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Mycoremediation Potential of *Pleurotus florida* (Oyster Mushroom) Mycelia to Treat Microplastic Contaminated Paddy Soil

Alfonso ME, Mislang R, Dela Cruz J, Aguilar C, Villafuerte C, Dungca GA, Tapnio L and David A

Biology Department, College of Arts and Sciences, Don Honorio Ventura State University, Bacolor, Pampanga 2001, Philippines

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Abstract

The study investigates the potential of the oyster mushroom (*Pleurotus florida*) to treat the microplastic-contaminated paddy soil. Three treatment groups, MP800, MP400, and MP200, were set into different concentrations of spawn and substrate to microplastic polluted soils, specifically in ratios of 4:1, 2:1, and 1:1 respectively. A total of seventeen microplastics with different size groups namely large (5 mm to 3 mm), medium (2.99 mm to 1 mm), and small (0.99 to 0.1 mm) were incorporated in each treatment set-up. The specific number of microplastics for each treatment set-up was four large MPs, five medium MPs, and eight small MPs. After the remediation process, the microplastic counts stayed the same, while the sizes increased slightly and decreased in average size. In terms of weight, only MP800 was seen as significant, with a *t*-value of 7.496, while other concentrations were shown to have slight differences in average weight. In MP800, the large and medium microplastics have noticeably large scars corresponding to the weight change. MP400 showed minimal scarring through large and medium, while MP200 showed in medium size. The visible scarring and tear in the treated microplastics can be correlated with the effects of mushroom enzymes on degrading the plastics. Using different amounts of substrate-to-soil ratio has no significant difference in the results of the effectivity of *P. florida* in the degradation of microplastics in terms of size and count. However, slight changes in the weight and abundance of holes and cracks in the surface of microplastics were more frequent in MP800 and MP400.

Keywords – bioremediation – explanatory mixed method – fungi – microplastic degradation – polymer scarring

Introduction

From sachet packaging to electronic materials humans heavily rely on plastics and these polymers are used everywhere. Only 9% of the 460 million tons of plastic items produced annually worldwide in 2019 were recycled; by 2060, that number is expected to rise to 1.2 billion tons (Lebreton & Andrady 2019). Plastics are known as durable and low-cost materials with excellent thermal and electrical insulating properties that make them strong and impossible to decompose (Thompson et al. 2009). As they break down, fracture, and crush during usage, tiny pieces or particles known as microplastics are created (Ziani et al. 2023).

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Corresponding Author: Louie S. Tapnio – e-mail – lstapnio@dhvsu.edu.ph

Microplastics are less than five (5) millimeters in size of fragment tiny plastics. Due to being widely used for commercial products, such as in packaging, the most abundant types of microplastic that exist in the environment are polyethylene and polypropylene (Ziani et al. 2023). Microplastic comes from macroplastics, which are broken down by natural processes in the environment. The transition of regular plastic into microplastic results from improper waste recovery and management. These contaminants accumulate in the environment, degrading into smaller particles (Barboza et al. 2018). Microplastics are becoming omnipresent in different ecosystems, especially soil environments (Yang et al. 2021).

A vast reservoir of microplastics may exist in soils, including sources such as precipitation, sewage sludge sprayed as fertilizer, and airborne fallout. Accordingly, microplastics may alter the function of the ecosystem and endanger soil biodiversity. Microplastics are composed of various polymers that can be detected in other land ecosystems, mostly in agroecosystems. Some agricultural land in Europe has been estimated to contain microplastics ranging from 63,000 to 430,000 tons per year. It has been reported in a study that the ratio of plastics per kilogram of soil ranges from 700 to 4000 plastic particles per kg of soil and 7% of dry soil weight is estimated to be microplastic fragments (Fuller & Gautam 2016, He et al. 2018).

Microplastic contamination in farmlands is assumed to originate from the spreading of sewage sludge, irrigation of land, and using plastic mulches which are common practices in managing agroecosystems. These practices are responsible for the accumulation and pollution of microplastics within the farmlands. These plastics were durable and partially non-biodegradable which stayed in the environment for long periods, usually a hundred of years or even longer (Peng et al. 2017).

Globally, the most intensively grown and cultivated mushroom is oyster mushrooms or *Pleurotus* species. They provide higher yields and cheaper cost of production among other mushroom species. They not only serve as food but they also use in their medicinal properties. They also efficiently turn agricultural waste into usable resources. *Pleurotus* species provide numerous potential and implementation as a biosorbent due to their large biomass, mycelial production and their ability to produce and synthesize enzymes, specifically Mn-peroxidase and laccase, that breakdown in the oxidation of polyethylene (Okal et al. 2023, Kapahi & Sachdeva 2017).

Pleurotus florida has used to treat crude oil-polluted soils in which this species has demonstrated remarkable results within 42 days (Stanley et al. 2018). In their study, *P. florida* accomplished a 92.09% removal in total petroleum hydrocarbon concentration. The experiments reduced the concentration from 13,286.3 mg/kg to 1 212 mg/kg. Two different studies showed that *P. florida* also displayed bioremediation capabilities in sites contaminated by heavy metals (such as arsenic, titanium, manganese, nickel, chromium, zinc, cadmium, and lead) and was found to have the ability to degrade all Hexachlorocyclohexane isomers in soil. Although γ-HCH was found to degrade the fastest, more persistent isomers were also found to be degraded by *P. florida*. (Arun Prasad et al. 2013, Mahopatra & Pandey 2015).

Roshandel et al. (2021) showed that *P. florida* effectively detoxified C13–C28 hydrocarbons, Phytane, and Pristane, according to gas chromatography (GC), suggesting a solid mycoremediation function. On the demonstrated toxicity test, in a 30-day duration, mycoremediation boosted germination by an average of 35.82% ± 8.89.

The potential of *Pleurotus* spp. on plastic remediation was also investigated in past research. In the study of da Luz et al. (2013), 10 grams of plastic were incubated with *P. ostreatus* at 25°C for 45 days. Over this period, indications of basidiocarp development, along with signs of plastic degradation, were observed. Moreover, *P. ostreatus* was used to degrade diapers composed of polyethylene (PE), polypropylene (PP), and absorbent polymers in a study by Espinosa-Valdemar et al. (2011). The fungus was inoculated with the diapers using three different methods. (a) with or without plastic. (b) either crushed or mechanically ground. (c) with or without grape marc supplement. and (d) wheat straw as a control. The samples were treated at different temperatures for 21 days under dark conditions. The results showed that up to 90% of the diapers' weight and

volume were degraded. Additionally, the collected mushrooms were free of human disease-causing pathogens and had a decent protein content and appearance, indicating potential for safe reuse.

