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MYCOFILTRATION OF SOIL LEACHATE USING *Lentinus squarrosulus* FOR BIOREMEDIATION OF SOILS FROM AUTO-MOBILE MECHANIC LEACHATE SOILS OF IKOKU MARKET, PORT HARCOURT

[1*]Ikechi-Nwogu, C. G., [2]Chukwu S. B and [1]Eke, P. C.

[1]Department of Plant Science & Biotechnology, Faculty of Science, University of Port Harcourt, Port Harcourt Zip code 500001, Rivers State, Nigeria

[2]Department of Biology and Biotechnology, School of Science Laboratory Technology, University of Port Harcourt, Port Harcourt Zip code 500001, Rivers State, Nigeria

***** Corresponding author E-mail**:** chinyerum.nwogu@yahoo.com /chinyerum.ikechi-nwogu@uniport.edu.ng

Tel.: +2348032325098, Orcid ID: https://orcid.org/0000-0003-4622-2744

ABSTRACT

This study was undertaken to investigate the mycofiltration of soil leachate from automobile repairs and servicing workshop Ikoku, Port Harcourt using *Lentinus squarrosulus.* To ascertain the contamination levels of the soil, sterilized sawdust was bagged, inoculated with mushroom spawn and allowed for 2 weeks to colonize the substrate. Then water extracted from the soil leachate was dispensed into the hole borne in the middle of the substrate then allowed to filter through into a sterile container which was subjected for physicochemical and microbiological analysis within 24 hours. Statistical analysis of data obtained after a 24-48 hours mycofiltration treatment, revealed a significant ($P<0.05$) reduction in the concentration of pH, Colour, Turbidity, Electrical conductivity, Total Dissolved Solid, Total Suspended Solid, Nitrite, Nitrate, Phosphate and Chemical Oxygen Demand. Electrical conductivity (µs cm^{-1}) was reduced from 751 to 297, total dissolved solid (mg L^{-1}) from 3.40 to 1.42, total suspended solid (mg L^{-1}) from 2.81 to 1.07, nitrate (mg L^{-1}) from 0.187 to 0.127, phosphate (mg L^{-1}) from 1.04 to 0.72, turbidity level from 1028 to 401, biological oxygen demand (mg L^{-1}) from 4.16 to 0, dissolved oxygen (mg L^{-1}) from 401 to 0 and chemical oxygen demand (mg L^{-1}) from 16.25 to 13.87. *L. squarrosulus* also exhibited a remarkable reduction in total heterotrophic count and total coliform count, which made it a potential purifying agent. The findings from this study showed that mycofiltration technique is a useful, efficient and affordable technology for removing pollutants from the soil in the automobile repairs and servicing workshop.

Keywords: Biological Treatment, Toxicity, Soil Leachate, *Lentinus Squarrosulus*, Mycofiltration, Automobile.

INTRODUCTION

The United Nations acknowledged conservation and sustainable use of the ocean, seas and marine resources for sustainable development as one of the seventeen Millennium Development Goals (MDGs). Its target by 2030, is to prevent and reduce marine pollution of all kind from land-based activities. In line with this goal, it is necessary to remove pollutants from the soil in the automobile repairs and servicing workshop.

Population growth in the past few decades, has increased rapidly due to development and has generated huge number of wastes, mounting pressure on the environment. Automobile repairs and servicing workshops are one of the main daily contributors of huge number of hazardous wastes to the soil. According to Adesuyi *et al.* (2016), Adesuyi *et al.* (2018a) and Njoku *et al.* (2018), a number of harmful and toxic chemical compounds, originating from workshops, industrial and agricultural activities are being released into our soil, water and air environment constantly and many of them have effects on humans, plants and animal resources. The dangers of automobile repairs and servicing workshop activities have been a general concern all over the world as these wastes have been reported to be extremely dangerous to humans and the environment (Imevbore and Adeyemi, 1981). Presently efforts are made in most developed countries to create a sustainable environment by imposing strict environmental regulations for automobile repairs and servicing workshop (Ekong *et al.*, 2012).

In Nigeria, the law provided for managing waste is not followed by automobile repairs and servicing workshop owners and staff. People set up their workshops indiscriminately and disposal of hazardous wastes such as brake fluid, greases, spent oil, radiator coolant, soot, fuel, metal scraps, chemicals and other volatile compounds into the soil and water drains. These waste, persist in the soil because they are not degradable and from auto mechanic workshops, they find their way into the air, water, soil, lakes and streams (Adeniyi and Afolabi, 2002; Adesuyi *et al.*, 2015).

