Isolation and characterization of *Aspergillus niger*: a plastic-inhibiting fungi isolated from different water bodies

[1]Joydip Nandi, [2]Jishu Haldar, [3]Indranil Chatterjee,[4]Sudipta Manik,[5]Bishan Sarkar.

[1]Dipertment of Pharmaceutical Technology, Birbhum Pharmacy School, Birbhum, West Bengal, India.
[2]Dipertment of Pharmaceutical Technology, Birbhum Pharmacy School, Birbhum, West Bengal, India.
[3]Dipertment of Pharmaceutical Biotechnology, Birbhum Pharmacy School, Birbhum, West Bengal, India.
[4]Dipertment of Inorganic Chemistry, Bankura university, Bankura, West Bengal, India.
[5]Dipertment of pharmacology, Birbhum Pharmacy School, Birbhum, West Bengal, India.

[1]nandijoydip2003@gmail.com, [2]jishuhaldar725@gmail.com, [3]indranil.chax@outlook.com,
[4]sudiptamanik75@gmail.com, [5]bishansarkar006@gmail.com

***Abstract* - *Aspergillus niger* was investigated for its potential to degrade low-density polyethylene (LDPE) plastic in aquatic environments. Water samples from ocean, lake, pond, and river sources in West Bengal, India, were analyzed for physical parameters (pH, TDS, conductivity, turbidity) and screened for fungal presence. *A. niger* was isolated from 8 of 36 colonies across seven samples and identified macroscopically (jet-black colonies) and microscopically (conidial heads, septate hyphae). LDPE sheets (2×2 cm) were pretreated (ethanol wash, UV sterilization), immersed in *A. niger* contaminated water, and incubated at 28°C for 30 days. Weight loss measurements revealed progressive degradation, averaging 15% mass reduction (0.00471 g) after 30 days. Optimal degradation occurred in pond samples (29% weight loss), while ocean and river samples showed 11–18% and 10% degradation, respectively. The study confirms *A. niger* role as a potent plastic-degrading fungus in diverse aquatic habitats, supporting its use in bioremediation strategies.**

***Keywords: Aspergillus niger,* Biodegradation, Environmental mycology, Fungal isolation, LDPE (low-density polyethylene), Mycoremediation, Plastic degradation, Water bodies, Weight loss.**

1.INTRODUCTION

Plastic pollution is an urgent environmental problem, with increasing production and resilience of plastic wastes outgrowing cleanup efforts to maintain ecosystems free from plastics (1). The pervasiveness of microplastics (MPs) in aquatic systems is a serious threat to ecosystems and human health since MPs are consumed by different marine species and subsequently by the human food chain (2). As a response to this increasing issue, scientists have been investigating bioremediation processes, specifically mycoremediation, to tackle plastic pollution in water bodies (3).

Fungi have also emerged as potential plastic degraders based on their metabolic ability to degrade complex molecules (3). Recent research has proven the existence of plastic-degrading fungi in different aquatic environments, including the marine environment (4). Certain fungi found in plastic waste have exhibited the ability to degrade plastic, providing the basis for new bioremediation systems in marine plastispheres (4).

The isolation and characterization of plastic-dwelling fungi in marine and freshwater environments play essential roles in determining their ecological functions and future applications in plastic waste treatment. Through the application of sophisticated molecular tools and cultivation strategies, scientists can investigate the diversity and metabolic potential of plastic-dwelling fungi, which could result in the generation of efficient and eco-friendly plastic pollution control methods in aquatic habitats (5,6).

1.1 Types of Plastics Present in our Environment

Plastics are synthetic or semi-synthetic products made up of polymers, which are repeating units that come in the form of long chains of molecules. They are used in a wide range of applications since they are flexible, long-lasting, and cheap to manufacture (7). These classes are widely applied in industrial and domestic use (8). There are a variety of plastics such as (Figure 1).

Figure 1: Types of Plastics.

➢ The production processes of plastic items are primarily composed of two processes:

A. Addition polymerization

Addition polymerization is a type of polymerization reaction in which monomers containing double or triple bonds (usually unsaturated compounds like alkenes) join together without the loss of any small molecules. This process involves the breaking of π (pi) bonds and the formation of σ (sigma) bonds, resulting in long-chain molecules called polymers.

EXAMPLE

Ethene (C_2H_4) undergoes addition polymerization to form polyethene (polyethylene)

$$nCH2=CH2 \rightarrow [-CH2-CH2-]n$$

- Here, 'n' represents the number of repeating units in the polymer chain.

B. Condensation polymerization

Condensation polymerization is a type of polymerization in which monomer units join together with the simultaneous elimination of small molecules such as water, HCl, or methanol. This process usually involves bifunctional or polyfunctional monomers containing two or more reactive groups.

EXAMPLE

Terephthalic acid reacts with ethylene glycol to form polyethylene terephthalate (PET) and water

$$n HOOC - C_6H_4 - COOH + n HO - CH_2CH_2 - OH \rightarrow [-OC-C_6H_4 - COO - CH_2 CH_2 - O -] n + 2n\ H_2O$$

➢ Here, 'n' represents the number of repeating units in the polymer chain.

These responses assemble tiny molecular building blocks into long chains of polymers (7).

Significantly, plastics are divided into two wide classes based on their molecular backbone and their potential to degrade biologically.

This categorization is pertinent in terms of their biodegradability and recyclability potential (9).

1. Hydrolyzable
2. Non-Hydrolyzable

1.2 Pollution made by Plastics

Plastic pollution manifests in various forms in various matrices of the environment and causes widespread ecological damage:

Plastic pollution is prevalent in water bodies, ranging from rivers, oceans, to remote areas like the Arctic (10,11). Macroplastics and microplastics are deposited in water bodies and affect marine life through entanglement and ingestion (10,12). Rivers, in particular, have the function of transporting plastic waste from land to oceans and therefore are channels and victims of plastic pollution (11).

Importantly, plastic pollution is not necessarily apparent as visible litter. It also includes chemical pollution from degrading plastics, with impacts on global warming, ecotoxicity, and air pollution (13). These impacts intensify over time, particularly under conditions of high ultraviolet radiation (13). Furthermore, plastics can act as sinks for persistent organic pollutants (POPs), adding to their difficulty of removal from the environment (14).

Plastic pollution exists on multiple levels, extending to water, soil, and air (15). It varies from macroplastics to microplastics and chemical emissions. The pollution extends not only to urban regions but also to distant points such as the Southern Ocean and the

deep Arctic seafloor (10,16). Such extensive pollution calls for extensive measures to combat plastic pollution at the regional and global scale.

1.3 Effect of Plastic Pollution on Flora and Fauna

Plastic pollution has severe effects on ocean and land flora and fauna. For the ocean, plastic pollution is a worldwide issue, which has effects on ocean health, wildlife, and human health (17). Plastic litter is ubiquitous in coasts worldwide, including remote and uninhabited islands, and has aesthetic and environmental problems (18).

The effect of plastic pollution on wildlife is of particular concern. Marine species are poisoned or disabled by entrapment in plastic waste, starvation or internal injury through ingestion, and impaired quality of life and reproductive potential (18). Land mammals are also affected, with 37 species having ingested plastic and four species using plastic waste as nesting or burrowing material (19). Plastic pollution in rivers directly affects aquatic animals, putting species at risk and causing economic loss (11).

Plastic debris surprisingly also serves as a substrate for colonization by other organisms, including phytoplankton and harmful algae (20). Such "plastisphere" may potentially increase the dispersal of invasive species, which is harmful to the protected and conservation island ecosystems (18,21). The relationship between plastic pollution and biodiversity is, however, complex, as plastic debris may sometimes increase local species diversity by providing new habitats for colonization (21). In short, plastic pollution has extensive effects on animals and plants in ecosystems. While some organisms adapt to or even live in plastic waste, the overall effect tends to be negative, undermining biodiversity and ecosystem functioning. Further studies are needed to fully understand the long-term effects of plastic pollution on world ecosystems and to formulate effective mitigation strategies.

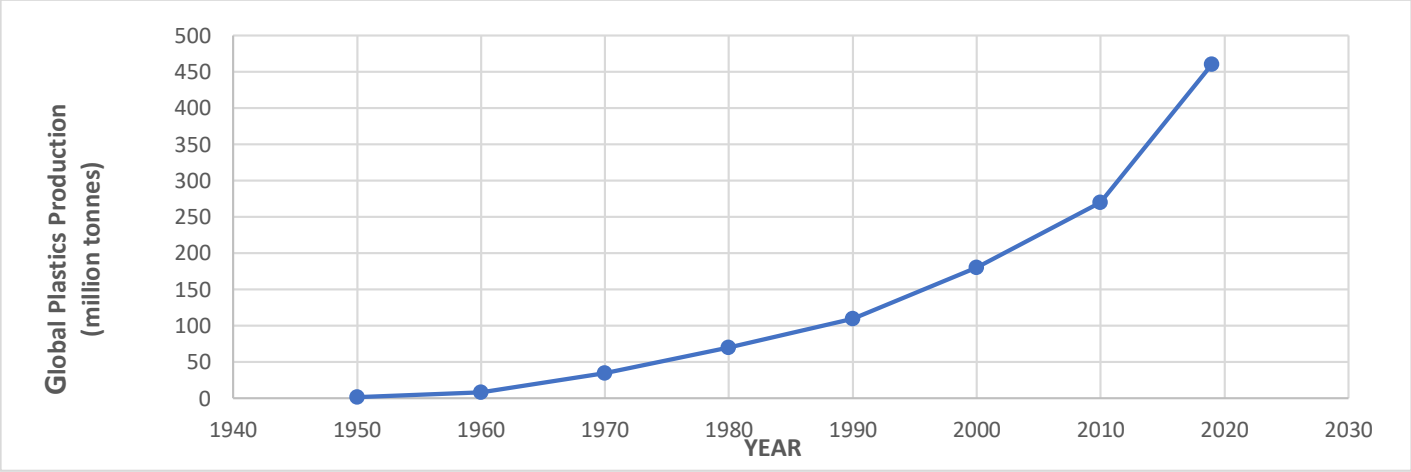
1.4 Statistical Data

Marine debris has peaked at levels of up to 580,000 items per km² in certain areas (22). Plastics production has been rising linearly since the 1950s, and projections indicate up to 12,000 million metric tons of plastic in the environment by 2050 (23).