This present study investigates the effectiveness of *P. florida* in remediating microplastic contaminants in rice paddy soil. The objectives of the study were to determine the difference in the size, count, and weight of microplastics after the remediation process characterize physical changes in the surface of microplastics, and; analyze the significance of using different concentrations of oyster mushrooms for each treatment group to the remediation result. A known amount of microplastics was incorporated into the soil and was treated with three different experimental groups.

Materials & Methods

The study had a total of seventeen (17) experimental set-ups and three treatment groups with five replicates each to ensure the accuracy of the results. Three replicates of each concentration were dedicated to the characterization and quantification of microplastics. At the same time, the other two replicates were used to examine the mycelium of *P. florida* after the remediation process. The three treatment groups were composite samples treated with a spawn substrate mixture, with three different concentrations, namely, 4:1 w/w, 2:1 w/w, and 1:1 w/w.

PHASE I: Preparation

Production of Microplastics

The treatment groups were spiked with microplastics based on the amount determined in previous studies (Elshafie et al. 2020). A study by Esguerra et al. (2023) found an average of 17 microplastic particles (originated from plastic straws, cups, bags, and packaging) in every 200 grams of soil in rural agricultural soil, originating from plastic straws, cups, bags, and packaging. Out of these particles, 46.46% were classified as small microplastics (ranging from 0.1 to 0.99 mm), while medium-sized microplastics (1-2.99mm), and large microplastics (3-5 mm) accounted for approximately 30.57% and 23.01%, respectively.

For the experimental set-ups, the same amount and material of microplastics as the study of Esguerra et al. (2023), were made and planted into the rice paddy soil. Moreover, the production and incorporation of microplastics were adapted from the study of Šunta (2021). The plastic straws, cups, bags, and parcel pouches were manually cut into the desired size using scissors, cutters, and a kitchen grater (Lambert & Wagner 2016). The microplastics were created into uniform shapes (quadlerateral) to easily distinguish the effects of the remediation on the surface of the pollutant.

Substrate Preparation

The main composition of the substrate used for the cultivation of the oyster mushroom was sawdust. The percentage of each ingredient is 85% of sawdust, 12% rice bran, and 3% Calcium Carbonate (CaCO3) mixture of the substrate. To meet the threshold of sufficient moisture for the oyster mushrooms, it was submerged overnight in water at room temperature. Any excess water was drained from the substrate before setting up the bagging of the substrate and soil (Owaid et al. 2015).

Polypropylene (PP) bags were chosen as the packaging medium for each treatment group due to their strength, rigidity, and heat resistance which can maintain their shape even when exposed to high temperatures (Maddah 2016). Treatment A, which contains 800 g of substrate, was packed in a 10 x 15 inch PP bag. Treatments B and C, which contained 400 g and 200 g were packed in 8 x 12, and 6 x 12 inch bags respectively.

Treatment Preparation and Bagging

The research consists of three setups with different ratios of substrate to soil. Treatment A and B each had five replicates while Treatment C had seven, totaling seventeen bags. In treatment A, a 4:1 ratio of substrate to soil was used with 800 grams of substrate and 200 grams of

soil. Treatment B utilized a 2:1 ratio with 400 grams of substrate and 200 grams of soil. Treatment C employed a 1:1 ratio with 200 grams each of substrate and soil.

The preparations were done in PP plastic bags with the substrates divided into five sections for each treatment group and the soil into four sections with 50 grams per layer. Treatments A, B, and C had layers containing 160 grams, 80 grams, and 40 grams of substrate respectively. The layering technique followed the method outlined by Seidu et al. (2015) starting with the substrate layer, at the bottom followed by a layer of soil. This was repeated thrice and topped off with the last section of the substrate. The same procedure was followed with Treatment B and Treatment C, accommodating the different concentrations of each treatment. Each bag was tied and sealed with rubber bands. A cotton was placed on top of the bag to seal in the moisture inside the bag (Bhattacharjya et al. 2014).

Pasteurization of the Soil and Substrate

The preparation and pasteurization of the substrate were adapted from the paper of Seidu et al. (2015) entitled "Mycoremediation of Diesel-Contaminated Soil with oyster mushroom (*P. ostreatus*) Using Maize (*Zea Mays*) as Test Crops." Certain modifications to the methods were initiated to accommodate the study's objective. Moreover, input from the mushroom expert from the Cabalen Mushroom Farmers Agriculture Cooperative was also considered in this study.

The bags were placed upside down in a drum half-filled with water. The drum was sealed with a lid and rubber to ensure proper enclosure. Using a wood fire, the substrate and soil mixture were pasteurized for eight (8) hours at 115℃temperature. The setups were cooled for five (5) hours afterwards.

PHASE II: Remediation

After pasteurizing the soil and substrate, 10 grams of Sorghum spawn were planted in each treatment group. The bags were stored in a regular room temperature room for the first fourteen (14) days of the experiment. After the two (2) weeks mark, the bags were transferred to a controlled room. The room was enclosed with plastic and mesh cloth to retain 55-70% moisture. The room was sprinkled with water thrice a day and maintained a temperature of 28℃ to 30℃ to retain moisture (Nongthombam et al. 2021).

The remediation process should last two (2) months when the mycelium has reached its growing limit and starts to grow its fruiting body (Ebenebe et al. 2013). However, due to time constraints, in this study, the remediation only lasted 45 days, starting on the 24th of March and ending on the 7th of May 2024. By this time, the mycelium covered the outer layer while having some growths in the soil where the microplastic contaminants were.

PHASE III: Extraction and Testing

Microplastic Extraction: Density and Oil Separation Method

Fewer studies are being conducted on microplastic in the soil environment than in aquatic ecosystems due to the complexity of soil components and characteristics. These are attributed to the lack of a standardized method for extracting MPs in soil (Kim et al. 2022). Density and oil separation techniques are the most common methods for extracting MPs in soil. These two separate methods produce the most optimal result (Radford et al. 2021). Additionally, a similar method to the study of Kim et al. (2022), entitled "Repeatable separation of microplastics integrating mineral oil extraction and a PDMS-Ni foam absorbent in real soil," was also used, resulting in a more efficient and faster approach in the extraction of MPs.

