Mycofiltration of urban derived raw stormwater using *Lentinus squarrosulus*

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Abstract

The physicochemical and microbial attributes of storm water samples prior to and after mycofiltration was determined using routine methods. The preparation of the substrate was done by supplementation of un-fermented sawdust with calcium carbonate, calcium sulphate, granulated sugar and wheat offal. The mixture was allowed to undergo composting for 7 days upon which, it was inoculated with *Lentinus squarrolsulus* spawn and incubated at room temperature. The sample was then passed through a network of mycelia for pollutant removal. The mean TDS and EC concentrations of the raw and mycofiltered samples was 1369.8 ± 4.5 and 516.4 ± 2.9 mg/l as well as 2785.0 ± 4.2 and 1251.0 ± 5.6 µS/cm. Pb, Cu, Cd, Cr and Fe readings for the raw samples were; 0.03±0.03mg/l, 0.07±0.03 mg/l, 0.01±0.01mg/l, 0.03±0.01mg/l and 1.00±0.11mg/l. For the mycofiltered samples, the Pb, Cd and Cr were reduced to nil while Cu and Fe decreased to 0.03 ± 0.03 mg/l and 0.47± 0.09 mg/l. The difference between the mean trace metal values recorded for the raw and filtered samples was insignificant (*p>0.05*). The results indicated that mycofilter derived from *L. squarrolsulus* mycelia was capable of purifying storm water sample.

Keywords: Lentinus squarrosulus, Mycofilters, Mycofiltration, Mycelia, Storm water, Substrate

INTRODUCTION

Bioremediation processes that utilize fungi and their extracellular enzymes for the breakdown of pollutants and reduction/removal of hazardous materials from environmental media have been described (Kulshreshtha *et al.*, 2010). However, despite the immense benefits which accrue from using fungi in remediation, little attention has been paid to it especially in comparison with techniques which utilize prokaryotic organisms such as bacteria (Pinedo-Rivilla *et al.*, 2009). Mycofilters have been described as eco-friendly solutions which find applications in handling the challenges encountered in the management of urban storm water, natural floods and the treatment of wastewater from industrial sources (Mnkandla and Otomo, 2021).

Storm water is sourced from high precipitation and can evaporate, form puddles and small ponds on the soil surface, infiltrate the soil or become a part of surface runoff which is then carried into water bodies through various forms of drainage (Stalter, 2018). Urban storm water is known to harbour a wide range of pollutants which may include sediments, faecal matter and microorganisms, pesticides, fertilisers and other agrochemicals, oils, grease and heavy metals (Taylor *et al.*, 2015). The addition of excessive nutrients from storm water could create dead zones in receiving water bodies (Stalter, 2018). As such, several Best Management Practices (BMPs) have been proposed for the treatment of pathogens present in these wastewater sources (Clary *et al.*, 2008; Taylor *et al.,* 2015). Mycofiltration is a filtration method which utilizes fungal species in the removal of water borne contaminants by putting it *via* a mycelial network with a high surface area (Stamets, 2005). There are a number of specific mechanisms which are made available for the removal of bacteria from wastewater by mycofiltration and these have made the process to attract attention as a potential BMP for the reduction of wastewater related pathogenic microorganisms. Fungi have shown the ability to tolerate adverse environmental conditions such as elevated trace metal concentrations, which permit them to take up and remove contaminants from wastewater through various mechanisms including chelation and biosorption (Singh *et al.,* 2011).

Mushrooms are known to naturally thrive on waste materials such as straws from rice and wheat (Akpaja and Olorunfemi, 2014). The edible mushroom, *L. squarrosulus* is predominantly found growing in the wild on decaying logs of trees in the wet season (De Leon *et al.*, 2017). Identical to other macrofungal species, *L. squarrosulus* can proliferate on a diverse range of substrates and habitats. Several *Lentinus* spp. have been documented to grow naturally on special substrates and can be cultured on pasteurized substrates (De Leon *et al.*, 2017). The efficacy of filtration of different water samples such as fish pond effluent and raw drinking water using *L. squarrosulus* based mycofilter has been reported by several authors (Ikechi-Nwogu *et al.*, 2020; Ikechi-Nwogu *et al.*, 2022). Normally, a mycofilter is known to comprise of a burlap sack stacked with substrate which include; straw or woodchips and saprophytic mycelium (Mnkandla and Otomo, 2021). Within the sack, the mycelium is known to proliferate in the form of filament-oriented network, prior to being placed in the water bodies for remediation purposes (Mnkandla and Otomo, 2021).