Studies have shown that 59% of seabird species examined from 1962 to 2012 had plastic in their body, with the average of 29% of the population having plastic in the gut (22). Plastic ingestion is projected to cover 99% of all seabird species by the year 2050, taking into account the current trend (22). For river-borne plastic pollution, more than 1000 rivers provide 80% of the global annual discharges into the ocean, and Asia provides the largest contribution (23).

Despite the sobering figures above, there is a difference between estimated and observed plastic pollution of the open ocean. The bulk load of plastic covering the open ocean was estimated to be tens of thousands of tons, significantly less than expected (24). This suggests the existence of massive size-selective sinks for the elimination of millimeter-sized fragments of plastic on a large scale, either through nano-fragmentation or export to the interior of the ocean (24). Even with quick and concurrent action, an estimated 710 million metric tons of plastic litter will be accumulated in aquatic and terrestrial systems globally by 2040 as soon in Graph no 1 (25). These figures make it more imperative to take global action to alleviate plastic pollution and its effects in marine systems strategies.

Graph 1: Plastic pollution in all over world.



1.5 Role of Microbes in Plastic Degradation

Microbes and fungi are key players in plastic degradation, and their potential to address the environmental problem of plastic pollution is on the increase. Fungi and bacteria have been found to be capable of plastic production and degradation, moving towards the circular economy approach (26).

Fungi, for example, have been promising in plastic degradation because of their wide range of enzymes with specialization in degradation of recalcitrant molecules (27). Some of the fungal enzymes such as laccases, peroxidases, cutinases, lipases, proteases, and ureases have been shown to degrade plastics such as polyethylene , polyvinyl chloride , polyethylene terephthalate, and polyurethane (PUR) (27,28). Some of the effective fungal strains include *Aspergillus spp., Penicillium spp., Bjerkandera adusta, Phanerochaete chrysosporium,* and *Pleurotus spp*. (28).

Endophytic fungi also exhibited the capability of biodegradation of plastic. Polyvinyl alcohol degradation was investigated where *Penicillium brevicompactum* OVR-5, derived from *Orychophragmus violaceus*, yielded an impressive 81% rate of removal of PVA (29). Fungi obtained from Antarctica also showed degradation capacity of polyurethane, polystyrene, and polyethylene, but *Penicillium spp*. yielded the best degradation rates (30). In brief, microbes and fungi offer promising avenues for mitigating plastic pollution with their diverse enzymatic activity. Disentangling the gene-protein-metabolite-environmental factor interactions associated with plastic biodegradation may reveal improved biotechnological strategies for plastic waste management (27,31). Advances in these microbial technologies support sustainable development goals and encourage the transition towards a circular plastic economy (31).

1.6 *Aspergillus niger* as a Plastic inhibiting Fungus

Aspergillus niger is a plastic-dwelling fungus that can degrade various types of plastic products. *Aspergillus niger* was isolated from plastic wastes and was found to have satisfactory plastic degradation ability (4,32).

A.niger has been reported to degrade polythene and plastic efficiently, as well as other fungal isolates such as *Aspergillus flavus, Aspergillus glaucus*, and *Penicillium species* (32). In comparative studies, *A.niger* has been used as a positive control to compare the plastic degradation potential of other fungal isolates (33,34).

Though *A.niger* has been proven to possess plastic degradation capabilities, there is also the consideration that some newly discovered fungal strains have been proven to degrade more quickly. For instance, in the degradation of polyester urethane, Embarria clematidis was discovered to surpass *A.niger* , with increased CO_2 production and increased percentages of clearance of the plastic material (34). *A.niger* is still a valuable species in the study of plastic biodegradation, however, and is a standard by which the performance of other fungal strains is measured.

1.7 Taxonomy of *Aspergillus niger*

Aspergillus niger is a thoroughly studied fungus that represents the genus *Aspergillus* and section *Nigri* (35). It is a member of the *A.niger clade*, whose members are *A.niger sensustricto* and *A.welwitschiae* (36). Black *aspergilli*, including *A.niger* , have been problematic in terms of taxonomy due to interspecific morphological similarities (35).

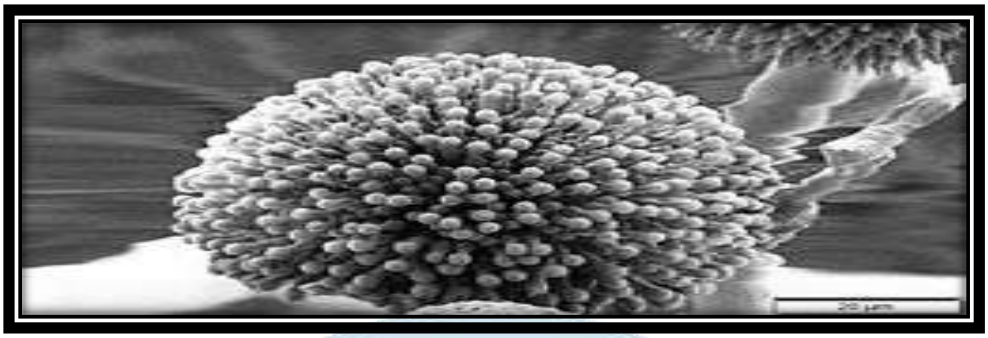
Surprisingly, new research has shown new information on the taxonomy of *A.niger* . *Aspergillus niger var taxi*, a new variant, was found to be a taxol-producing endophytic fungus from *Taxus cuspidata* in China (13). A new phylogenetic species, *Aspergillus vinaceus sp. nov*., was found in the *A.niger clade*, differentiated by its potential to produce asperazine and sclerotia (36).

Lastly, despite *A.niger 's* specificity as a separate species, it continues to shift in its classification. Modern techniques of gene sequencing from multi-loci and phylogenetics have taken precedence in delineating the otherwise near-kin members of *A.niger* species (35,36). Through all these updates to its classification, more becomes understood about *A.niger* species and that which is allied, their biodiversity as well as function within given environs.

Aspergillus niger is included in *Aspergillus subgenus Circumdati*, section *nigri*. The section *nigri* includes 15 related black-spored species that may be confused with *A.niger* , including *A.tubingensis, A.foetidus, A. carbonarius,* and *A.awamori.*

Figure 2: Photomicrograph Showing the Conidial head (Conidiophore) Of Aspergillus niger (Access on 06/07/2025 https://en.wikipedia.org/wiki/Aspergillus_niger).

Figure 3: Details Of the Head by Scanning Electron microscope of A.niger (Access on 06/07/2025 https://en.wikipedia.org/wiki/Aspergillus_niger).



1.8 Scientific Classification

Table 1: Scientific classification of Aspergillus niger.

Domain:	*Eukaryota*
Kingdom:	*Fungi*
Division:	*Ascomycota*
Class:	*Eurotiomycetes*
Order:	*Eurotiales*
Family:	*Aspergillaceae*
Genus:	*Aspergillus*
Species:	*A.niger*

2. LITERATURE REVIEW

2.1. Anusha h ekanayaka, *et al.,* reviews the current knowledge on fungi that can degrade various synthetic plastics and analyses the phylogenetic relationships of these plastic-degrading fungi. Plastic-degrading fungi belong to three major fungal phyla*: Ascomycota, Basidiomycota*, and *Mucoromycota*. The *Eurotiomycetes* class with in *Ascomycota* contains the highest number of recorded plastic-degrading fungi. There is a need for more research on plastic-degrading fungi to address the global problem of plastic accumulation. In this paper containing lack of sequence data directly from the plastic degrading fungal strains used in the studies. Need for more comprehensive genomic and evolutionary analyses of plastic-degrading fungi lack of confirmation of fungal degradation abilities on large sized plastic waste in natural environments need for more detailed in situ studies to understand plastic-fungi interactions.

2.2. Fatimah alshehrei, *et al.,* Investigate that the study isolated several fungal strains from red sea water and found that the *penicillium sp*. Strain showed the highest ability to biodegrade low-density polyethylene, reducing its weight by 43.4%. *Penicillium sp*. Showed the highest percentage of LDPE degradation at 43.4%. Other Aspergillus species also showed moderate LDPE degradation activity. Fungal growth and physical association with the LDPE surface was observed through microscopy, indicating the fungi were able to colonize and degrade the plastic. In this paper consisting with lack of sequence data directly from the plastic-degrading fungal strains used in the studies. Need for more comprehensive genomic and evolutionary analyses of plastic-degrading fungi. Lack of confirmation of fungal degradation abilities on large-sized plastic waste in natural environments need for more detailed in situ studies to understand plastic-fungi interactions and degradation mechanism.