In this study, eleven (11) 200 grams of soil samples underwent microplastic extraction. For the density and oil separation method, 20 mL of canola oil, 00.33g/mL of laboratory-grade Sodium Chloride (NaCl) solution, and 400 mL of distilled water were mixed and combined with every 200 grams of soil. The NaCl solution was prepared and assembled by dissolving 33 grams of NaCl which was stirred in 100 mL of distilled water, then mixed with the oil and distilled water. To

guarantee and reach homogeneity, the soil was progressively introduced and blended with an electric mixer for 15 minutes. The mixture was then kept undisturbed for 24 hours to enable low density particle separation and settling of the soil (Liu et al. 2018).

Microplastic Manual and Vacuum Filtration

To eliminate large particles, the mixture was initially filtered through 3 inches opening sieve. The primary filtration process removed large microplastics. For this study, the tissue was used as a replacement for the PDMS-Ni foam. A two layer of a two-ply facial tissue was folded into a square shape and stamped in the layer of oil of the mixture. The oil was hindering the vacuum filter from performing faster, and with the tissue method, it was able to cut down the filtration process to 30 minutes per 200 grams of soil. Upon further observation, the tissue was able to attract microplastics to its surface.

Mycelium Extraction

Three methods were utilized to extract the mycelium from the soil, which were adapted from the study of Awad and Pena (2023), and Kuo (2019). The filtration separation technique was tested out with no promising results. Two hundred (200) grams of soil with detected mycelium was mixed with four liters of distilled water and was mixed thoroughly with the use of a magnetic stirrer. The mixture was stirred at 500 rpm for 30 minutes before it was subjected to vacuum filtration. 0.22 μm filter paper and a vacuum filtration system were used. Upon observation of the filter paper, only soil residue was extracted, and the mycelium was suspected to be dissolved during the stirring process (Awad & Pena 2023). The second method was based on Kuo (2019) study. A 20% solution of Potassium Hydroxide (KOH) was combined with soil and distilled water. KOH degrades organic compounds in soil and turns mycelia in to a transparent color. The mixture remained undisturbed for 24 hours. Vacuum filtering was used to harvest mycelium, but yielded similar results as the first procedure. Finally, manual physical separation of mycelium from soil was carried out. Mycelium was removed from the soil with tweezers and analyzed under a microscope. The same procedure allows for the extraction of microplastics with mycelia adhering to their surface possible.

Microplastic Quantification and Microscopy

Following extraction, the microplastics were dried in petri dishes. Once dried, the MPs were quantified and characterized using a compound microscope. Microplastic particles were carefully counted. Their weight was determined with an analytical balance (Li et al. 2019). Furthermore, a microscopic study of microplastics was performed to detect scarring, holes, abrasions, and cracks on their surface (da Luz et al. 2013). The contaminants were measured by placing each particle under a compound microscope and photographing the image. The image was subsequently processed in Image J.

Image J was used with the compound microscope's specific calibration. The ocular lens has 10x magnification, with 4x magnification for the scanner, with a total magnification of 40 x. The field of view has a diameter of 5 mm. The microplastics were measured vertically and horizontally to accommodate each side (Schnepf 2023). The large microplastic particles were captured in the table surface and then were plugged into Image J. The measurement scales in Image J were calibrated using the known length of a ruler beside the microplastics.

Statistical treatment

The data, sizes, counts, and weight were described using mean, standard deviation, and percent. Moreover, average reduction analysis was used to compare the size of the microplastic before and after treatment. Comparative analysis of different substrate-to-soil ratios was treated using analysis of variance (ANOVA) and a two-sample (independent sample) T-test. After running the test of normality, analysis of variance (ANOVA), and two-sample (independent sample) T-test. Significant differences between the means of independent variables (different substrate-to-soil ratios) were determined using ANOVA. ANOVA, or f-test, was used to determine if there was a

significant difference between each of the treatments in terms of the length, width, and weight of the microplastics. The *f*-value and *p*-values were calculated to determine whether to accept or reject the null hypothesis in terms of the significant difference between varying substrate-to-soil ratios. Further, a paired t-test was used to determine if there was a significant difference in the size, count, and weight of the microplastics before and after treatment. The significance level of the data was presented in *p*-values (0.05). A result with a *p*-value higher than 0.05 signifies a statistically insignificant result, accepting the null hypothesis; also, if the *p*-value is less than 0.05, the null hypothesis will be rejected.

Results

The 4:1 w/w treatment group had 800 grams of substrate. At the same time, there were 400 grams and 200 grams of substrate in the 2:1 and 1:1 w/w ratios, respectively. The soil weight was 200 grams for all the treatment groups. To better represent, 4:1 w/w ratios were labeled as MP800, 2:1 w/w ratios were labeled as MP400, and MP200 represented the 1:1 w/w ratio. Each replication from a treatment group was labeled as Treatment A: MP801, MP802, MP803; Treatment B: MP401, MP402, MP403; Treatment C: MP201, MP202, MP203, and Treatment C.1: MP204, and MP205. MP204 and MP205 contained four irregularly shaped microplastics, four small MPs, five medium, and four large-size microplastics. Changes in the sizes and counts of microplastics were analyzed to indicate degradation in the contaminants. The characteristics of microplastic degradation were observed through weight loss, size decrease, and the production of holes and cracks on the microplastic surface.

Qualitative Characterization of Microplastics

Characterization of Microplastics by Size

a. *Average Initial Microplastic Sizes*

Table 1 Average initial sizes of the microplastics used in the study.

Category	X (length in mm)	Y (width in mm)
Small	0.7202	0.7708
Medium	1.5777	2.0200
Large	4.2440	4.3532

Each bag contained 17 microplastics; eight were small sizes, five were medium, and four were classified as significant. The average size of the small microplastics is 0.72 by 0.77 mm. The medium and large sizes average 1.58 by 2.02 mm and 4.24 by 4.35, respectively. The average initial sizes of the microplastics were used to determine whether there was an expansion or shrinkage in the sizes of the microplastics after the remediation process.

b. *MP800 Sizes After Remediation*

Table 2 Average sizes of the microplastics in MP800 after the remediation process.

