Mycelium-producing Mushroom *Calocybe indica* (Milky Mushroom) as Bio-antagonist Against the Bacteria present in Marikina River

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Abstract

Mushroom forming fungi are one of the nature's most powerful decomposers and degrades a wide variety of environmentally persistent pollutants and organic contaminants by secreting strong extra cellular enzymes due to their aggressive growth and biomass production. This study assesses the potential of *Calocybe indica* (Milky mushroom) as bio-antagonist against microorganism serves as pollutants on Marikina River through Heterotrophic plate count (HPC). The use of *Calocybe indica* (Milky mushroom) as the medium of mycoremediation effectively lessens the count of bacterial colonies in Marikina River's water by creating a bio-antagonistic relationship with the bio-pollutants present on the water samples. Increasing the period of treatment and observation. Using of water samples from different depth from surface level down to aphotic region of the Marikina River. Identifying the strain of microorganisms present on the water. Using other mushroom to compare the potential of *Calocybe indica* to kill bio-pollutants should be done for the improvement of the study.

Keywords: Fungi, Mycoremediation, Bio-antagonism, Bio-pollutant

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Introduction

Nowadays, most cases of pollutants is not easily to determine, there are various ways to determine the status of the water. First, through chemical analysis which is very difficult and expensive. Second, through biological testing wherein it helps to determine different environmental factors affect various physiological processes. Thus, the biological testing, as a first stage in ecological monitoring system, enables reduce expenditures. In addition it makes it possible to detect the combined effect of a several pollutants (Gupta, R.K. and Doshi, A.2014)

Mushroom forming fungi are one of the nature's most powerful decomposers and degrades a wide variety of environmentally persistent pollutants and organic contaminants by secreting strong extra cellular enzymes due to their aggressive growth and biomass production (Packa, D. Wachowska, U. Wiwart, M. 2017). Nowadays, various species of mushroom forming-fungi use for mycoremediation. Mycoremediation is a tool to remove the contaminants using different species of mushrooms. It effectiveness depends on the enzymes produced by the mushroom to remove the pollutant (Adenipekun C. O. and Lawal R. 2012).

In the study of Kumar G. et al (2016) they found out that *Trichoderma sp.* is an effectively bio-control to enhance the crop production without bringing any negative effects to the

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crops. In the study of Shekhar, S. Maurya, C. Srivastava, J. (2017) they used Oyster mushroom as mycofilter and removed the organic contaminants present in the wastewater by approximately 20% per cubic foot more effectively than mycofiltration by wood mulch alone. This mushroom produces a fungus-like bacterial colony called Mycelium, the vegetative part of a fungus or, consisting of a mass of branching, thread-like hyphae that shows a great potential in killing bacteria and using it as food for the mushroom. In the study of Landum M. C. et al (2015), fungi has an antagonistic activity by producing inhibitory volatile compounds that inhibit the growth of bacteria present on olive tree.

Calocybe indica (Milky mushroom) a tropical edible fungus, a protein source without cholesterol have an ability to maintain the blood cholesterol (Nita, B. 2002). Since same with *Pleurotus sp*. it is used for bioremediation but this mushroom is easy to cultivate, less investment very attractive fruiting body. Pleasing milk white color, long shelf life, more nutritious and less time to grow (Alam et al.,2008).

This study assess the potential of *Calocybe indica* (Milky mushroom) as bio-antagonist against microorganism serves as pollutants on Marikina River through Heterotrophic plate count (HPC).

The other factors contributing on the number of bacterial colony were not investigated in this study. Identifying the specific strains of bacteria were not included. This paper describes only the ability of the *Calocybe indica* as bio-antagonist to eliminate the bio-pollutants.

Methods

The Research Design

The method of research used by the researchers is experimental. It is a research design wherein the effects of a treatment on a variable is determined. The term experimental design refers to a plan for assigning experimental units to treatment condition.

Statistical Analysis

The researchers used the weighted mean count (WMC) is expressed as colony forming units (CFU) per mL sample (American Public Health Association 2012) and is calculated following the formula:

$$WM = \frac{n}{(fa \times 1) + (fb \times \_\_\_\_)}$$

Where: n – total number of colonies in all plates counted

fa – number of plates in the lowest dilution with countable colonies

fb – number of plates in the next higher dilution with countable colonies

Gathering of Raw Materials

The researchers collected the following materials: 3 aquarium, 3 aerator, 4 pairs of rubber gloves, 4 masks, 12 scouring pad, 6 six Liter water containers, cooler, marker, tape, plastic cover, milky mushroom (fruiting bag, sterilized glass bottle (for the water sampling), sticks, flour, scissors.

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Setting of Materials

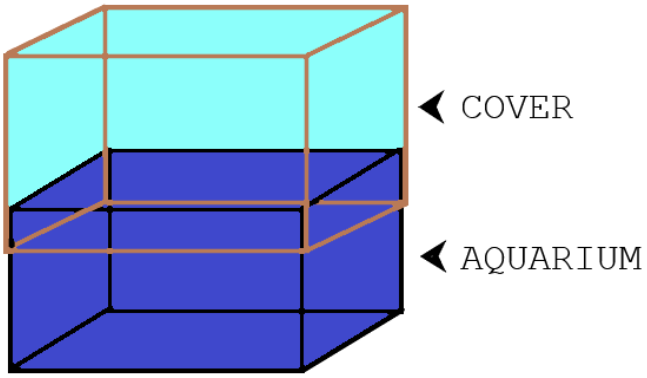

Figure 1. *Set-up of Aquarium with water samples*

The figure above shows the set-up of the materials wherein the water samples are placed in the aquarium. At the top of it, the *Calocybe indica* was placed.

