), Landfill leachate contains pathogenic microorganisms, mostly coliform bacteria, organic (acids, alcohols, aldehydes and sugars, and the inorganic components such as calcium, magnesium, sulphate, chloride and ammonia) and inorganic

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pollutants (ammonium, phosphorous, sulphate and heavy metals such as; Fe, Pb, Ni, Cd, As, Cr, Cu and Hg). As reported by Golden and Inichinbia, (2020) that groundwater around landfill contaminated area contains highly conductive leachates like sulphur, methane and ammonia gas. It may also contain other toxic pollutants such as the aromatic hydrocarbons (Benzene, Toluene, Ethylbenzene, and Xylene), phenols, pesticides, polyethylene, plasticizers, and halogenated organic compounds like PCBs and dioxins. Landfill leachates cause serious environmental issues polluting the groundwater, soil and air (Salam and Nilza, 2020). Groundwater is the main source of drinkable water supply (Peiyue *et al.*, 2021) and its contamination cause environmental and health hazards. They can impact human health, environmental quality and socioeconomic development. High levels of these pollutants are a health risk for human and could have carcinogenic effects on them. Another example is high concentrations of nitrate in the drinking water, causing "blue baby syndrome" (Peiyue *et al.*, 2021). The United Nations acknowledged availability and sustainable management of water and sanitation for all as one of the seventeen Millennium Development Goals (MDGs). Its target by 2030, is to improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally. In line with this goal, it is necessary to remove pollutants from leachate. There are three conventional leachate treatments methods known: chemical, physical and biological treatments (Wiszniowski *et al.*, 2006). According to Dugan, biological treatment has the potential to remove problematic water quality constituents while minimizing the production of treatment residuals (Chawaga, 2016). Biological treatment according to Fulekar and Pandey (2012), is the use of biological means/ agents such as microorganisms (yeast, fungi or bacteria) and plants to degrade or detoxify substances hazardous to human health or the environment. Researches and studies have shown the effectiveness of a mycelium-permeated substrate (a biological treatment) in removing pollutants from contaminated water sources in some rural communities around the world (Stamets, 2005). This method is known as mycoremediation and Šašek and Cajthaml (2005) stated that mycoremediation, is a form of bioremediation and it is the application of fungi for example, Phanerochaete sp., Pleurotus sp., Trametes versicolor, Nematoloma frowardii, and Irpex lacteus in the remediation of polluted soils and aqueous effluents. Mycoremediation is an eco-friendly and cost-effective method of managing wastes (Azubuike *et al.*, 2016; Sharma and Bhattacharya, 2017). According to Wandle Trust (2014), Mycoremediation or mycofiltration is the pioneer technique of using fungi to filter out pollutants from water. Several mycoremediation studies indicate potentials of mushroom mycelium to treat polluted water (Nahid and Mannan, 2020). White-rot fungi a famous group of natural degraders are among the few fungi that can digest lignin and enable fungi to break down pollutants (Rhodes, 2014). An example is Lentinus *squarrosulus* Mont. an edible mushroom commonly found in the wild. It belongs to the division Basidiomycota, class Agaricomycetes, order Polyporales, and family Polyporaceae. This study was therefore undertaken to investigate the efficacy of mycoremediation capacity of edible mushroom (*Lentinus squarrosulus* Mont.) for reduction of physicochemical properties and microbial load of landfill leachate obtained from Choba dumpsite, Rivers State, Nigeria.

MATERIALS AND METHODS

Description of the Study Area/ Sampling of Leachate: A dumpsite leachate sample was collected on the 20th of September, 2019 for the study. The sample for the study was collected at Choba Dumpsite located at Choba extension close to Odums Junction, Port Harcourt, Rivers State (Fig. 1), stored at 4^{o}C and immediately transported to the lab same day.