This preliminary study was carried out to ascertain the possible effectiveness of mycofiltration using *L. squarrolsulus* as a method of reducing the organic and inorganic pollutant load associated with raw urban storm water.

MATERIALS AND METHODS

Sample c

ollection

The urban storm water sample used in the study was sourced in August 2022 from Isihior quarters located at the outskirts of Benin city, Edo State. The sample was rapidly poured into a prepared sampling bottle and transported to the laboratory for analysis. An already existing pure spawning culture of *L. squarrolsulus* applied for the water filtration was supplied by Prof. E. O. Akpaja of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin.

Mycofiltration procedure

The mycofilter utilized in this study was a modification of a *L. squarrosulus* based filter earlier described by Ikechi-Nwogu *et al.* (2022). A quantity of sawdust was obtained from a local sawmill and further milled into dust-like particles. These were dried in the sun to ensure moisture reduction and obtain a constant weight. The substrate used contained the following components: un-fermented sawdust (77% w/v), calcium carbonate (1% w/v), calcium sulphate (1% w/v), granulated sugar (1% w/v) as well as wheat offal (20% w/v). The addition of water was conducted and the water additive was properly mixed with these nutritional components. Tarpaulin was used to cover the mixture which was then allowed to undergo composting for seven days. At 48-hour intervals, the mixture was turned making a total of three times during the composting period, to ensure homogeneity of the fermentation process. Upon completion, the substrate was placed into 15 x 30cm bags of 2kg weights and subjected to pasteurization using steam for 4 hours to decrease any contaminants present, then it was cooled

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in an aseptic room. Upon cooling of the substrate, a viable inoculum of *L. squarrosulus* was centrally placed and incubated at ambient temperature (20 ± 2°C) until the substrate was totally colonised by the fungal mycelia. This substrate was then placed in a perforated bowl with a hole made in the centre and the storm water samples obtained were passed through the substrate.

Physico-chemical tests

A HANNA™ pH meter was used to determine pH of the samples. Total dissolved solids (TDS) and electrical conductivity (EC) were ascertained with the aid of a HACH™ TDS/Conductivity meter. The values for turbidity, colour, total suspended solids (TSS), nitrate, phosphate and sulphate were determined through spectrophotometry as described by Dirisu *et al.* (2016). Dissolved oxygen (DO) and biochemical oxygen demand (BOD) were determined using modified Winkler titration procedure as detailed by Ademoroti (1996). The COD content of the samples was determined using HACH™ COD reactor. Atomic Absorption Spectrophotometry (AAS) (Buck Scientific model 210 VGP USA) was utilized in the determination of the heavy metal concentrations of both the raw and treated samples. All analyses were done in duplicates and the mean results recorded.

Microbiological analyses

Serial dilution and the pour plate procedure as described by Cappuccino and Welsh (2020) was utilized in determining the total heterotrophic bacterial and fungal counts of both the raw and filtered waste water samples. Commercially available culture media such as Nutrient agar (NA), Peptone water and Potato Dextrose agar (PDA) were utilized and incubation of the seeded labeled NA and PDA plates was done at 35 °C for 48 hours and room temperature for five days respectively. The resultant discrete colonies were manually enumerated and the cfu/ml values were derived using a formula provided by Cappuccino and Welsh (2020).

Statistical analysis

The physico-chemical data obtained from the duplicate raw and mycofiltered samples were expressed in mean and standard deviation with the aid of Microsoft excel 2016 version. The mean trace metal values were subjected to Mann Whitney non-parametric T test to ascertain if the difference between the mean trace metal content of filtered and raw storm water samples was statistically significant or insignificant (α = 0.05).