Trace of these pollutants within and around automobile mechanic workshops implies that according to Nwachukwu *et al.* (2010) water bodies (surface and groundwater) within and away from their vicinity may equally be polluted with trace metals due to continuous interactions between soil and water and high dispersion rate in the tropical rain forest belt). Heavy metals easily accumulate in the topsoil to toxic levels due to their persistence and eventually make their way to humans through the food chain, where they perturb biological processes (Adesuyi *et al.*, 2015). The rains carry non-degradable pollutants and wastes into the soil, groundwater and nearby streams (Maduka, 2004). Groundwater pollution (also called groundwater contamination) occurs when pollutants are released and make their way down into the groundwater. There are different types of groundwater pollutant such as arsenic and fluoride and they have been recognized by the World Health Organization (WHO) as the most serious inorganic contaminants in drinking-water on a worldwide basis (WHO, 2011).

Volatile Organic Compounds (VOCs) are a dangerous contaminant of groundwater. They are generally introduced to the environment through careless industrial practices examples include aromatic hydrocarbons such as BTEX compounds (benzene, toluene, ethylbenzene and xylenes), and chlorinated solvents including tetrachloroethylene (PCE), trichloroethylene (TCE), and vinyl chloride (VC) *(*Smith *et al.*, 2016). To avoid the potential environmental burden of waste disposal, waste minimization and recycling have received growing attention.

There are number of methods of landfilling leachate treatment such as chemical precipitation, membrane filtration, aerobic and anaerobic biological treatment but they do not reach the desired level of results due to complex composition of leachate. Chemicals have been deployed in water treatment but in a green-conscious world, there have been growing concerns on the use of these chemicals. It is better to use natural process that will cause no harm biological treatment is that natural process that will cause no harm (Chawaga, 2016). For this reason, it is very important to seek new methods for leachate treatment such as mycoremediation used in the case study of this work.

Bioremediation is an eco-friendly and cost-effective method of managing wastes (Azubuike *et al.,* 2016; Sharma and Bhattacharya, 2017). According to Fulekar and Pandey (2012), it 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. 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, involves mixing fungi mycelium into contaminated soil, aqueous effluents, placing mycelium mats over toxic sites, or a combination of these techniques, in one time or successive treatments (Stamets, 2005). Mycoremediation is similar to mycofiltration. Mycofiltration is simply the use of mycelial mats to sieve toxic waste and microorganisms from polluted water. Stamets (2005) applied mycofiltration membranes to filter pathogens including protozoa, silt, chemical toxins, bacteria and viruses.

According to Wandle Trust (2014), 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). However, the potential of mycelium for the treatment of water is supported by an established evidence base on mycoremediation, the use of fungi to clean polluted land. Scientists in the 1980s have observed how fungi break down and remove persistent pollutants such as petrochemicals, heavy metals and pesticides from the environment (Green, 2018).

White-rot fungi are among the few fungi that can digest lignin and enable fungi to break down pollutants (Rhodes, 2014). An example is *L. 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 is aimed at removing pollutants from the soil in the automobile repairs and servicing workshop using *Lentinus squarrosulus.*

MATERIAL AND METHODS

The soil sample for the study was collected on the 9th of September, 2019 at the Ikoku Automobile Market in Diobu, Port Harcourt, Rivers State (Fig. 1). To obtain auto-mobile mechanic leachate, soil extraction was performed according to the modified method of DSMZ, (2007).

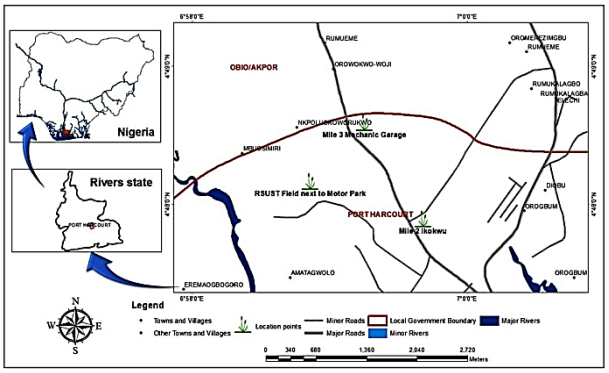

Figure 1: Map of Port Harcourt City Local Government Area, Rivers State, Nigeria Showing Location of Sample Collection

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 (Fig. 2). Holes were bored in the middle of the substrate to enable it hold the liquid using a

sterile stainless spoon and the auto-mobile mechanic 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.

Fig. 2: Mycofiltration using spawn

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.

Data Analysis

Quantitative data for pH and other 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

The values of physioco-chemical characteristics of the auto-mobile mechanic leachate sample revealed that the auto-mobile mechanic leachate was contaminated to varying degrees (Table 1).

Table 1: Effect of mycofiltration treatment of auto-mobile mechanic leachate

Parameters	Before	After
pH	8.4	6.6
Colour (pt-Co unit)	1304	726
Turbidity (NTU)	1028	401
Electrical Conductivity (µS/cm)	751	297
Total Dissolve Solid (m/l)	3.40	1.42
Total Suspended Solid (m/L)	2.81	1.07
Nitrite (m/L)	0.187	0.127
Nitrate (m/l)	0.194	0.133
Phosphate (m/l)	1.04	0.72
Dissolve Oxygen(m/l)	401	0
Biological Oxygen Dissolve (m/l)	4.16	0
Chemical Oxygen Dissolve (m/l)	16.25	13.87

After 24–48 hours of mycofiltration treatment of water samples, there was change in colour and reduction in pH, turbidity, electrical conductivity, total dissolved solid, total suspended solid, sulphate, nitrite, nitrate, phosphate, dissolved oxygen, biological oxygen demand, chemical oxygen demand and ammonium-nitrogen.