		X			Y	
MP	**Small**	**Medium**	**Large**	**Small**	**Medium**	**Large**
801	0.8016	1.9046	4.7420	0.8066	1.9812	4.5725
802	0.7025	1.8264	4.1566	0.7136	1.7252	4.1805
MP	**Small**	**Medium**	**Large**	**Small**	**Medium**	**Large**

Table 2 Continued

		X			Y	
803	0.6404	1.4490	4.6510	0.8516	1.9190	4.2683
Average	**0.7148**	**1.7267**	**4.5165**	**0.7906**	**1.8751**	**4.3404**

The small microplastics sizes range from 0.64 to 0.85 mm. The average size for this concentration's small microplastics is 0.71 by 0.79 mm. The size range for the medium MPs was 1.45 to 1.98, with an average size of 1.73 by 1.88 mm. The smallest size from large MPs was 4.16 mm, and the largest was 4.74. The average size in large MPs was 4.52 by 4.34 mm.

c. ***MP400 Sizes After Remediation***

Table 3 Average sizes of the microplastics in MP400 after the remediation process.

		X			Y	
MP	**Small**	**Medium**	**Large**	**Small**	**Medium**	**Large**
401	0.7213	1.8642	4.0050	0.6640	1.7694	4.4130
402	0.8660	1.3992	4.0866	0.8149	2.0732	4.3770
403	0.8365	1.7480	4.3045	0.7713	1.7660	4.3830
Average	**0.8079**	**1.6705**	**4.1320**	**0.7501**	**1.8695**	**4.3910**

The average size of the microplastics from MP400 treatments for small, medium, and large sizes are 0.81 by 0.75 mm, 1.67 by 1.87 mm, and 4.13 by 4.39 mm, respectively. The range sizes for small MPs are 0.66 to 0.87 mm. The medium MPs range from 1.40 to 2.07 mm, while the large sizes range from 4.01 to 4.38 mm.

d. ***MP200 Sizes After Remediation***

Table 4 Average sizes of the microplastics in MP200 after the remediation process.

		X			Y	
MP	**Small**	**Medium**	**Large**	**Small**	**Medium**	**Large**
201	0.6538	1.7434	3.6748	0.7270	2.1170	4.5415
202	0.6406	0.8538	4.4770	0.7494	0.8868	4.2795
203	0.6113	1.6266	4.0203	0.8205	2.1422	4.0690
Average	**0.6352**	**1.4079**	**4.0574**	**0.7656**	**1.7153**	**4.2967**

As presented on the table, small- sized MPs range from 0.61 to 0.82 mm. Medium-sized MPs range from 0.88 to 2.14 mm. Large-sized MPs range from 3.67 to 4.54 mm. The average sizes of all small, medium, and large MPs in this treatment group are 0.64 by 0.76 mm, 1.41 by 1.72 mm, and 4.06 by 4.30 mm.

Table 5 Average sizes of the microplastics in MP200 w/ irregular shapes after the remediation process.

		X			Y	
MP	**Small**	**Medium**	**Large**	**Small**	**Medium**	**Large**
204	0.7899	1.6938	3.6935	0.0806	1.9066	4.3593

Table 5 Continued

		X			Y	
205	0.9754	2.1142	4.6233	1.1548	2.0618	5.2033
Average	**0.8827**	**1.9040**	**4.1584**	**0.9927**	**1.9842**	**4.7813**

The small MPs had an average size of 0.88 by 0.99 mm, while the medium and large-sized MPs had an average size of 1.90 by 1.98 mm and 4.16 by 4.78 mm, respectively.

e. ***Average Size Reduction of Microplastics After Treatment***

Table 6 Average size reduction of microplastics in MP800 after treatment.

MP800				
Size	**X Reduction (mm)**	**X %**	**Y Reduction (mm)**	**Y %**
Small	0.0054	0.75%	-0.0198	-2.57%
Medium	-0.149	-9.44%	0.1449	7.17%
Large	-0.2725	-6.42%	0.0128	0.29%

The average size reduction of the microplastics after remediation in MP800 is 0.0054 by -0.0198 mm, -0.149 by 0.1449 mm, and -0.2725 by 0.0128 mm in small, medium, and large sizes, respectively. It can be observed that all sizes (length in medium and large, while width in small size) had both shrinkage and expansion in size. Small MPs had a 0.75% reduction in length and a 2.57% expansion in width. Medium and large sizes also exhibit the same expansion in length at 9.44% and 6.42%, respectively. However, in the width of the medium and large MPs, there is a 7.17% and 0.29% reduction in their sizes.

Table 7 Average size reduction of microplastics in MP400 after treatment.

MP400				
Size	**X Reduction (mm)**	**X %**	**Y Reduction (mm)**	**Y %**
Small	-0.0877	-12.18%	0.0207	2.69%
Medium	-0.0928	-5.88%	0.1505	7.45%
Large	0.112	2.64%	-0.0378	-0.87%

It can be observed that on the length of small and medium, and the width of large MPs, there was an increase in sizes at 12.8%, 5.88%, and 0.87%, respectively. There was an average 2.69%, and 7.45% reduction in the width of small and medium MPs, respectively. In the length of the large MPs, there was an average size reduction of 2.64%.

Table 8 Average size reduction of microplastics in MP200 after treatment

MP200				
Size	**X Reduction (mm)**	**X %**	**Y Reduction (mm)**	**Y %**
Small	0.085	11.80%	0.0052	0.67%
Medium	0.1698	10.76%	0.3047	15.08%
Large	0.1866	4.40%	0.0565	1.30%

The microplastics in MP200 had shown size reduction in both their length and width. The small MPs had an average of 11.80% by 0.67% size shrinkage, while the medium and large sizes had reduced by 0.17 by 0.30 mm and 0.19 by 0.06 mm, respectively.

Table 9 Average size reduction of microplastics in MP200 (irregular) after treatment.

	MP200 (Irregular)			
Size	**X Reduction (mm)**	**X %**	**Y Reduction (mm)**	**Y %**
Small	-0.1625	-22.56%	-0.2219	-28.79%
Medium	-0.3263	-20.68%	0.0358	1.77%
Large	0.0856	2.02%	-0.4281	-9.83%

Contrary to the MP200, the microplastics in the MP200 (Irregular) had increased and decreased in size. The small MPs had an average of 0.16 by 0.22 mm increase in its length and width. Medium MPs had an average of 0.33 mm increase in length and a size reduction of 0.04 mm in width. Large MPs also exhibit both shrinkage and expansion in size. The length of large MPs had an average decrease of 0.09 mm in length and an increase of 0.43 mm in width.

f. *Test of Signifiant Difference Between Before and After Treatment in Terms of MP Size - X (length)*

Table 10 Test of significant difference between before and after treatment in terms of X (length).