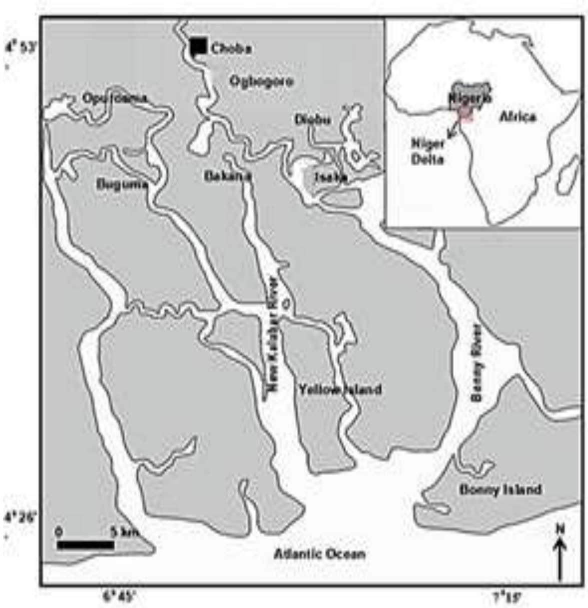

Fig 1: Map Showing Choba Environs the Location of Sample Collection

Physicochemical Analysis of Leachate before and after treatment: The samples were analyzed for relevant physicochemical parameters according to internationally accepted procedures of APHA, (1994).

Method for Mycofiltration: Sterilized sawdust was bagged, inoculated with the mushroom spawn and allowed for 1-2 weeks to colonize the substrate. After the third week, the colonized substrate (Fig. 1) was ready for mycofiltration.

Fig. 1: Substrate colonized by mushroom.

They were placed on a funnel and covered with nylon bags to prevent contamination. Holes were borne in the middle of the substrate (to allow the substrate hold the liquid) using a sterile stainless spoon and the dumpsite leachate sample was dispensed into the substrate and allowed for 24 to 48 hours to filter through into a sterile container. The collected filtrates were taken to the laboratory for analysis.

Preparation of Media: Powdered agar was appropriately weighed in grams into desired volume of sterile distilled water, allowed to dissolve completely and autoclaved at a temperature of 121 ºC for 15 minutes. After sterilization, it was dispensed into sterile Petri dish and allowed to solidify.

Microbial Analysis of leachate: To achieve this, Miles and Mishra enumeration or Surface Drop method, modified by Reshma 2022. 9ml sterile dilution blanks were each marked 10^1, 10^2, …. 10^n (desired dilution). 1ml of the sample using sterile pipette was taken and mixed with the first dilution blank measured as represented in Fig. 2. 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, 0.02ml of the suspension was transferred onto the nutrient agar plate. The inoculated agar plates were then Incubated at 35-37ºC for 18-24 hours.

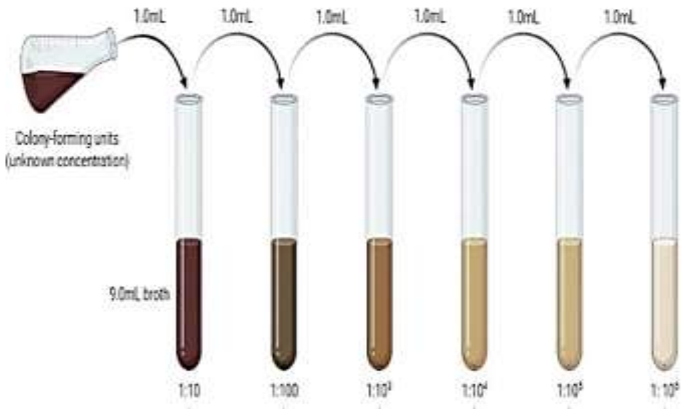

Fig 2: Serial dilution (Source: Bioender- https://biorender.com)

Data Analysis: Quantitative data for the physicochemical parameters were summarized as means ± standard errors, which were then subjected to Duncan multiple comparison and Dunetts tests in a one-way ANOVA, using SPSS version 15.0 for Windows 2007. Significant differences were set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Physicochemical Analysis of Leachate: The values of physicochemical characteristics of the dumpsite leachate sample represented in Table 1 above, revealed that leachate was polluted. The table 1 showed that after 24-48 hours of mycofiltration treatment of leachate samples, there was reduction in pH, Colour, Turbidity, Electrical conductivity, Total Dissolved Solid, Total Suspended Solid, Nitrite, Nitrate, Phosphate, Dissolved Oxygen, Biological Oxygen Demand, Chemical Oxygen Demand and total elimination of microbes. From the results of the microbial examination of the effluent, there was a total elimination of microbes. Data obtained revealed a ($p<0.05$) significant.