2.3. Sagar kittur, *et al.,* Analyse that *Aspergillus niger* can biodegrade polyethylene bags, and the optimal pre-treatment method is a combination of UV and nitric acid, which resulted in 27.33% degradation in 30 days when using a molasses-based media. The combination of UV and nitric acid pretreatment, when used with a molasses-based media, was the most effective method for the biodegradation of 40-micron polyethylene bags. The optimal exposure time was 4 days of UV treatment followed by 5 days of nitric acid treatment. The pretreatment method involving 4 days of UV exposure and 5 days of nitric acid treatment was more effective than other pre-treatment methods tested. In this paper limited FTIR analysis, focusing only on a few specific wave numbers testing a limited number of media formulations, with no comparison to other potentially more effective media lack of quantitative data on the extent and rate of plastic degradation achieved using the optimized molasses media.

2.4. E munir, *et al*., determined that two fungal strains, *Trichoderma viride* and *Aspergillus nomius*, isolated from landfill soil were able to degrade LDPE, as evidenced by a reduction in the weight and tensile strength of LDPE film after incubation with these fungal isolates. The two fungal isolates, rh03 and rh06, were able to degrade LDPE plastic, reducing its weight by 5.13% and 6.63% respectively after 45 days of cultivation. The two fungal isolates were also able to significantly reduce the tensile strength of the LDPE film, with rh03 reducing it by 58% and rh06 reducing it by 40%. the two fungal isolates were identified as *Trichoderma viride* and *Aspergillus nomius* through molecular analysis. The hydrophobic nature and limited functional groups of LDPE may have limited the ability of the fungi to degrade it, resulting in slower growth and degradation. The degree of degradation observed (5-6% weight loss) was relatively low, suggesting the degradation ability of the fungi may be limited. There is limited prior research on

the ability of *Trichoderma viride* and *Aspergillus nomius* to degrade LDPE, compared to other more commonly reported fungi, which may limit the generalizability of the findings.

2.5. Sakshi Varshney, *et al.,* provides an overview on the role of fungi in the systematic degradation of plastic, discussing the various fungal strains, biodegradation mechanisms, and assessment techniques involved. Various fungal strains, including *aspergillus nomius*, *Trichoderma viride*, *Cephalosporium sp*., *Stagonosporopsis citrulli*, *Colletotrichum fructicola*, *Diaporthe italiana*, and others, have been found to successfully and efficiently degrade various plastic polymers. The mechanism of biodegradation includes biodeterioration, biofragmentation, assimilation, and mineralization, which involve different enzyme functions and bond cleavage. Numerous assessment techniques, such as SEM, atomic force microscopy, fourier-transform infrared spectroscopy, and gas chromatography, have been used to measure the degree of plastic biodegradation. The current understanding of the specific biodegradation mechanisms and enzymes involved in plastic degradation is limited and requires further investigation. The paper does not provide information on specific potent microbial consortia or enzymes that could be used for effective plastic biodegradation.

2.6. R Pramila, *et al.,* isolated two fungal strains, *Aspergillus versicolor* and *Aspergillus sp*., from marine water that were able to degrade LDPE as the sole carbon source. Two fungal isolates, sb and sd, were able to utilize LDPE as the sole carbon source for growth. The fungal isolates sb and sd were able to degrade LDPE, as evidenced by the production of carbon dioxide during their growth. SEM analysis of the LDPE films colonized by the fungal isolates showed structural changes such as the formation of pits, cracks, and holes, indicating biodegradation of the LDPE. The fungi isolated can degrade LDPE, but the process takes a relatively long time (3-4 months), so further research is needed to find microbes that can degrade LDPE more quickly. While the fungi were able to colonize the LDPE surface, this did not necessarily correlate with their ability to efficiently degrade the LDPE, suggesting the need for further research on the relationship between surface colonization and biodegradation. The authors state they have shown LDPE can be completely degraded into carbon dioxide, but acknowledge that the "right conditions" for this to occur still need to be determined through further research.

2.7. N raaman, *et al.,* found that their study found that the fungal strains *Aspergillus niger* and *Aspergillus japonicus* were able to degrade LDPE plastic by 8-12% over a 4-week period, with *Aspergillus japonicus* showing a higher degradation potential of 12% as compared to 8% for *A.niger*. The study found that the fungal strains *Aspergillus niger* and A. japonicus were able to degrade low-density polyethylene (LDPE) plastic by 8-12% over a 4-week period, with A. japonicus showing a higher degradation potential of 12% compared to 8% for *A.niger*.

2.8. Glen cletus Dsouza, *et al*., Evaluated that the biodegradation of LDPE using a consortium of *Aspergillus species* under controlled laboratory conditions, with the extent of biodegradation analysed through weight loss, SEM, and FT-IR analysis. The fungal consortium of *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus oryzae* was able to degrade LDPE under controlled laboratory conditions, with a maximum weight loss of 26.15% over 55 days using potato dextrose broth media.The use of the fungal consortium resulted in higher degradation rates compared to using individual fungal species. The addition of the surfactant tween 80 did not significantly improve the degradation rates. The study focused on using fungal species in controlled environments for LDPE biodegradation, which is a relatively new and underexplored area compared to using bacterial cultures in natural environments. The study briefly explored using shredded LDPE as the sole carbon source, suggesting this is a promising area for further investigation. The specific growth rate values obtained are only applicable to the particular *Aspergillus* consortium used in this study, and further research would be needed to determine growth rates for other fungal consortia.

2.9. Kohinoor kaur, *et al.* Has elaborate a comprehensive review of the diverse microorganisms that can biodegrade PET plastic, discussing their enzymatic mechanisms, influencing factors, and potential applications in waste management and bioremediation. A diverse range of microorganisms, including bacteria, fungi, algae, and wax worms, have been found to be capable of naturally degrading pet plastic. The *Bacterium Thermobifida fusca* is a key PET degrading microorganism, with its enzymes exhibiting high stability and activity under a wide range of environmental conditions. The bacterium *Ideonella sakaiensis*, also known as the "first plastic-eating bacteria," can break down pet plastic using specialized enzymes that cleave the polymer into its monomeric components. The challenge of preventing plastic pollution, which the researchers acknowledge as "quite impossible" the possibility that the fungal isolates came from external sources rather than being native to the ohiakwu river, which could limit the generalizability of the findings. The complexity of microbial communities in the river, which the researchers describe as "very challenging", potentially limiting the full understanding of the degradation potential of the isolated fungi.

2.10. Ivano brunner, *et al.,* Had isolated over 100 fungal strains from plastic debris floating in a lake, identified 12 different fungal species, and found that none could degrade polyethylene but 4 could degrade polyurethane. None of the fungal strains isolated from plastic debris were able to degrade Polyethylene. 4 fungal strains isolated from plastic debris were able to degrade polyurethane. Two additional fungal strains not isolated from plastic debris, *Agaricus bisporus* and *Marasmius oreades*, were also able to degrade PU. Guild of the fungi (e.g. saprotrophs, pathogens, symbionts) would help identify fungi with potential to degrade plastics the paper proposes that if fungi or other microorganisms capable of degrading plastics could be identified, their spores or enzymes could potentially be incorporated into plastic materials during manufacturing to enable degradation when the plastic is discarded.

2.11. Bs banko, *et al.,* Describe the paper describes a screening method to identify fungal strains with the potential to degrade various plastic polymers, focusing on strains from hydrocarbon-rich and hypersaline environments. Screening of 146 fungal strains identified 83 strains that produced more CO_2 when exposed to a mixture of plastic polymers compared to the control. Further testing of 9 top-performing strains showed that 5 strains (*Wickerhamomyces anomalus, rhodotorula sp., Rhodotorula dairenensis, Cladosporium sp.,* and *Rhodotorula diobovata*) produced more CO_2 when exposed to individual plastic polymers. FTIR-ATR and raman spectroscopy analysis confirmed that the presence of these fungi resulted in changes to the chemical structure of the plastic polymers. Respirometry can only detect mineralization of the polymer, and cannot detect microorganisms that are only capable of depolymerization without further utilization of the oligomers or monomers high variability between biological replicates, potentially caused by differences in initial inoculum or incubation conditions, leading to some cases of decreased CO_2 production the

requirement for additional confirmation tests beyond respirometry increases the complexity of the screening approach, though it also provides more insight into the degradation capacity of individual microorganisms.

3. RESEARCH METHODOLOGY

3.1. Materials and Method

3.1.1. Instruments & Materials

Sterile glass bottle, tds meters, digital ph meter, digital electrical conductivity meter, micro processer nephelometry turbidity meter, bod incubator, autoclave, hot air oven, laminar air flow, conical flask, test tube, beaker, petri dish, inoculating loop, uv sterilizer, digital weighing machine, low density poly ethylene sheets, glass slide, microscope (compound binocular), micropipette and thermometer.

3.1.2 Chemicals Used

Potato Dextrose Ager with Chloramphenicol, Lactophenol, Cotton Blue solution, Ethanol, Agar agar, and distil water.

3.2 Collection and Incubation of Water Samples

Water samples were collected from four distinct aquatic environments: Ocean, lake, pond, and river. From each source, three different samples were obtained, resulting in a total of twelve water samples. The collection was carried out using sterile glass bottles to prevent any external microbial contamination. Immediately after collection, the samples were securely sealed and transported to the laboratory under controlled conditions. Upon arrival, all samples were incubated at 28 °C for 14 days to promote the growth for isolation of potential plastic-inhibiting fungi. This incubation period aimed to allow sufficient microbial development, facilitating the subsequent isolation and characterization of *Aspergillus niger*. The geographic coordinates (latitude and longitude) of each sampling site were recorded (Table 2).

Table 2: Location mapping of Samples collected.