Before	**After**	**t**	**p**	**Significance**
Initial	**800**	0.541	0.59	Insignificant
	400	0.996	0.322	Insignificant
	200	1.659	0.101	Insignificant
	200 (Irregular)	-0.019	0.985	Insignificant

It can be gleaned from the table that with a *t*-value of 1.659 (MP200), 0.996 (MP400), 0.541 (MP800), and -0.019 (Irregular 200), there is no significant difference between before and after treatment in terms of the size in the x-axis (length) of the microplastics. Similarly, with a *p*-value of 0.101, 0.322, 0.59, and 0.985, respectively, there is also no significant difference between before and after remediation in terms of the size of MPs.

g. *Test of Significant Difference Between Before and After Treatment in Terms of MP Size - Y (width)*

Table 11 Test of significant difference between before and after treatment in terms of Y (width).

Before	**After**	**t**	**p**	**Significance**
Initial	**800**	1.018	0.321	Insignificant
	400	0.575	0.209	Insignificant
	200	1.66	0.100	Insignificant
	200 (Irregular)	-0.212	0.833	Insignificant

As presented from the table, with a *t*-value of 1.66 (MP200), 0.575 (MP400), 1.018 (MP800), and -0.212 (Irregular 200) and a *p*-value of 0.100, 0.209, 0.321, and 0.833, respectively, there is no significant difference between before and after the remediation process in terms of the size in the y-axis of the microplastics.

The average length and width of the microplastics were measured and compared before and after the remediation to assess whether the treatment worked. Although there were minor changes, the size alterations were deemed statistically insignificant.

1.2 Characterization of Microplastics by Counts

Table 12 Microplastics counts before and after the remediation process.

	Initial			After		
Size	**MP800**	**MP400**	**MP200**	**MP800**	**MP400**	**MP200**
Small	8	8	8	8	8	8
Medium	5	5	5	5	5	5
Large	4	4	4	4	4	4
Irregular	-	-	4	-	-	4

Treatments A, B, C, and C.1 had 17 microplastics in each bag initially. After the extraction method, all microplastics from each bag and treatment can still be found. In conclusion, the remediation treatment is statistically insignificant in reducing the concentration of microplastics in contaminated areas.

1.3 Characterization of Microplastics by Weight

Table 13 Average weight reduction of microplastics after treatment.

Treatment Groups	Average Weight Loss (mg)	Percentage
MP800	1.70	19.73%
MP400	1.60	18.09%
MP200	-0.34	-4.57%
MP200 (Irregular)	-0.60	-8.37%

The microplastics in treatment MP800 and MP400 showed weight loss with an average of 1.70 and 1.60 mg, respectively. MP800 had a 19.73% weight reduction from its initial weight, with the highest percent reduction among the four treatment groups. MP400 had the second-highest weight loss at 18.09%. At the same time, MP200 and the treatment with irregular shapes of MPs showed an increase in the average weight of the contaminants of 0.34 and 0.60 mg, respectively. These are equivalent to 4.57% increased weight in MP200 and 8.37% in MP200 (irregular).

Table 14 Test of significant difference between before and after treatment in terms weight.

Before	After	t	p	Significance
Initial	**MP800**	7.496	<0.001	Significant
	MP400	1.399	0.234	Insignificant
	MP200	-0.714	0.515	Insignificant
	MP200 (Irregular)	-0.92	0.455	Insignificant

It can be gleaned from the table that, with a *t*-value of -0.714 (MP200), 1.399 (MP400), and -0.0.92 (Irregular 200) and a *p*-value of 0.515, 0.224, and 0.455, respectively, there is no significant difference between before and after treatment in terms of weight. However, the weight after the remediation in MP800 (Treatment A) significantly differs from the initial weight, with a value of t= 7.496 and p- <0.001.

2. Qualitative Characterization of Microplastics via Visible Scarring

The qualitative characterization of the visible scarring of the microplastics after the remediation process was carried out by observing microplastics under a compound microscope.

2.1 MP800

a. Medium Size MPs

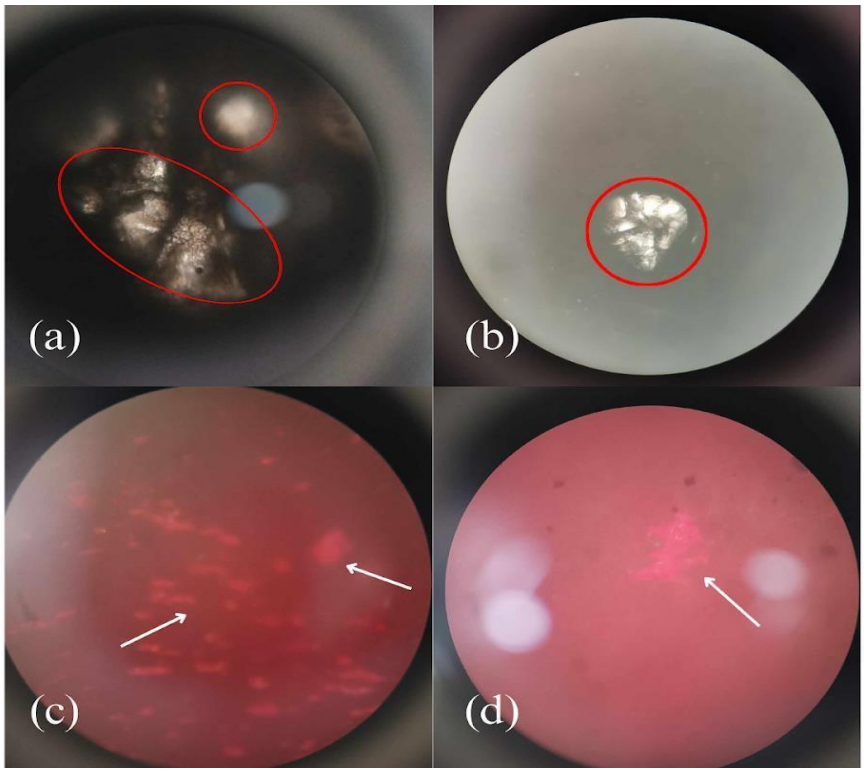

Fig. 1 – Visible scarring in MP800 - medium size MP. a, b, c, d The visible scarring of the oyster mushroom in the medium size microplastics from treatment MP400. The MPs were viewed under a compound microscope with the high-power objective (HPO). Images a, b show holes in the microplastics, while c, d show scarring on the surface of the microplastics.