Lentinus squarrosulus is a promising bioremediation agent because of its ability to filter large variety of pollutants that can reach soil and ground water, thus reducing their toxicity as reported in this current study. Several researches have drawn conclusions from their results about mycofilters being capability of removing contaminates. In a study conducted by Ikechi-Nwogu *et al.* (2020), the mycofilters produced by *Lentinus squarrosulus,* was effectively used to remove contaminants from drinking water. *Lentinus squarusulus* demonstrated the capability to reduce parameters such as pH, conductivity, turbidity, TDS and TSS to be within the recommended limits of World Health Organization (WHO). White-rot fungi (WRF) as known bioremediation agents are capable of degrading a broad spectrum of pollutants.

Table 1: Effect of Mycofiltration Treatment of Dumpsite Leachate

Parameters	BEFORE	AFTER
pH	7.4	7.7
Colour (Pt-Co Unit)	227	108
Turbidity (NTU)	152	50
Electrical conductivity (µS/cm)	415	201
Total Dissolved Solid (mg/l)	560	218
Total Suspended Solid(mg/l)	22	20
Nitrite (mg/l)	0.124	0.120
Nitrate (mg/l)	0.130	0.125
Phosphate (mg/l)	1.4	1.1
Dissolved Oxygen, DO (mg/l)	8.64	1.52
Biological Oxygen Demand, BOD (mg/l)	7.2	1.52
Chemical Oxygen Demand, COD (mg/l)	4.96	1.32

Table 2: Microbial Analysis of dumpsite leachate showing the Percentage Effect of Mycofiltration

Description	Count before Mycofiltration	Count after Mycofiltration	Percentage change (%)	Significant difference
Heterotrophic count	5.9×10^5	0	100%	S
Total coliform count	6.0×10^3	0	100%	S
Fecal count	5.0×10^3	5.0×10^2	10%	NS
Total fungal/yeast count	6.3×10^5	6.3×10^5	100%	S

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

This is confirmed by the work of Jurado *et al.* (2011), where the authors used used white-rot fungi species to transform and detoxify soil pollutant under different environmental conditions? Several *Pleurotus* species such as *P. eryngii, P. ostreatus, P. pulmonarius,* and *P. sajor-caju*, similarly showed themselves to be highly effective at degrading aromatic pollutants like: 2,4-dichorophenol, benzo pyrene and chlorinated biphenyls in either submerged cultures or under solid-state fermentation (SSF) conditions. White-rot fungi in the same way, appeared to be a promising tool in endocrine-disrupting chemicals elimination during wastewater treatment processes (Grelska and Noszczyńska, 2020). Zahmatkesh *et al.* (2018), equally applied (WRF) in wastewater treatment under non-sterile conditions. Confirming the efficiency of WRF, others like the *P. spadiceum* (MUT 1585) strains is another powerful agent of bioremedation for coloured effluent from the treatment of landfill leachate. Similar research was carried out by Diaz *et al.* (2022) using WRF such as *P. chrysosporium* to treat a biologically and physically pre-treated landfill leachate. In another investigation by Ren and Yuan (2015), these famous group of natural degrading, white rot fungi, showed efficient removal of recalcitrant compounds.

Microbial Analysis of leachate: Microbial examination of the dumpsite leachate was equally carried out and from the outcomes, there was a total elimination of microbes (Table 2). Data obtained revealed a ($p<0.05$) significant.

Conclusion: This study revealed the effectiveness of mycofiltration in reducing the physicochemical properties and microbial loads of landfill leachate. It is expected that mycofiltration process will help to keep the environment healthy for humans and animals, as an eco-friendly process that will not threaten the environment or biodiversity.

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