Sample Source	Sample No.	Sample Code.	Location Name	Latitude (°N)	Longitude (°E)
Ocean	1	O1	DIGHA MOHONA	21.629413N	87.548505E
Ocean	2	O2	TALSARI, DIGHA	21.603836N	87.462158E
Ocean	3	O3	TALSARI, DIGHA	21.603592N	87.465172E
Lake	1	L1	GANGDUA, AMARKANAN	23.407900N	87.087105E
Lake	2	L2	KHANTA, BANKURA	23.826242N	87.087105E
Lake	3	L3	CHINPI, BIRBHUM	23.826242N	87.416824E
Pond	1	P1	PRATAPBAGAN, BANKURA	23.240292N	87.061653E
Pond	2	P2	CHINPI, DUBRAJPUR	23.8243113N	87.4370936E
Pond	3	P3	DUBRAJPUR, BANKURA	23.828940N	87.428717E
River	1	R1	GANDHESWARI RIVER	23.264252N	87.070793E
River	2	R2	SALI RIVER	23.405352N	87.085620E
River	3	R3	DARKESWAR RIVER	23.211073N	87.078484E

3.3 Physical Tests of The Water Samples

3.3.1 Determination of pH

As part of the physical analysis in this study, the pH of twelve water samples collected from various aquatic environments including rivers, oceans, ponds, and lakes was measured to assess the acidity or alkalinity of the water in which *Aspergillus niger* was potentially present. The pH testing was conducted using a calibrated digital pH meter following standard protocols to ensure accuracy and reliability. Before measurement, the pH meter was properly calibrated using standard buffer solutions (pH 4.0, 7.0, and 10.0). Each water sample was collected in a clean, sterilized container and brought to room temperature before testing. The electrode of the pH meter was rinsed with distilled water and gently blotted dry between readings to avoid cross-contamination. The pH values recorded ranged across a spectrum, reflecting the natural variations in water chemistry across the different sources. These pH readings play a critical role in understanding the environmental conditions that may influence the growth and plastic-degrading potential of *Aspergillus niger* in these habitats (37).

3.3.2 Determination of Total Dissolve Solid

To better understand the environmental conditions where *Aspergillus niger* might grow, I had measured the TDS in twelve water samples collected from rivers, oceans, ponds, and lakes respectively. This test was done by using a digital TDS meter, and all necessary steps were followed to ensure accurate results. Before testing, each water sample was brought to room temperature, and the meter was properly calibrated according to the standard procedure. The probe was carefully rinsed with distilled water between each measurement to avoid any cross-contamination. The TDS readings helped to determine the amount of dissolved substances like minerals, salts, and organic matter present in each sample. These values are important because they give us an idea of the water's quality and how suitable the environment might be for the growth and plastic-degrading activity of *Aspergillus niger* (38).

3.3.3 Determination of The Electrical Conductivity of Water Samples

The electrical conductivity (EC) test was performed using a digital conductivity meter, following all necessary steps to ensure reliable results. Before testing each sample, the device was properly calibrated, and the probe was carefully rinsed with distilled water to avoid contamination. The water samples were allowed to settle at room temperature, as temperature can affect Electrical conductivity readings. Electrical conductivity gives an idea of how many dissolved salts or ions are present in the water. These values are useful because they help indicate how suitable the water might be for the growth and plastic-degrading activity of *Aspergillus niger* in different types of aquatic environments.

3.4 Isolation and Identification of Fungal Strains from Water Samples

3.4.1 Preparation of Potato Dextrose Agar (PDA) Media with Chloramphenicol for Fungal Isolation.

To isolate *Aspergillus Niger* and other fungi from water samples, Potato Dextrose Agar (PDA) supplemented with chloramphenicol was prepared following standard microbiological protocols. The commercially available PDA powder with chloramphenicol (manufactured by Redymed Company) was used to ensure uniformity in media composition. Briefly, 40 g of PDA powder with chloramphenicol was dissolved in 1 L of distilled water and sterilized by using autoclave at 121° C for 15 minutes. After cooling to approximately 45–50° C, thereby facilitating selective fungal isolation without any bacterial growth in the media. The medium was then dispensed into 12 different sterile Petri plates. At a time 3 sterile petri plates are inoculated with water samples under laminar airflow hood to prevent contamination. This selective PDA medium was employed to culture fungi from water samples collected across diverse aquatic environments, including lakes, ponds, rivers, and oceans respectively, to isolate and characterize *Aspergillus niger*, a plastic-degrading fungal species. Strict aseptic techniques were maintained throughout the procedure to ensure the reliability of the results (39).

3.4.2 Inoculation of Water Samples on the Culture Media

For the isolation of *Aspergillus Niger*, a total of 12 water samples were collected from four different aquatic sources rivers, oceans, ponds, and lakes respectively. With three replicates from each site to ensure representative sampling. The samples were collected aseptically using sterile containers, maintaining proper sterility to prevent contamination. Immediately after collection, the samples were transported to the laboratory under cold conditions to preserve microbial viability. For fungal isolation, 100 µl from appropriate dilutions were spread onto prepared PDA plates supplemented with chloramphenicol (50 mg/L) to inhibit bacterial growth. The plates were then incubated at 28±2° C for 5–7 days to allow fungal growth. Distinct colonies resembling *Aspergillus niger* (based on morphological characteristics such as dark sporulation and velvety texture) were subculture onto fresh PDA plates for pure cultures and further characterization. This standardized procedure ensured the effective isolation of plastic-inhibiting fungi from diverse aquatic environments for subsequent identification and study (40).

3.4.3 Determination of NTU Value

The nephelometric turbidity unit (NTU) value serves as an indirect indicator of microbial contamination, as higher turbidity levels often correlate with increased suspended particles that harbour microorganisms. In this study, 12 different water samples were incubated for 7 days with soft agar broth to assess microbial growth in relation to NTU values. Each sample is analysed using a calibrated turbidity meter to determine turbidity levels. Elevated NTU readings corresponded with greater particulate matter, which provided attachment sites and nutrients for bacteria, fungi, and other microbes, indicating higher microbial loads. Conversely samples with low NTU values exhibited clearer water with fewer suspended solids, suggesting reduced microbial loads. The incubation process with soft agar broth allowed for microbial proliferation, reinforcing the observed correlation between turbidity and microbiological burden. These findings align with established water quality standards, where high turbidity is linked to elevated health risks due to potential pathogen proliferation. By combining NTU measurement with soft agar incubation, this method provides a rapid, preliminary assessment of both water clarity and microbial contamination (41).

3.4.4 Macroscopic Identification of *Aspergillus niger*

After incubation, the fungal colonies grown on Potato Dextrose Agar (PDA) plates which were examined for macroscopic features to preliminarily identify *Aspergillus niger*. The colonies were observed with the naked eye using magnifying glass for key characteristics such as colour, texture, growth rate, and colony morphology. *Aspergillus niger* typically exhibited rapid growth, covering the plate within 3–5 days at 28±2°C Initially, the colonies appeared white or pale yellow, but as sporulation began, they developed a distinctive jet-black or dark brown coloration due to the dense production of conidial heads. The surface texture was velvety to powdery, while the reverse side of the colony (underside of the agar) often appeared pale yellow to cream-colored. The colony margins were usually entire (smooth), and in some cases, a slight wrinkled or radial furrowed pattern was observed. These macroscopic traits provided a strong preliminary indication of *Aspergillus Niger*, which was later confirmed through microscopic analysis. And After Matching With The Reference Sample Of *Aspergillus niger* (42).

Colony Appearance

1.Color:

- Young colonies (2–3 days): White or pale yellow due to initial hyphal growth.
- Mature colonies (5–7 days): Jet black or dark brown spore mass (conidial heads), often with a velvety or powdery texture.
- Reverse side: Pale yellow to cream-colored (some strains may show dark pigment diffusion into the agar).

2. Texture & Growth Pattern

- Surface: Velvety, cottony, or granular due to dense conidiophores.
- Edge: Entire (smooth) or slightly irregular.
- Growth rate: Rapid (colonies typically reach 3–5 cm diameter in 5–7 days at 25–30°C).

3. Pigmentation Variations

- Some strains may produce yellowish zones (due to secondary metabolites like ochratoxins).
- Rare variants may appear greyish if sporulation is delayed (43).

3.4.5 Preparation of The Staining Solution

1. Mixing the Solutions
   - In a clean glass beaker, combine the readymade Lactophenol solution and Cotton Blue solution in a 10:1 ratio (e.g., 10 mL Lactophenol + 1 mL Cotton Blue).
   - Stir gently (using a magnetic stirrer or glass rod) to ensure uniform mixing. Avoid vigorous shaking to prevent bubble formation.
2. Adjusting Concentration
   - If the Cotton Blue solution is highly concentrated (e.g., >0.1%), dilute it slightly with distilled water before mixing with Lactophenol.
   - The final solution should appear clear and deep blue without undissolved particles.
3. Storage
   - Transfer the prepared LPCB solution into an amber bottle to protect it from light degradation.
   - Label with the preparation date and store at room temperature (43).

3.4.6 Microscopic Identification of *Aspergillus niger* Using Staining Technique

Following macroscopic observation, definitive identification of *Aspergillus niger* was conducted through detailed microscopic examination using lactophenol cotton blue (LPCB) staining and light microscopy (100X-400X magnification). The procedure involved aseptically transferring fungal growth to a glass slide, staining with LPCB, and gently teasing the sample to observe key diagnostic structures under a compound microscope. A small portion of the fungal colony was carefully lifted with a sterile inoculating needle and placed on a clean glass slide. A drop of LPCB stain was added, followed by gentle teasing of the mycelium with needles to separate the structures (44).

➢ Distinct Microscopic Features of *Aspergillus niger.*

1. Conidiophores

- Structure: Long, smooth, hyaline (transparent), and thick-walled.
- Origin: Unbranched, arising from foot cells embedded in the mycelium.