b. Large Size MPs

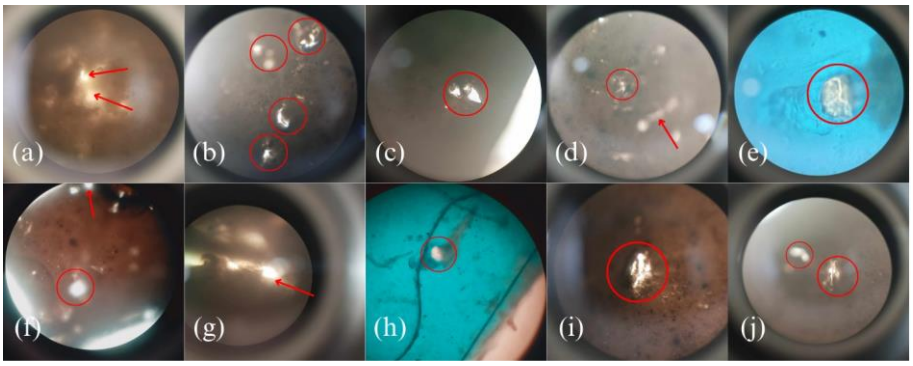

Fig. 2 – Visible scarring seen in MP800 - large size MPs. a, b, c, d, e, f, g, h, i, j The microplastics under compound microscope at low power objective (LPO). It is evident that out of the 16 large microplastics subjected to the MP800 treatment, 10 of them exhibited reaction from the remediation process. The MPs appeared to have scarring and holes, indicating abrasion from the remediation process.

2.2 MP400
a. Medium Size MPs

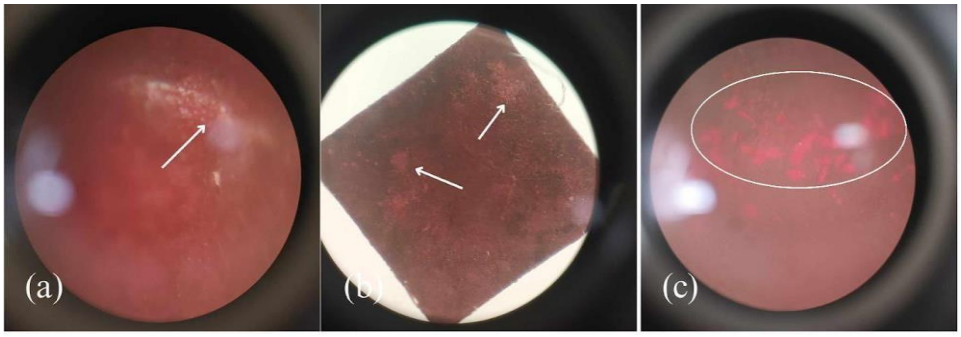

Fig. 3 – Visible scarring seen in MP400 - medium size MP.a, b, c The high-power microscopic observation of medium size microplastics from Treatment MP400. The microplastics appear to have abrasion in their surface.

b. Large Size MPs

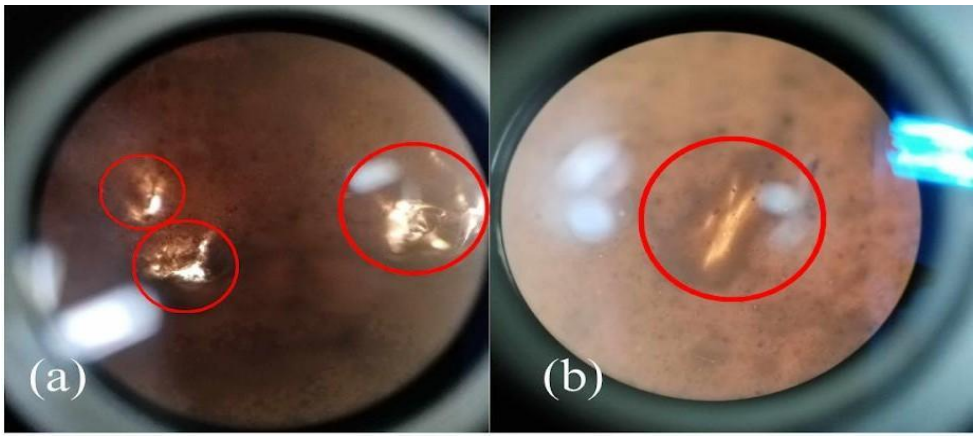

Fig. 4 – Visible scarring seen in MP400 - large size MP. a, b Large microplastics from treatment MP400 under the low power objective. The microplastics appeared to have roughness and pitting in their surface after the remediation process.

2.3 MP 200
a. Medium Size MPs

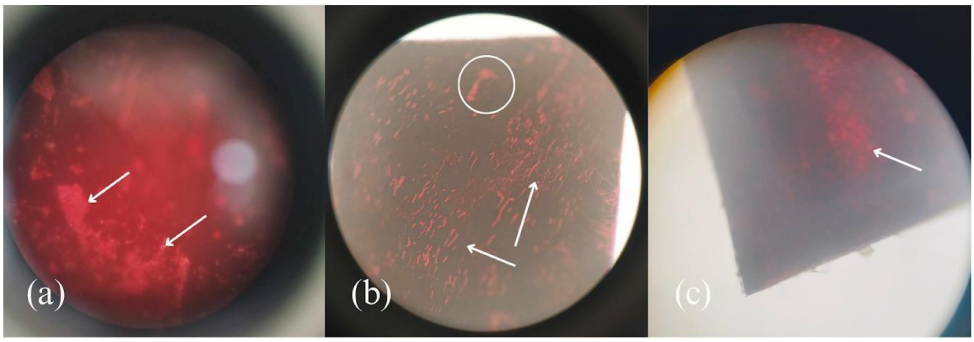

Fig. 5 – Visible scarring seen in MP200 - medium size MP. a, b, c Medium size microplastics from treatment MP200. Similarly with the medium MPs from MP400, the microplastics appear to have abrasion after the remediation process.