RESULTS

The physico-chemical assessment of the raw and mycofiltered storm water were shown in Table 1. The mean pH of the raw and filtered samples was 7.5 ± 0.07 and 6.7± 0.02. The mean TDS and EC concentrations of the raw and mycofiltered samples was 1369.8 ± 4.5 and 516.4 ± 2.9 mg/l as well as 2785.0 ± 4.2 and 1251.0 ± 5.6 µS/cm (Table 1). The mean TSS and colour readings for both raw and filtered samples was 1822.5 ± 1.3 and 344.4 ± 5.4 mg/l as well as 5333.5 ± 3.5 and 1272.5 ± 3.5 PtCo units. The mean turbidity and phosphate concentrations of the raw and mycofiltered samples was 3863.0 ± 3.5 and 886.5± 0.7 FTU as well as 1.5± 0.06 and 0.7 ± 0.06 mg/l. The mean nitrate and sulphate readings of the raw and mycofiltered samples was 0.03 ± 0.01 and 0.5 ± 0.2 mg/l as well as 5.0± 0.7 and 8.2 ± 0.2 mg/l. The mean DO and BOD readings of the raw and mycofiltered samples was 8.4± 0.05 mg/l and 9.5 ± 0.4 as well as 5.2 ± 0.05 and 2.6 ± 0.04 mg/l. The mean COD of the unfiltered and mycofiltered samples was 2.4 ± 0.01 and 1.7± 0.09 mg/l. Table 2 revealed the mean heterotrophic bacterial counts (THBC) and total fungal counts (TFC) for the samples prior to and after mycofiltration. The mean THBC data for the raw and filtered samples was $5.1 \times 10^6 \pm 4.2$ and $0.7 \times 10^6 \pm 1.4$ CFU/ml while TFC was nil prior to and after mycofiltration of the sample.

Table 1: Physico-chemical parameters of the raw and mycofiltered storm water samples

Parameter	Raw sample	Filtered sample
pH	7.5 ± 0.07	6.7 ± 0.02
TDS (mg/l)	1369.8 ± 4.5	516.4 ± 2.9
EC (µS/cm)	2785.0 ± 4.2	1251.0 ± 5.6
TSS (mg/l)	1822.5 ± 1.3	344.4 ± 5.4
Colour (PtCo Units)	5333.5 ± 3.5	1272.5 ± 3.5
Turbidity (FTU)	3863.0 ± 3.5	886.5± 0.7
Phosphate (mg/l)	1.5± 0.06	0.7 ± 0.06
Nitrate (mg/l)	0.03 ± 0.01	0.5 ± 0.2
Sulphate (mg/l)	5.0± 0.7	8.2 ± 0.2
DO (mg/l)	8.4± 0.05	9.5 ± 0.4
BOD (mg/l)	5.2 ± 0.05	2.6 ± 0.04
COD (mg/l)	2.4± 0.01	1.7± 0.09

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Table 2: Microbial counts of the raw and mycofiltered storm water samples

Parameter	Total count (cfu/ml)	
	Raw sample ($\times 10^6$)	Filtered sample ($\times 10^6$)
THBC	5.1 ± 4.2	0.7± 1.4
TFC	0.0 ± 0.0	0.0± 0.0

Keys: THBC - Total heterotrophic bacterial count; TFC - Total fungal count. The heavy metal result for analyses prior to and after treatment is shown in Table 3. The concentrations of the trace metals in raw samples were low prior to filtration. Prior to treatment, the amounts of Pb, Cu, Cd, Cr and Fe were; 0.03 ± 0.03mg/l, 0.07± 0.03 mg/l, 0.01± 0.01mg/l, 0.03 ± 0.01mg/l and 1.00±0.11mg/l. After treatment, the levels of Pb, Cd and Cr were reduced to nil while Cu and Fe decreased to 0.03 ± 0.03 mg/l and 0.47 ± 0.09 mg/l respectively. The difference between the mean trace metal values recorded for the raw and filtered samples was insignificant ($p>0.05$).