2. Vesicles

- Shape: Globose to sub globose, covering the entire apex of the conidiophore.
- Fertile Surface: Bears phialides in a biseriate (two-layered) arrangement, with metulae supporting phialides (45).

3.Phialides

- Primary Metula: Radiate from the vesicle, forming the first layer.
- Secondary Phialides: Produce chains of conidia.

4.Conidia (Spores)

- Appearance: Dark brown to black, spherical, with echinulate (spiny) ornamentation.
- Arrangement: Form dense, radiating chains, giving the colony its characteristic black colour.

5. Supporting Structures

- Hyphae: Septate, with porous septa allowing cytoplasmic streaming.
- Hulle Cells: Thick-walled sterile cells occasionally present, though more commonly associated with Aspergillus sexual structures(46).

3.4.7 Pretreatment of LDPE Samples

After carefully examining the collected samples under the microscope and confirming the distinct features of *Aspergillus niger* like its dark spores and radiating conidial heads we identified this fungus in eight of the twelve environmental samples. To test how well *A.niger* could break down plastic, we prepared small squares of LDPE (2 cm × 2 cm) as test materials. First, we softened the LDPE sheets by boiling them at 50°C, making the plastic more accessible to fungal enzymes. Then, we washed the sheets with ethanol to remove any contaminants and sterilized them under UV light for 14 minutes to ensure no unwanted microbes interfered with the experiment. This step-by-step preparation was crucial to create a controlled environment where *A.niger* could interact with the plastic without competition from other organisms (47).

3.5. Inoculation of The LDPE Sheet in Water samples Contaminated with *A.niger*

After preparing our little plastic squares, we set out to see just how well our *A. ngier* fungi could break them down. We took five large test tubes like the ones you'd see in a mad scientist's lab and filled each one with a different sample of our contaminated water, each teaming with their own population of *A. nigier*. Before we let the fungi go to work, we carefully weighed each LDPE sheet on our precision scale, recording these initial weights like scientists taking baseline measurements before an important experiment.

Then came the exciting part we submerged each plastic square into its designated test tube, watching them disappear beneath the murky surface where hungry fungi waited. It felt a bit like launching tiny ships into uncharted microbial waters Now, safely tucked away in their incubation chambers, these test tubes will become miniature worlds where we'll observe the silent battle between fungi and plastic. Every few days, we'll rescue our plastic squares, gently dry them, and weight them again, watching for those telltale signs of weight loss that indicate our fungal friends are winning the fight against pollution.

The beauty of this simple experiment lies in its clarity by comparing those initial weights to the final numbers, we'll get hard evidence of just how effective *A.niger* can be at tackling our plastic waste problem. It's amazing to think that these microscopic organisms might hold the key to solving one of our biggest environmental challenges.

Before the Introducing The of Low-Density Polyethylene sheet In *A.niger* contaminated Water we measure the initial weight of LDPE sheets (47).

3.6. Incubation of Water Samples Containing LDPE Sheet

Sterilized low-density polyethylene (LDPE) sheets (2 × 2 cm) were aseptically introduced into test tubes containing *A.niger* contaminated water sample (representing ocean, lake, pond, and river sources). The tube were incubated in a BOD incubator at 28° C under static conditions to promote fungal colonization. At 10-day intervals, LDPE sheets were carefully extracted, rinsed with sterile distilled water to remove adherent biomass, and oven-dried at 50° C to constant weight. Percentage weight loss was calculated using the formula:

$$\text{Weight loss (\%)} = \left(\frac{Wi - Wf}{Wi}\right) \times 100$$

Wi = Represent Initial Weights.

Wf = Represent Final Weights.

And to assess structural degradation, LDPE sheets were gold-coated and analysed via SEM at 5-15 kV, with particular attention to surface pitting, hyphal penetration, and biofilm distribution. Control samples (LDPE in sterile water) were processed identically to account for abiotic degradation (48).

4. DISCUSSION

4.1 Physical Parameters of The Water Samples

The water samples wear collected from different environments oceans, lakes, ponds, and rivers showed distinct physical characteristics that could influence the growth and activity of *Aspergillus niger*. From (table 3), Ocean water had the highest mineral content, with TDS levels ranging from 862 to 911 ppm and Electrical conductivity between 1326 and 1401 µs/cm, along with a slightly alkaline pH (7.82–8.43). This makes sense, as seawater naturally contains more dissolved salts. In contrast, freshwater sources like lakes and rivers had much lower TDS (64–475 ppm) and electrical conductivity (98–732 µs/cm), with pH levels closer to neutral or slightly acidic (6.09–7.22). Ponds fell somewhere in the middle, with moderate TDS (328–682 ppm) and a mildly acidic pH (6.45–6.79). Since *A.niger* is known to grow best in slightly acidic to neutral conditions, these findings suggest that freshwater environments particularly lakes and ponds might be more suitable for this fungus compared to the highly saline ocean. The lower mineral content and favourable pH in these water bodies could create better conditions for *A.niger* to thrive and potentially break down plastic. However, further analysis linking these physical parameters with fungal isolation data will help confirm this relationship.

Table 3: Physical Parameters of The Water Samples.

Sample Source	Sample No.	Sample code	T.D.S	Electric Conductivity(µS/cm)	pH
Ocean	1	O1	862	1326	8.10
Ocean	2	O2	863	1328	8.43
Ocean	3	O3	911	1401	7.82
Lake	1	L1	304	468	7.01
Lake	2	L2	64	98	6.32
Lake	3	L3	122	188	7.22
Pond	1	P1	328	505	6.45
Pond	2	P2	425	655	6.62
Pond	3	P3	682	1050	6.79
River	1	R1	161	248	6.09
River	2	R2	90	139	6.98
River	3	R3	475	732	6.92

4.2 Determination of The Microbial Load in The Water Samples

As shown in table 4, The ocean samples showed moderate cloudiness (5-9 NTU), which isn't surprising since seawater typically contains plenty of microscopic life and organic matter. The ponds presented an interesting mix, ranging from quite clear (2 NTU) to moderately cloudy (8 NTU). Rivers told the most dramatic story while two samples were relatively clear (3-6 NTU), one sample (R2) was exceptionally cloudy with 20 NTU, suggesting either heavy microbial activity or possibly runoff from land. The lake samples remained consistently clear (2-4 NTU), painting them as the most pristine-looking of all the water bodies tested. What does this mean for our plastic-degrading fungi? The varying turbidity levels suggest different microbial ecosystems across these environments. The cloudier waters (like river sample R2 and some pond/ocean samples) might be richer feeding grounds for *A.niger* , while the clearer lake waters could represent more challenging environments. However, appearances can be deceiving sometimes clear water still hosts significant microbial life. That's why we'll need to combine these observations with our actual fungal isolate results to get the full overview of where *A.niger* prefers to grow. Moreover, River sample R2 stood out as exceptionally cloudy at

(20 NTU), Lakes consistently showed the clearest water with (2-4 NTU) while Oceans and ponds fell somewhere in between (2-9 NTU). Turbidity gives us clues, but we'll need culture results to confirm fungal presence.

Table 4: NTU Value of The Water Samples.

SAMPLE SOURCE	SAMPLE NO	SAMPLE CODE	NTU
Ocean	1	O1	5
Ocean	2	O2	8
Ocean	3	O4	9
Pond	1	P1	8
Pond	2	P2	2
Pond	3	P3	3
River	1	R1	3
River	2	R2	20
River	3	R3	6
Lake	1	L1	3
Lake	2	L2	4
Lake	3	L3	2

Figure 4: Fungal colony Formation in Water samples- O1, O2, O3, L1, L2, L3.