Collection of Water Samples

The water samples were collected from three different sites of Marikina River namely: Barangka (S1), Sto. Nino (S2), Tumana (S3) on August 31, 2018 following the standard procedure in collecting water samples (Water Quality Monitoring Manual Volume 1).

Water Analysis: Pre-treatment

Heterotrophic Plate Count (HPC) were used to determine the number of bacterial colony. The water samples were serially diluted up to 10^{-5}. One ml portion of the dilutions were pour-plated in duplicates using Plate Count Agar. The plates were incubated at room temperature for 4 days and colonies were observed under Quebec colony counter (American Public Health Association 2012).

Observation and Treatment

The researchers observed the set-up every 24 hours for twelve days. The researchers put flour on the aquarium, it served as the food for the bacteria present on the water samples and measure the temperature of the water on each aquarium. The temperature readings were recorded on Table 1.

Water Analysis: Post-Treatment

Heterotrophic Plate Count (HPC) were used to determine the number of bacterial colony. The water samples were serially diluted up to 10^{-5}. One ml portion of the dilutions were pour-plated in duplicates using Plate Count Agar. The plates were incubated at room temperature for 4 days and colonies were observed under Quebec colony counter (American Public Health Association 2012).

Results and Discussion

Table 1. Daily Temperature of Water Samples

DAYS	**AQUARIUM 1 (Site 1)**	**AQUARIUM 2 (Site 2)**	**AQUARIUM 3 (Site 3)**
	Temperature (^{0}C)	**Temperature (^{0}C)**	**Temperature(^{0}C)**

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1	28	28	28
2	28	29	29
3	28	29	28
4	29	28	29
5	28	28	28
6	28	28	28
7	28	29	28
8	28	28	29
9	29	28	28
10	29	28	28
11	28	29	29
12	28	28	28

Source: Primary Data, 2020

Table 1 shows the temperature of water samples on three aquariums. The data shows that there is no tremendous change on the temperature of water during the twelve-day observation period.

Table 2. Heterotrophic Plate Count (HPC) of Environmental Water Sample: Pre-Treatment

Sample Code	Dilution					HPC, CFU per ml
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
S1	TNTC[a]	TNTC	112[b];88[b]	16;12	2;0	$1.0x10^5$
S2	TNTC	TNTC	86[b];68[b]	16;6	0;0	$7.7x10^4$
S3	TNTC	TNTC	288[b];248[b]	26;24	3;2	$2.7x10^5$

Source: Primary Data, 2020

a. Too numerous to count
b. Considered in the computation
c. Growth of spreader (bacteria that rapidly spread) observed; not counted

Table 2 shows the result of Heterotrophic plate count (HPC) test before the treatment period. It indicates that Samples 1, 2, 3 contained $1.0x10^5$, $7.7x10^4$, $2.7x10^5$ CFU per mL.

Table 3. Heterotrophic Plate Count (HPC) of Environmental Water Sample: Post Treatment

Sample Code	Dilution				HPC, CFU per ml
	10^0	10^{-1}	10^{-2}	10^{-3}	
S1	TNTC[a]	TNTC	378;336	24;35[b]	$3.5\ x10^4$
S2	TNTC	TNTC	Spr[c];141[b]	30[b];27	$1.6x10^4$
S3	TNTC	TNTC	155[b];122[b]	33[b];56[b]	$1.7x10^4$

Source: Primary Data, 2020

a. Too numerous to count
b. Considered in the computation
c. Growth of spreader (bacteria that rapidly spread) observed; not counted.

Table 3 shows the result of Heterotrophic Plate Count (HPC) test after the treatment period. It indicates that S1, S2, and S3 contained $3.5x10^4$, $1.6x10^4$, and $1.7x10^4$ CFU per mL.

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Table 4. Comparison of Weighted Mean Count (WMC) of Heterotrophic Plate Count (HPC) Before and After the Treatment

Sample Code	Before the Treatment (HPC, CFU per mL)	After the Treatment (HPC, CFU per mL)	Difference
S1	1.0×10^{5}	3.5×10^{4}	6.5×10^{-1}
S2	7.7×10^{4}	1.6×10^{4}	6.1×10^{-1}
S3	2.7×10^{5}	1.7×10^{4}	2.53×10^{-1}

Source: Primary Data, 2020

Table 4 shows the difference of weighted mean of Heterotrophic plate count of Water samples before and after the treatment period. It shows that, the *Calocybe indica* lessen siginifcantly the number of bacterial colonies in Sites 1 and 3.

Based from the data gathered there's no tremendous changed on the temperature of the water from Day 1 to Day 12 of treatment period. It means that the environment of the set-up were all well maintained. Based from the weighted mean count of Heterotrophic plate count before and after the treatment period it shows that the bacterial colony in Site 1: Barangka was lessen by 6.5×10^{-1}, while in Site 2: Sto. Niño was lessen by 6.1×10^{-1}, while on Site 3: Tumana was lessen by 2.53×10^{-1}. Among the three sites, the bio-pollutants in Site 1: Barangka were lessen. The results of the study showed that *Calocybe indica* (Milky mushroom) really served as an effective bio-antagonist to the bacteria present in Marikina River's Water. The heterotrophic plate count of the water samples unveiled that *Calocybe indica* (Milky mushroom) was not statistically significant in decreasing the amount or count of bacteria present in the water samples.

Conclusion

This study the use of *Calocybe indica* (Milky mushroom) as the medium of mycoremediation effectively lessen the count of bacterial colonies in Marikina River's water by creating a bio-antagonistic relationship with the bio-pollutants present on the water samples. The researchers recommend the following for the improvement of the study. Increase the period of treatment and observation. Use water samples from different depth from surface level down to aphotic region of the Marikina River. Identify the strain of microorganisms present on the water. Use other mushroom to compare the potential of *Calocybe indica* to kill bio-pollutants.

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