b. Irregular MPs

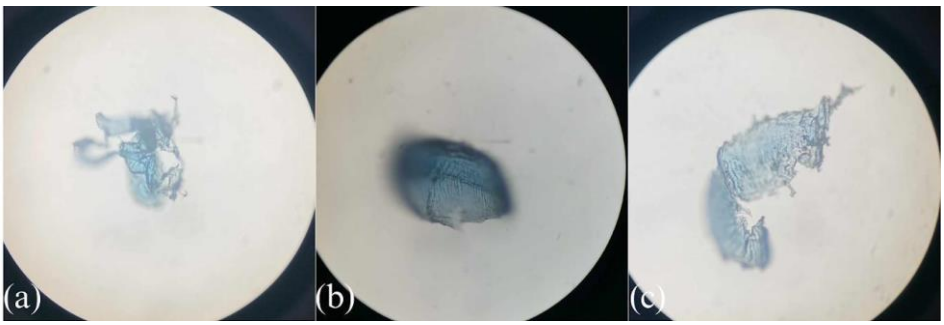

Fig. 6 – Irregular MPs in treatment MP200. a, b, c The irregularly shaped microplastics had no obvious alterations under a compound microscope.

3. Comparison of different concentrations of substrate-to-soil ratio in the effectivity of the remediation process

3.1 Test of Significant Difference Between Treatment in Terms of Size

Table 15 Test of significant difference between treatment in terms of size - X (length).

Treatment	F	P	Significance
MP800			
MP400	0.571	0.566	Insignificant
MP200			

It is evident from the table that, with a *f*-value of 0.571 and a *p*-value of 0.566, there is no significant difference between treatment in terms of x.

Table 16 Test of significant difference between treatment in terms of size - Y (width).

Treatment	F	P	Significance
MP800			
MP400	0.166	0.89	Insignificant
MP200			

Refering to Table 16, it shoes that, with an *f*-value of 0.166 and a *p*-value of 0.89 in which there is no significant difference between treatment in terms of y.

3.2 Test of Significant Difference Between Treatment in Terms of Count

There is no significant difference in the initial and after count of microplastics in every treatment group. The microplastic counts for treatments stayed the same throughout the remediation process.

3.3 Test of Significant Difference Between Treatment in Terms of Weight

Table 17 Test of significant difference between treatment in terms of weight.

Treatment	F	P	Significance
MP800			
MP400	1.931	0.231	Insignificant
MP200			

It can be gleaned from the table that, with an *f*-value of 1.931 and a *p*-value of 0.231, there is no significant difference between treatments in terms of weight.

There is no significant difference between using varying amounts of concentration in testing the effectivity of *P. florida* in the degradation of microplastics in terms of size, counts, and weight. However, the changes in the surface appearance of microplastics result from degradation by the oyster mushroom.

4. Mycelium Analysis

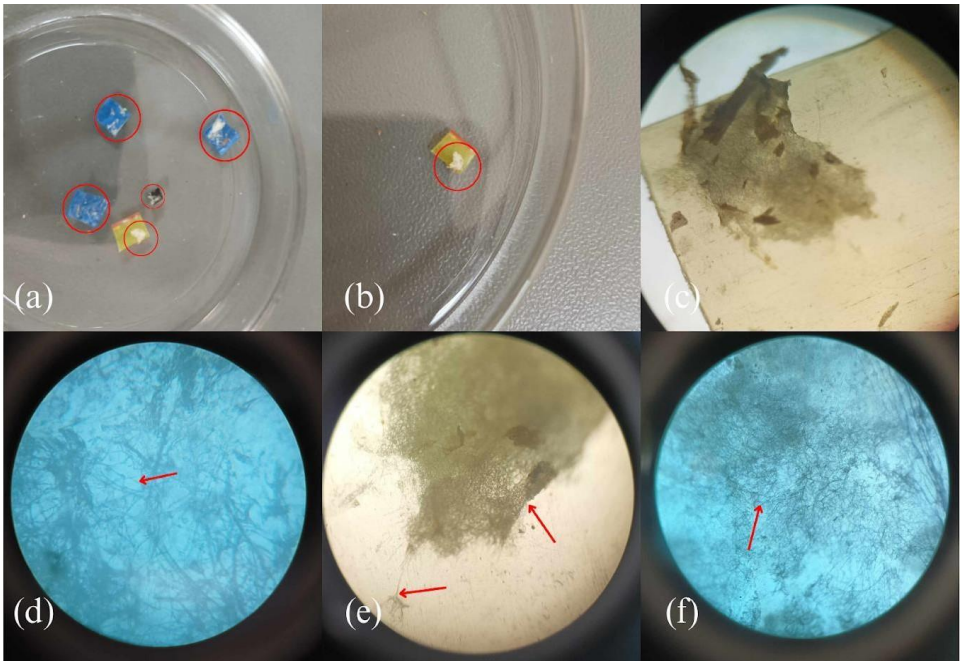

Fig. 7 – Mycelium binding in the microplastics. a, b are the raw photographs of mycelium wrapping in the microplastics, c, d, e, f the microscopic view.

Several extraction methods were carried out for harvesting the mycelium of the oyster mushroom, however, due to the lack of equipment for the analysis of the mycelium, manual extraction was utilized

Discussion

Absorption of enzymes by the pollutants and contaminants, such as microplastics, is the key stage in their degradation process. The shift in the size of the contaminants can be explained by the engagement of enzymes produced by the mushroom with microplastics (Mohanan et al. 2020). Moreover, the microplastic material can influence the degradation rate of the contaminant (Monkul & Ozhan 2021). The exact cause of the increase and decrease in the sizes of microplastics cannot be determined; however, based on the study of Owaid et al. (2015) and Chamas et al. (2020), it can be concluded that the fluctuation in the sizes of microplastics after the remediation process can be associated with the production of enzymes, and degradation rate of microplastics.

ANOVA and t-tests have been applied to evaluate the significant differences in the size, count, and weight of microplastics before and after remediation. With a *p*-value of greater than 0.05 on both length and width measurements, there was no statistically significant reduction in the dimension of microplastics by any treatment group (MP800, MP400, and MP200), used for this experiment. It implies that the observed changes within surface characteristics such as scarring and holes were not large enough to statistically reduce the size and count of the microplastics.