During the rainy seasons, the rains fall and wash these contaminants from the soils into groundwater threatening the ecosystem and human health if not decontaminated. In this study, the mycofilters produced by the fungus Lentinus squarrosulus was effectively used to remove soil leachate from automobile repairs and servicing workshop. The permissible limits of these parameters in Nigeria, was used as reference. According to NESGQCR (2011), the permissible limits of pH of surface and groundwater is between 6.5-8.5. The level of the soil pH could be as a result of the various activities carried out in the workshops. All these activities involve the use of one chemical or the order that affect the pH composition of soil (Adelekan and Abegunde, 2010). This in turn, affects the pH of groundwater over a period of time rendering it acidic and unsafe for domestic purposes. L. squarrosulus was effectively used to reduce the pH to a permissible level.

Electrical Conductivity was measured in microsiemens per centimeter (µS/cm) to be 751 and was reduced to 297, which is almost at the permissible level of between 0 to 200 µS/cm. The results of the Turbidity are reported in units called Nephelometric Turbidity Units (NTU). According to DataStream (2022), high turbidity will decrease underwater light availability thereby causing a negative impact on aquatic life.

When these contaminants from the soils with high Total suspended solids (TSS) values are washed into groundwater, the water becomes foggy making it difficult sun to travel through the water. When this occurs, plants and algae have difficulty growing. Aside this, the gills of fishes are clogged and eggs buried when TSS is high. TSS was reduced from 2.81 to 1.07mg/L although not up to the 0.75 mg/L permissible limits recorded by NESGQCR (2011) but BOD, is within the permissible limits of NESGQCR.

The results of the microbial examination of soil leachate sample after mycofiltration, revealed total elimination of microbes (table 2).

Table 2: Microbial Analysis of Soil Leachate Showing the Percentage Effect of Mycofiltration

Description	Count before Mycofiltration	Count after Mycofiltration	Percentage change (%)	Significant difference
Heterotrophic count	1.5×10^5	1.3×10^4	0.123%	NS
Total coliform count	9.3×10^3	0	100%	S
Fecal count	5.3×10^3	0	100%	S

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

The white-rot fungi have been mostly used to transform many environmental organo-pollutants, including pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, wood preservatives, synthetic dyes and

waste materials from paper producing plants (Robles-Hernandez *et al*., 2008).

Abioye (2011) specified that it is mandatory to remediate hydrocarbon polluted soil in order to have a harmless and healthy environment which will in turn result to a healthy lifestyle across the globe. Biological remediation of hydrocarbon and metal polluted soil is a better and environmentally friend procedure.

According to Šašek and Cajthaml, (2005), fungi have the capability to degrade sundry compounds and materials has been utilized in the remediation of polluted soils. Stamets, (2005), likewise stated that fungi can hyperaccumulate metals and also have the ability to trap and digest many other organisms.

In this study, the mycofilters produced by *Lentinus squarrosulus* was effectively used to remove contaminants and microbes from auto-mobile mechanic leachate.

The use of white rot fungi for decontamination of auto-mobile mechanic leachate polluted sites is not new and aforementioned researches have shown that this group of fungi hold a lot of potential in this regard. Ikechi-Nwogu *et al*. (2020) in their research, also specified the ability of *L. squarrosulus* to control and reduce water-borne bacterial diseases. Also, the findings from studies of Akpaja and Olorunfemi (2014), shows that mycofiltration technique is a useful, efficient and affordable technology for toxicity reduction.

In agreement with this study, other white rot fungi like *Lentinus squarrosulus, Pleurotus tuber-regium, Pleurotus pulmonarius, Pleurotus florida* have been successfully cultivated or grown in petroleum hydrocarbon contaminated sites or media with noticeable decontamination (Ogbo & Okhuoya (2008); Ogbo *et al.* (2010); Adenipekun *et al.* (2013) and Igbojionu *et al.* (2013)).

CONCLUSION AND RECOMMENDATION

This study has pointed out that Lentinus squarrosulus substrate applied as remediating substance, has the capacity of reducing colour, pH, turbidity, electrical conductivity, total dissolved solid, total suspended solid, sulphate, nitrite, nitrate, phosphate, dissolved oxygen, biological oxygen demand, chemical oxygen demand and ammonium-nitrogen thereby balancing the ecosystem. The reduction of auto-mobile mechanic leachate by this fungus indicates that the fungus is a promising mycoremediation agent and should be properly harnessed for that purpose. Future research and developments should focus on establishing in-site removal of leachate from auto-mobile mechanic workshops.

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