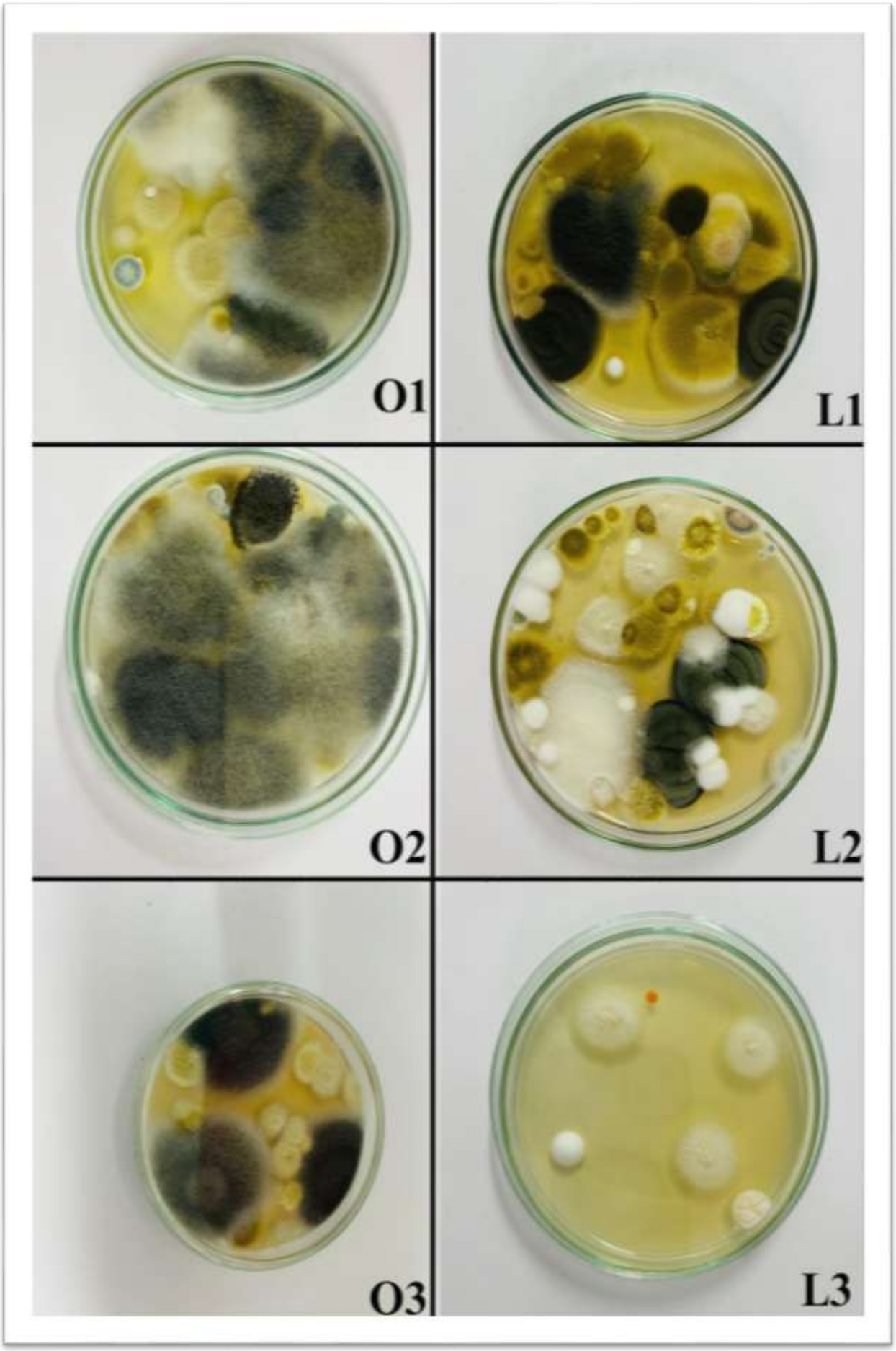
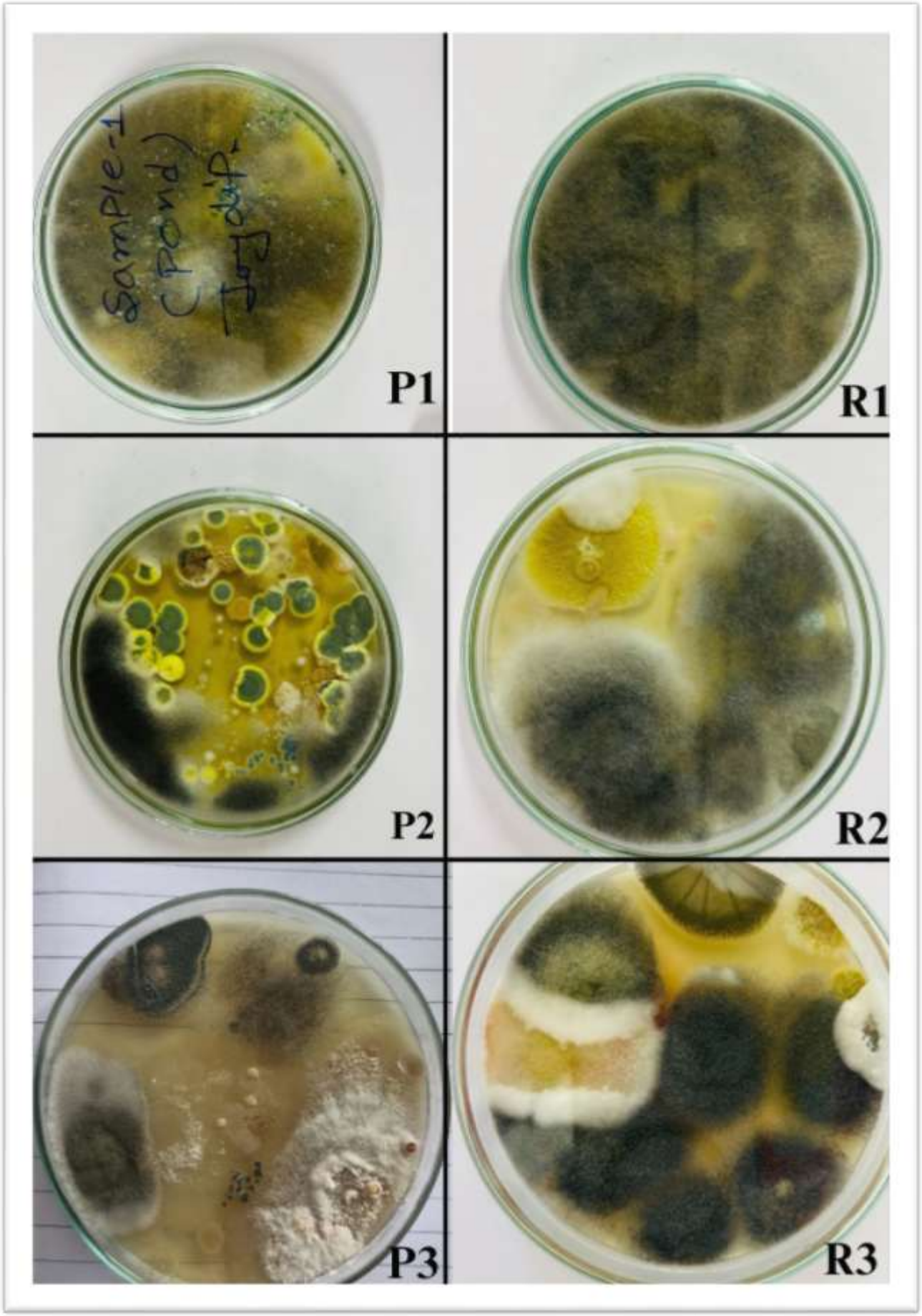


Figure 5: Fungal colony Formation in Water samples- P1, P2, P3, R1, R2, R3.



4.3 Determination of The Fungal Variety Present in Different Water Samples

When we cultured fungi from different water sources, we uncovered a vibrant tapestry of microbial life. Ocean samples grew 10 colonies total (O1: 5, O2: 2, O3: 3), lakes yielded 8 (L1: 5, L2: 2, L3: 1), ponds produced 9 (3 per sample), and rivers totalled 8 colonies (R1:1, R2:4, R3:3) respectively as shown in table 5. The plates bloomed with striking diversity jet-black, pale yellow, brown, green, white, and gray colonies painting each water body with its own "fungal fingerprint."

Table 5: Quantification and colony morphology of Fungal growth on PDA plates.

Sample source	Sample no	Sample code	No of fungal Colony found	Colony code	Colour of the Colony	Identify as *Aspergillus Niger*
Ocean	1	O1	5	A1	Jet black	Yes
				A2	Pale yellow	No
				A3	Brown	No
				A4	Gray	No
				A5	White	No
Ocean	2	O2	2	B1	Gray	No
				B2	Jet black	Yes
Ocean	3	O3	3	C1	Dark black	No
				C2	Gray	No
				C3	Pich yellow	No
Lake	1	L1	5	D1	Greenish Black	No
				D2	Dark Black	No
				D3	Pich yellow	No
				D4	Black	No
				D5	White	No
Lake	2	L2	2	E1	Black	No
				E2	White	No
Lake	3	L3	1	F1	White	No
Pond	1	P1	3	G1	White	No
				G2	Pale yellow	Yes
				G3	Green	No
Pond	2	P2	3	H1	Jet black	Yes
				H2	Greenish yellow	No
				H3	White	No
Pond	3	P3	3	I1	Gray	No
				I2	Jet black	Yes
				I3	White	No
River	1	R1	1	J1	Gray	No
River	2	R2	4	K1	Brown	Yes
				K2	Pich yellow	No
				K3	Sap green	No
				K4	White	Yes
River	3	R3	4	M1	Dark brown	Yes
				M2	White	No
				M3	Black	No
				M4	Yellow	No

Figure 6: Microscopy of The Fungal Colonies- (J1, K1, K2, K3, K4, M1, M2, M3, M4).