Table 3: Mean trace metal values for raw and mycofiltered samples

Sample	Pb	Cu	Cd	Cr	Fe
Raw	0.03±0.03	0.07±0.03	0.01±0.01	0.03±0.01	1.00±0.11
Filtered	0.00±0.00	0.03±0.03	0.00±0.00	0.00±0.00	0.47±0.09
P value (Exact Sig.)	0.222	0.222	0.222	0.222	0.222

DISCUSSION

There were reductions in the mean pH, TDS, TSS, COD, BOD, turbidity, colour, TSS, TDS, and EC values recorded for the filtered samples in comparison with the raw sample. These phenomena could be directly attributed to the interactions between the raw storm water and the mycofilter comprising of *L. squarrosulus* mycelia and substrate which served as an anchorage for the fungal mycelia. The reduction of pH of water by mycofiltration has been observed by earlier researches (Okuo *et al.,* 2009; Akpaja and Olorunfemi, 2014). Also, reductions in several parameters such as; pH, COD, BOD and TDS values after mycofiltration was also reported by Shekhar *et al.* (2017). The reduction of pH and electrical conductivity of the storm water upon treatment can be attributed to the fungal ability to scavenge ions and radicals from environmental media (Paria and Chakraborty, 2022). Additionally, the reduction of COD and BOD may be due to the decomposition of organic matter initially present in storm water by the activity of the secretions of *L. squarrosulus* (Kunzler, 2018). The DO, nitrate and sulphate values were comparatively higher for the mycofiltered storm water sample. This trend was in tandem with a report by Bhatnagar *et al.* (2021) which documented an increment in DO from 2.4mg/l to 4.9mg/l. The increased nitrate and sulphate levels observed in the treated sample could be attributed to the metabolic (degradative) interactions between the mycofilter and the organic matter content of the raw storm water which culminated in the elevated levels of these dissolved nutrients in the treated sample.

The results of the microbial analysis of the treated storm water sample indicated that there was a reduction in the THBC for the mycofiltered sample. Taylor *et al.* (2015)

reported a decrease in *E. coli* counts in storm water by 20% upon treatment by mycofiltration. Similar reductions in THBC have also been reported by Akpaja and Olorunfemi (2014). The reduction of microbial population by fungi has been reported to occur through a chemical defence mechanism which is affected through the secretion of a toxin/enzyme such as a protein, peptide or a secondary metabolite, to inhibit the proliferation of the prokaryotes and thereby decrease competition for nutrients (Kunzler, 2018).

The amounts of all the trace metals (Pb, Cd, Cr, Cu, Fe) decreased in the mycofiltered storm water sample. The reduction in the trace metal concentrations *via* mycofiltration are identical to those recorded in previous studies (Akpaja and Olorunfemi, 2014; Bhatnagar *et al.,* 2021). The reduction in the concentration of the trace metals in the mycofiltered sample can be attributed to the ability of fungal species to scavenge and accumulate metals (biosorption) as reported by several researchers (Cordero and Casadevall, 2017; Maurya *et al.,* 2019). This trend has been attributed to the chelation of these metals by the hydroxyl groups present in fungal cell walls which comprise of β-glucans and chitin to which these metals become absorbed (Salazar-Ramirez *et al.,* 2020). This mechanism has been applied in the removal of Cd and Se using *Pleurotus tuberregium* (Okuo *et al.,* 2008) and Pb using *Pleurotus florida* (Prasad *et al.,* 2013) from wastewater streams.

CONCLUSION

This study revealed the efficacy of *L. squarrosulus* derived mycofilter in improving selected physico-chemical and microbial qualities of raw urban storm water. The results obtained showed marked reductions

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in the pH, BOD, COD, phosphate, TSS, TDS, EC and colour parameters. The mean heterotrophic bacterial counts also decreased, as did the concentrations of selected heavy metals after treatment *via* mycofiltration. Based on the observed trends, mycofiltration can be suggested as a bioremediatory approach for the reduction of contaminant concentrations in storm water streams or receptacles. Although in this study, the mycofilter was applied to a sole raw sample which was a limitation, the results reported would suggest the potential of developing and commercializing *L. squarrosulus* derived mycofilters that can be applied in the treatment of different wastewater streams for eventual re-use of the treated water for different anthropogenic purposes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR'S CONTRIBUTION

AD conceptualized the research and conducted the experiments. NOO and OO were also involved in the experimental studies and developed the primary draft of the manuscript. All the authors reviewed and corrected the final manuscript draft prior to submission.

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