Purification of untreated drinking water using *Lentinus squarrosulus*

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Abstract

A large percentage of sicknesses and diseases in the world are caused by polluted water. This study examined the water purifying property of mushroom *Lentinus squarrosulus* Mont. Untreated water samples were obtained from a bottling water factory in Rivers State, Nigeria and subjected to physicochemical and microbiological analysis within 24 hours of collection to ascertain the contamination level. Sterilized sawdust was bagged, inoculated with mushroom spawn and allowed for 2 weeks to colonization the substrate. After the third week, the colonized substrates were ready for mycofiltration. Holes were bore in the middle of the substrate using sterilized stainless spoon and the untreated water sample was dispensed into the holes and allowed to filter through into a sterile container. Collected filtrates were taken to the laboratory for analysis. From the results obtained, the filtrate with *L. squarrosulus,* reduced the electrical conductivity (µs cm^{-1}) from 804.5 to 225.5, total dissolved solid (mg L^{-1}) from 378 to 114, total suspended solid (mg L^{-1}) from 7 to 2, nitrate (mg L^{-1}) from 0.1955 to 0.116, phosphate (mg L^{-1}) from 0.945 to 0.49, turbidity level from 10 to 4, biological oxygen demand (mg L^{-1}) from 6.72 to 5.12, dissolved oxygen (mg L^{-1}) from 7.36 to 5.12 and chemical oxygen demand (mg L^{-1}) from 6.29 to 6.08. *L. squarrosulus* exhibited a remarkable reduction in turbidity, total heterotrphic count and total coliform count, which made it a potential water purifying agent. The ability of *L. squarrosulus* to control and reduce water-borne bacterial diseases is beneficial to those who cannot afford or have access to clean drinking water in developing countries.

Keywords: Biological treatment, Impurities, *Lentinus squarrosulus,* Mycofiltration, Water-borne diseases.

Introduction

Water is important for life and should be adequatelysupplied to all. Although Nigeria is blessed with abundant water resources, access to safe drinking water is a problem. As a result of this problem, citizens depend on unsafe water that has direct effect on their health. The World Health Organization estimated that up to 80% of all sicknesses and diseases in the world are caused by inadequate sanitation, polluted water and unavailability of water (WHO, 1997). The United Nations acknowledged improving water quality as one of the eight Millennium Development Goals (MDGs). Its target by 2015, was to reduce the number of people without access to safe drinking water to 50% (WHO, 2011).

In line with this goal, it is necessary to purify water to make it fit for human consumption. Water treatment removes or reduces contaminants so that the water becomes fit for use. 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 than ingesting water dosed with inorganic compounds. Biological treatment is that natural process that will cause no harm (Chawaga, 2016). According to Dugan, biological treatment has the potential to remove problematic water quality constituents while minimizing the production of treatment residuals (Chawaga, 2016). It also has the ability to treat a broad range of contaminants and increasing water stability within the distribution system.

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*, *Nematolomafrowardii*, and *Irpexlacteus* 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 simplythe 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.In the southern part of Nigeria, fruiting occurs at the beginning and end of the rainy seasons (Ayodele *et al.,* 2007). This study explored the potentials of *Lentinus squarrosulus*(Mont.)in reducing high levels of microbial constituent,organic matter, nutrients and solids in untreated drinking water.

Untreated drinking water sample was collected from a bottling water company in Rivers State. Sterile colonized mushroom substrate, sterilized funnel, sterilized plastic container, nylon bag, disinfectant (methylated spirit), cotton wool, marker, masking tape, sterilized stainless spoon, petri dishes, pasteur pipette with a rubber teat, ¼ ringer solution, agar medium (NA, MCA, PDA, EMB), micropipette with plastic teat, universal bottles, autoclave, hot air oven, incubator, disinfectant, cotton wool, HANNAH field pH meter, HACH DR 2000 spectrophotometer, HACH TDS/conductivity meter (Model No. C0150), HACH Digital Titrator (Model 16900).

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. 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 using a sterile stainless spoon and the untreated water 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.

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 average values of physioco-chemical characteristics of the untreated water sample revealed that the water sources were contaminated to varying degrees (Table 1). After 24–48 h 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, ammonium-nitrogen (Table 1).

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).

Today, the use of biological methods in the treatment of drinking water is moderately a new concept. Biological treatment techniques have been a backbone in water treatment for generations. During this process, solids are separated out while dissolved organics and other compounds are consumed by the biological treatment techniques. Conventional water treatment technologies, such as coagulation /sedimentation and lime softening, involve chemical addition that results in the removal of target contaminants as they are consumed by the biomass (Bell-Games, 2015).

Conclusion

In this study, the mycofilters produced by the fungus *Lentinus squarrosulus,* was effectively used to remove contaminants from drinking water. *Lentinus squarusulus* demonstrated the capacity to reduce bacteria count as well as the physicochemical properties of water. The values of water quality parameters such as pH, conductivity, turbidity, TDS, and TSS from the water sample collected were found to be within the recommended limits of WHO.

The fungus *L. squarosulu s*played a promising role in the process of mycofiltration. The process of mycofiltration is environmentally friendly and cost effective. Based on the results obtained after filtration, it was resolved that all physico–chemical parameters in the drinking water sample, were consistent with World Health Organization standard for drinking water (WHO).

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Fig. 1: Substrate colonized by mushroom.

Fig. 2: Mycofiltration usingspawn.

Table 1: Effect of mycofiltration treatment of drinking water.