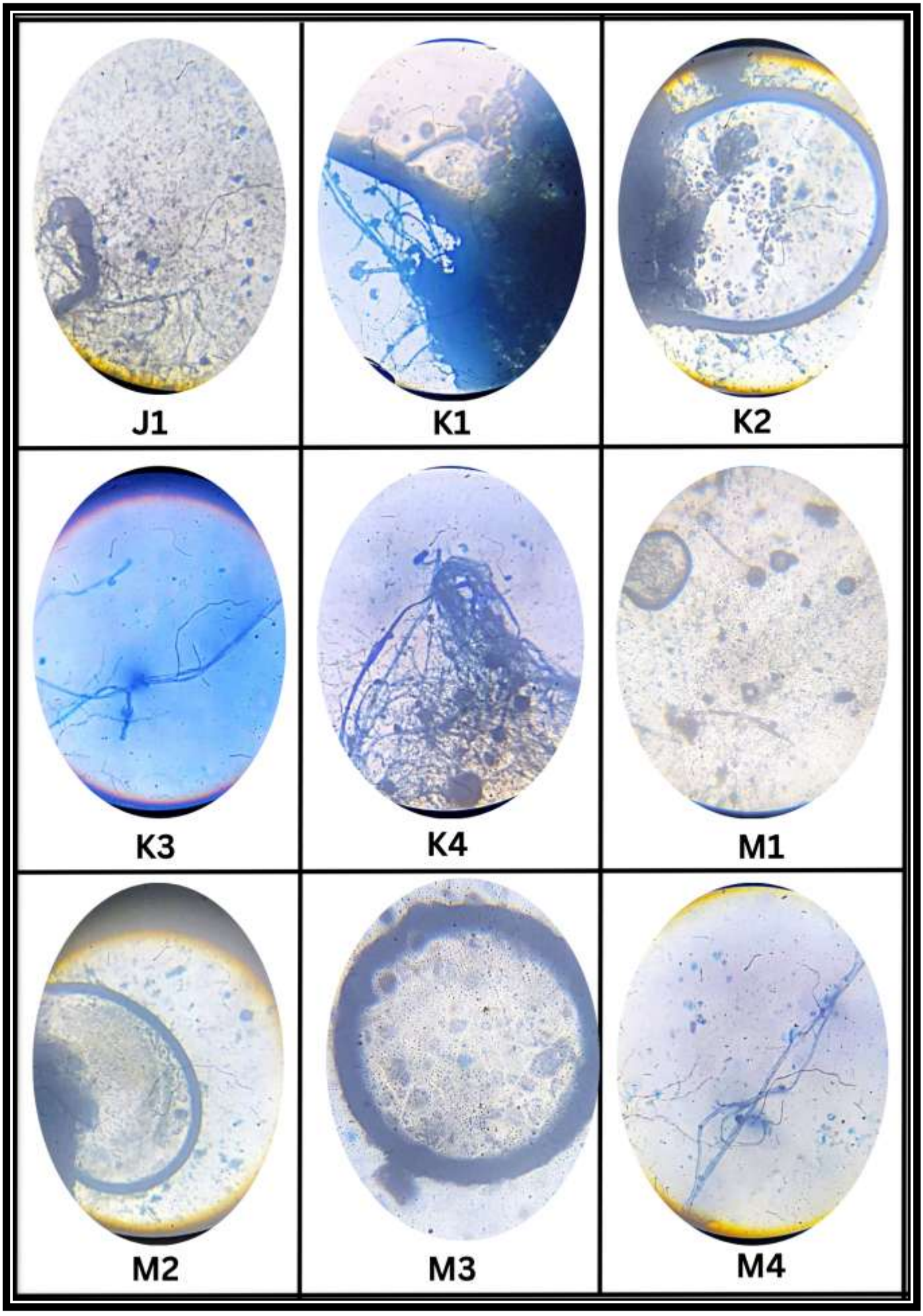


Figure 7: Microscopy of The Fungal Colonies- (G1, G2, G3, H1, H2, H3, I1, I2, I3).

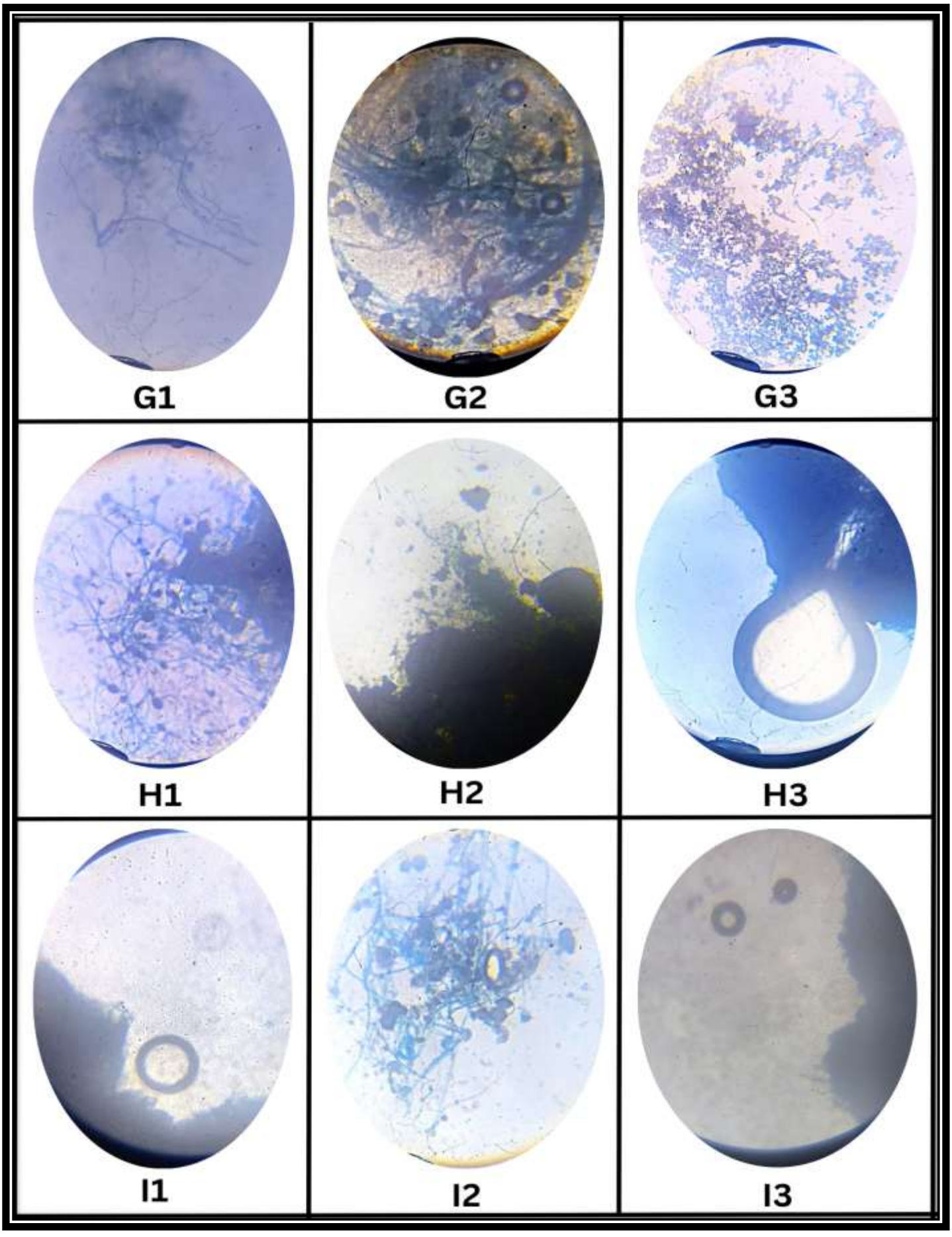


Figure 8: Microscopy of The Fungal Colonies- (C3, D1, D2, D3, D4, D5, E1, F2, F1).