Contrastingly, the outcome of weight analysis was much larger. The MP800 treatment exhibited a statistically significant reduction in the microplastic mass at a *t*-value of 7.496, and a *p*-value of <0.001. The high substrate concentration used in this treatment supports the idea that it favoured degradation. Surface damage, including abrasions and holes, contributed to the weight loss observed. The ANOVA analysis showed that there was no statistical difference among the treatment groups concerning changes in size and number, hence confirming that although surface-level degradation was observed, it was not sufficient to meaningfully impact the size or quantity of microplastics.

The degradation of microplastics can be divided into four stages: deterioration, fragmentation, assimilation, and mineralization (Mohanan et al. 2020). Based on the physical observation of the microplastics, the contaminants reached the assimilation stage, in which the mushroom released enzymes that degrade weak parts of the polymer (da Luz et al. 2013). The pollutants were not reduced in quantity as the mineralization stage of the degradation was not complete. In this stage, microplastics would have completely degraded and become by-products such as water and carbon dioxide (Mohanan et al. 2020).

In the results, the figures 1-5 show the alteration of the surface of the microplastics. Holes and abrasions can be observed in the contaminants, suggesting the effectivity of the mushroom in degrading microplastics (da Luz et al. 2013). Although these changes were also observed in MP400 and MP200 treatments, the abundance of these were seen in MP800. With these alterations, it can be concluded that the abrasions caused a significant difference in the weight of microplastics in treatment MP800. Moreover, the study of Owaid et al. (2015) suggests that the type and amount of substrate used affects the growth and performance of the oyster mushroom. The higher the substrate concentration used, the better the oyster mushroom grows and performs. This could explain the higher weight difference in treatment MP800, as the treatment contained the most substrate among others.

The observation from Figures 1 to 5 shows the effectivity of *P. florida* in the remediation process in terms of causing visible scarring on the surface of microplastics. The remediation process mainly affected the large-sized microplastics, while it did not have any visible effect on the small-sized microplastics. Both medium and large-sized microplastics from MP800, MP400, and large-sized MPs from MP200 treatment exhibited significant surface alteration, including holes and abrasion after the remediation process. Moreover, the amount of substrate used affects the growth and performance of the mushroom (Owaid et al. 2015). The abundance of scarring in treatment MP800 can be explained by having a higher substrate concentration.

The degradation rate of the contaminants relates to the proportion of the surface area of the plastics and the direct contact with the organism that degrades them (Chamas et al. 2020). The abundance of large microplastics having been degraded compared to the small size can be explained by the surface area of the contaminants. The large MPs showed better results in degradation as they were mostly the sizes that made direct contact with the mycelia (Fig. 7).

According to the study of da Luz et al. (2013) and Okal et al. (2023), *Pleurotus* spp. is capable of producing enzymes that help them attach to several surfaces, including plastics. Studies suggest *Pleurotus* spp. utilizing plastics as carbon and energy sources results in the contaminants' degradation (da Luz et al. 2013, Mohanan et al. 2020). The holes and cracks after the treatment were caused by the formation of the hydroxyl groups (-OH) and carbon-oxygen bonds. These formations also explain the degradation of microplastics in terms of abrasion and discoloration (da Luz et al. 2013).

Increased, decreased, or no weight reduction can be observed during the degradation of plastics. The mass might rise during brief exposure periods due to the attachment of microorganisms and/or the incorporation of oxygen. Surface pits and fractures that form during degradation can also get clogged with adhering biomass and other debris (Chamas et al. 2020). It is important to note that the weight increase of microplastics can be due to the debris from soil, substrate, and/or mycelium attached to the surface of the contaminants.

The mycelial growth on the surface of the microplastics suggests fungal biomass production

that is responsible for the degradation and oxidation of plastics (Fig. 7). Oxidative enzymes such as depolymerase depolymerase, esterases, peroxidases, laccases, and oxygenases are proven to be one of the enzymes responsible for the binding of mycelium on the surface of plastics. (da Luz et al. 2013, Khatua et al. 2023, Okal et al. 2023). Hydrophobins from oyster mushrooms can help anchor the fungi on the surface of microplastics. Degradation of microplastics results from the production of enzymes and proteins (Khatua et al. 2023).

Temperature can also affect the development and performance of oyster mushrooms (Hoa & Wang 2018). Based on the reports of Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA 2024), the month of April and May, the Philippines experienced an intense heat index. The mushrooms were kept in the growing house at a temperature of 28-30℃, which was higher than the ideal temperature of 20-25℃ (Nongthombam et al. 2021). According to Aditya and Kumud (2023), high temperatures inhibit the growth and performance of oyster mushrooms. Furthermore, extended periods of observation and testing are recommended to achieve meaningful outcomes from microplastic degradation (Chamas et al. 2020). The heat and duration of the study may have had an impact on the oyster mushroom's ability to remediate pollutants.

The filtration setup developed by the study of Radford et al. (2021) was not efficient enough for the separation of mixtures of soil, oil, and microplastics. The tissue technique was employed to speed up the filtration process. The use of tissue as an absorbent was similar to the method used by Kim et al. (2022), in which they used PDMS-Ni foam to collect MPs that were floating in the oil section of the mixture. Both materials are highly absorbent of oils and suspended microplastics. Both methods serve as baselines for extraction methods and are combined to apply as much efficiency as possible.

The result of the research still varies; this is due to numerous factors it needs to monitor and consider that was discovered through the research's duration, namely the type of plastics, mushroom species, environmental conditions, substrate, soil composition, and the duration of the remediation process, which affects the overall result produced by the study and set up. The study may serve as a baseline for researching similar topics like tackling plastic waste or mushroom research in general. The discussed results may inform future researchers of what crucial factors and conditions need to be considered. Moreover, the research methodology can be replicated in a different setup, such as the usage of other types of fungi or organisms for treatments of soil-contaminated soil. The modified environment and setup may produce more efficient and significant results in the treatments of microplastics.

As numerous factors are required to be considered to implement the treatment procedure in a controlled environment, mainly the temperature and humidity, these two elements affect the growth rate, which in turn affects the effectiveness of the treatment. These elements were unpredictable in a natural environment and could prevent the process of treatment. Other critical factors also include sunlight exposure, wind, and soil composition. The setup cannot be directly replicated and is similar to an off-site treatment to promote an onsite treatment. It may need some certain modification or further research to conclude it will guarantee success in an onsite treatment. To ensure the effectiveness of treatments in diverse environments, it is necessary to conduct a survey of an area or trials to refine procedures and make adjustments. Moreover, further research is needed to conclude it may perform similar results in a natural environment.

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