Parameters	Before	After
PH	5.65	5.4
Color	17.5	5.5
Turbidity	10	4
Electrical Conductivity (µs cm^{-1})	804.5	225.5
Total Dissolved Solid (mg L^{-1})	378	114
Total Suspended Solid	7	2
Dissolved Oxygen	7.36	5.12
Biological Oxygen Demand	6.72	5.12
Chemical Oxygen Demand	6.29	6.08
Phosphate	0.945	0.49
Nitrite	0.1875	0.1115
Nitrate	0.1955	0.116

Table 2: Microbial analysis of untreated water showing the percentage effect of mycofiltration.

Description	Count Before Mycofilteration	Count after Mycofilteration	Significant difference ($P \leq 0.05$)
Total heterotrphic count (cfu mL^{-1})	3810000	400	S
Total coliform count (cfu mL^{-1})	40000	0	S
Total fungal count (cfu mL^{-1})	13470000	1070000	S

References

American Public Health Association (APHA) 2005. Standard methods for the examination of water and wastewater (21st ed.). pp. 32-33.

American Public Health Association (APHA) 1998. Standard methods for the examination of water and wastewater (18th ed.). Washington DC: pp. 45-60.

Ayodele SM, Akpaja EO, Anyiador F, 2007. Evaluation of the yield of *Lentinus squarrosulus* (Mont) Singer on selected economic tree species. *Pak. J. Biol. Sci.,* **10**: 4283-4286.

Azubuike CC, Chikere CB, Okpokwasili GC, 2016. Bioremediation techniques classification based on site of application: principles, advantages, limitations and prospects. *World J. Microbiol. Biotechnol.,* **32**: Article 180.

Barcelona M, Gibb JP, Helfrich JA, Garske EE, 1985. *Practical guide for groundwater sampling*. Illinois State Water Survey ISWS Contract Report, Illinois.

Bell-Games T, 2015. Key considerations for biological drinking water treatment. https://www. wateronline.com/doc/key-consi derations-for-biological-drinking-water-treatm ent-0001. Accessed 16 October 2019.

Brown J, Summers R, Lechevallier M, Collins H, Roberson A, Hubbs S, Dickenson E, 2015. Biological drinking water treatment? *J. Am. Water Works Assoc.,* **107**: 20-30.

Chawaga P, 2016. The Benefits of biological treatment for drinking water. https://www.wateronline.com/doc/the-benefits -of-biological-treatment-for-drinking-water-00 01. Accessed 17 October 2019.

Claaasen HC, 1982. Guidelines and techniques for obtaining water samples that accurately represent the quality of an aquifer. *US Geological Survey Open File Report* 821024.

Edward JB, Patricia BC, 1988. Biological processes in drinking water treatment. *J. Am. Water Works Assoc.*, **80**: 82-93.

EPA, 2017. National primary drinking water regulations. United States Environmental Protection Agency. https://www.epa.gov/ ground-water- and-drinking-water/national-pri mary-drinking-water-regulations. Accessed 17 March 2020.

Fulekar MH, Pandey B, 2012. Bioremediation technology: A new horizon for environmental clean-up. *Biol. Med.*, **4**: 51-59.

Green M, 2018. Mycofiltration: Harnessing fungi to clean polluted water. The environment magazine for the chartered institution of water and environmental management. pp. 16-17.

KRS, 2020. What are total dissolved solids (TDS) & how to reduce them? https://www.kent.co.in/blog/what-are-total- dissolved-solids-tds-how-to-reduce-them/. Accessed 17 March 2020.

Nahid A, Mannan MA, 2020. Mycoremediation: Expunging environmental pollutants. *Biotechnol. Rep.*, **26**: Article e00452.

Rhoda CJ, 2014. Mycoremediation (bioremediation with fungi) - growing mushrooms to clean the earth bioremediation with fungi. *Chem. Speciat. Bioavailab.*, **26**: 196-198.

Robles-Hernandez L, Gonzàlez-Franco AC, Crawford DL, Chun WWC, 2008. Review of environmental organo-pollutant degradation by white-rot basidiomycetes mushrooms. *Tecnocien. Chihuah.,* **2**: 3239.

Šašek V, Cajthaml T, 2005. Mycoremediation: Current state and perspectives. *Int., J. Med. Mushrooms,* 7: 360-361.

Sensorex, 2017. Conductivity monitoring for reverseosmosis https://sensorex.com/blog /20 17 /07/12/conductivity- monitoring- revers eo smosis/. Accessed 9 April 2020.

Sensorex, 2019. Sensorex https://sensorex.com/blog/ 2019/10/08/electrical-conductivity-water-imp ortant- industrial-applications/. Accessed 9 April 2020.

Sharma S, Bhattacharya A, 2017. Drinking water contamination and treatment techniques. *Appl. Water Sci.*, **7**: 1043-1067.

Stamets P, 2005. Mycelium running: How mushrooms can help save the world. Tenspeed Press, Berkeley, Toronto. pp. 54-64.

The Wandle Trust 2014. Mycofiltration: Using mushrooms to clean the river. https://www.wandletrust.org/mycofiltration/. Accessed 13 May 2020. Washington DC.

Ward MH, JonesRR, Brender JD, De Kok TM, van Breda SG, 2018. Drinking water nitrate and human health: An updated review. *Intl. J. Environ. Res. Public Health*, **15**: Article 1557.

WHO, 1997. Basic environmental health, Geneva. Third edition.

WHO, 2011. Guidelines for drinking-water quality, Fourth Edition.