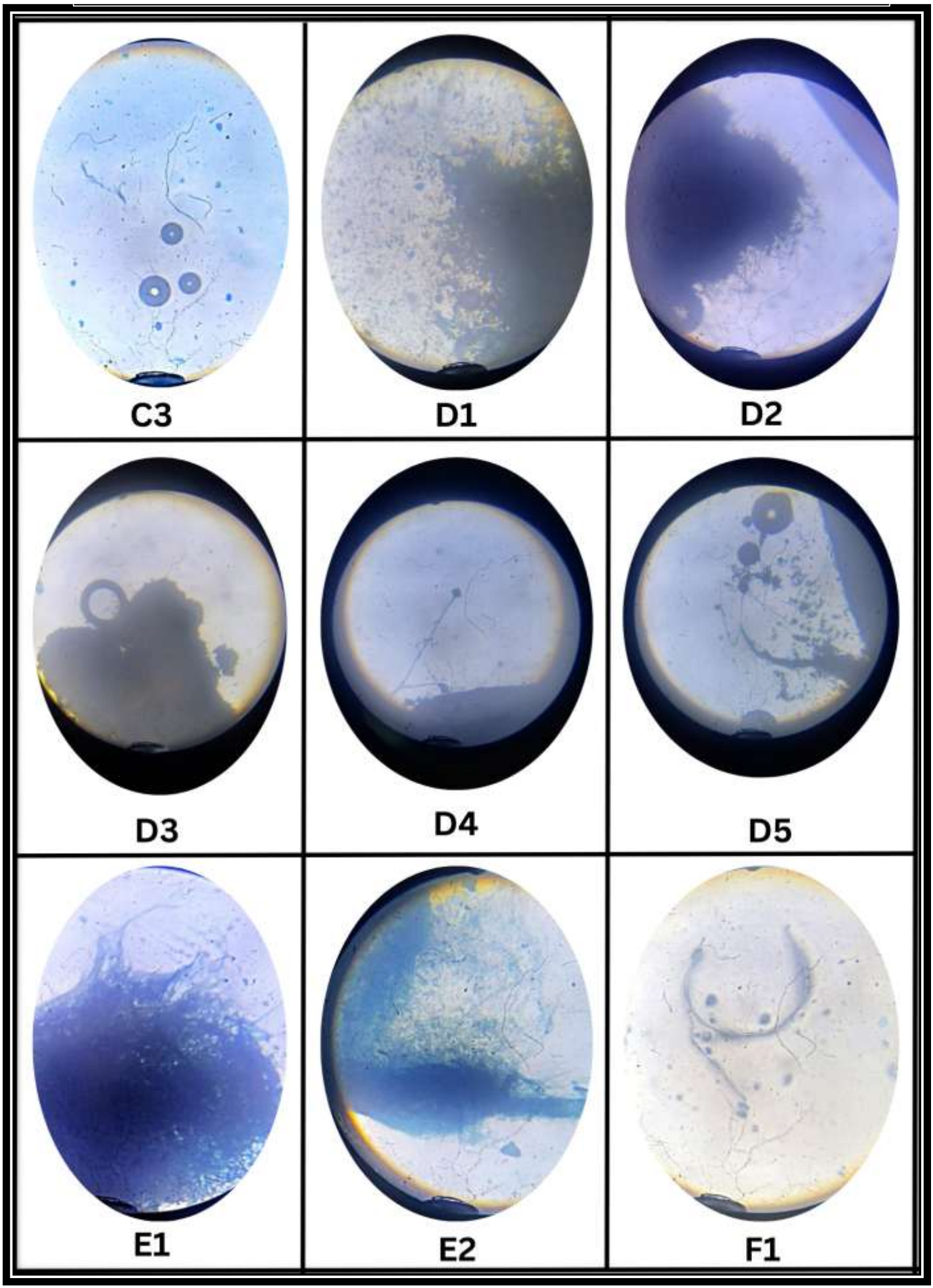
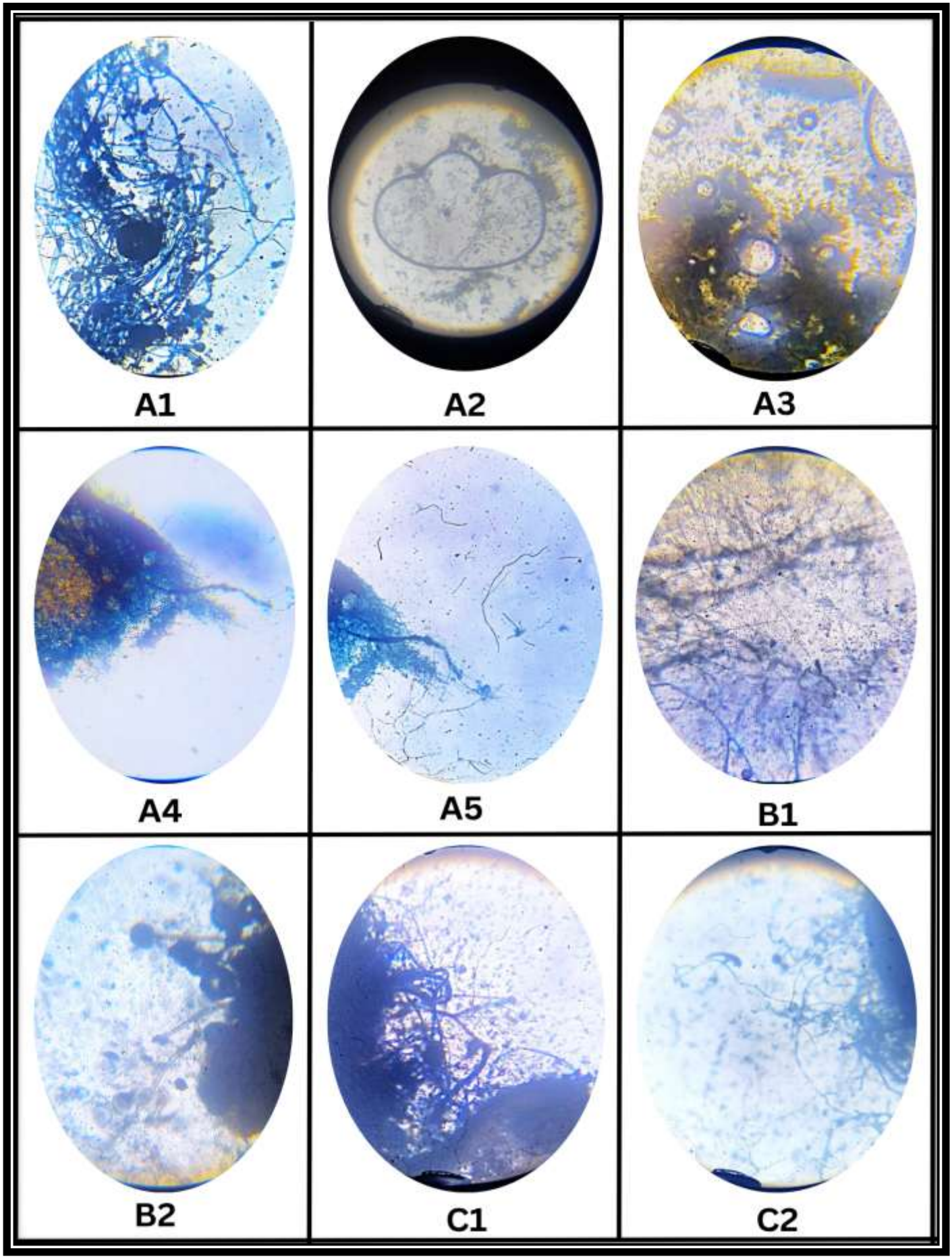


Figure 9: Microscopy of The Fungal Colonies- (A1, A2, A3, A4, A5, B1, B2, C1, C2).



4.4 Identification of *A.niger* From Different Types of Fungal Isolate Grown on PDA plates

After staining with lactophenol cotton blue solution, critically we identified *Aspergillus niger* by comparing colonies to established literature references. Macroscopically, we tracked classic jet-black morphology (as in ocean samples O1/A1 and O2/B2, and pond sample P3/I2 and P2/H1). Microscopically, we confirmed characteristic features: dark conidial heads, septate hyphae, and radiate conidiophores. This dual approach resolved surprises like river samples where *A.niger* appeared as brown (R2/K1) and white (R2/K4) and Dark brown(P3/M1) colonies and one Pale yellow colony(P1/G1) proving morphology alone can be deceptive. As shown in (fig 6-9).

Ultimately, only 8 of 36 total colonies were confirmed as *A.niger*, found in just 7 samples as shown in fig 10-11. In Ocean sample 1 (O1) Containing One Jet-Black Colony Whose Colony Code is A1, sample 2 (O2) Containing one jet-black colony whose colony code is B2. In Pond sample 1 (P1) Containing one Pale yellow colony whose colony code is G1, Pond sample 2 (P2) Containing one jet black colony whose colony code is H1 and Pond: sample 3 (P3) Containing one jet black colony whose colony code is I2. Lastly River sample 2 (R2) Containing two different types of fungi one is Brown in colour and one is white in colour whose colony codes are K1 and K4 respectively. where is River sample 3 (R3) Containing one Dark brown colony whose colony code is M1.

Figure 10: Microscopy of A.niger Fungal Colonies- (A1, B2, G1, H1).

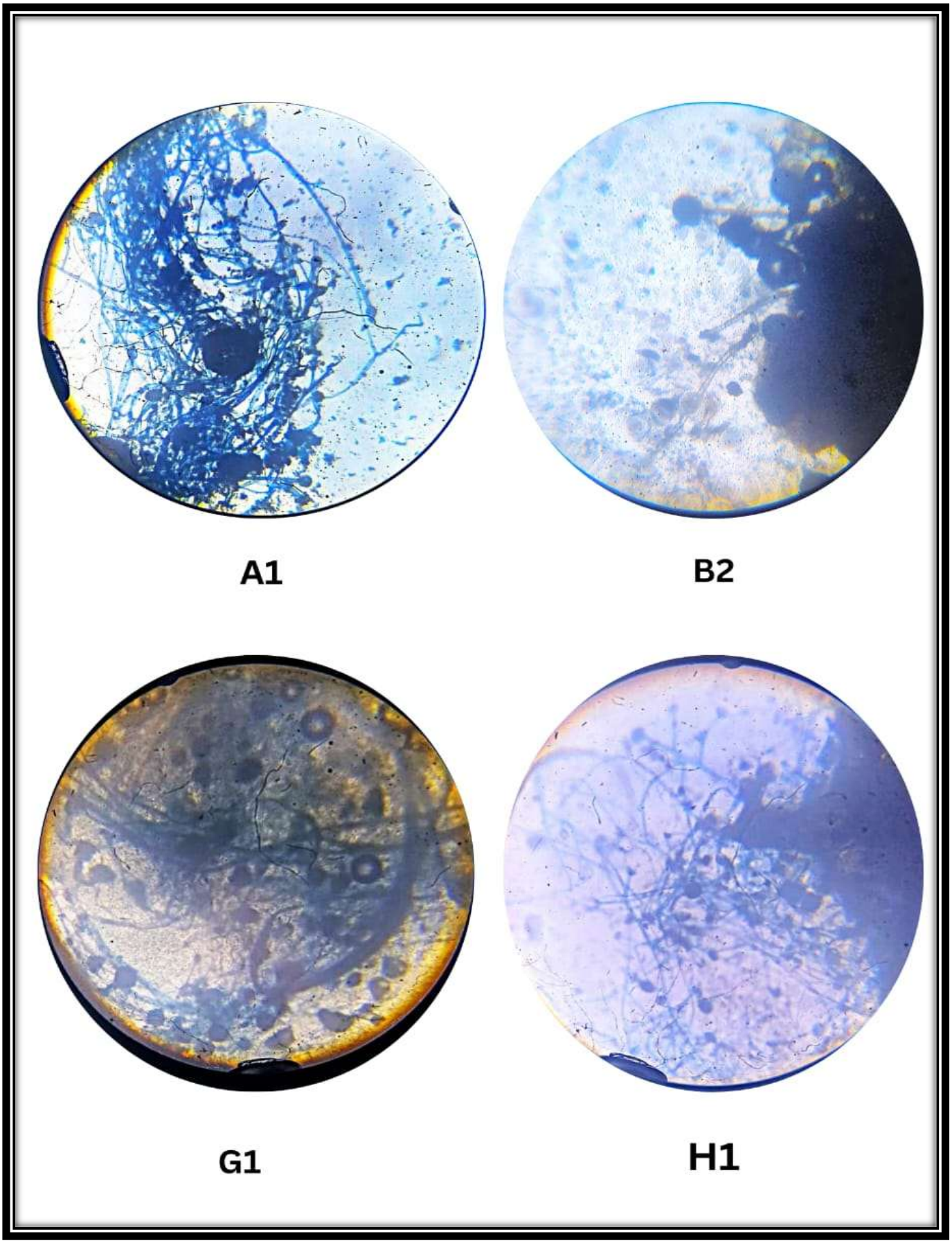
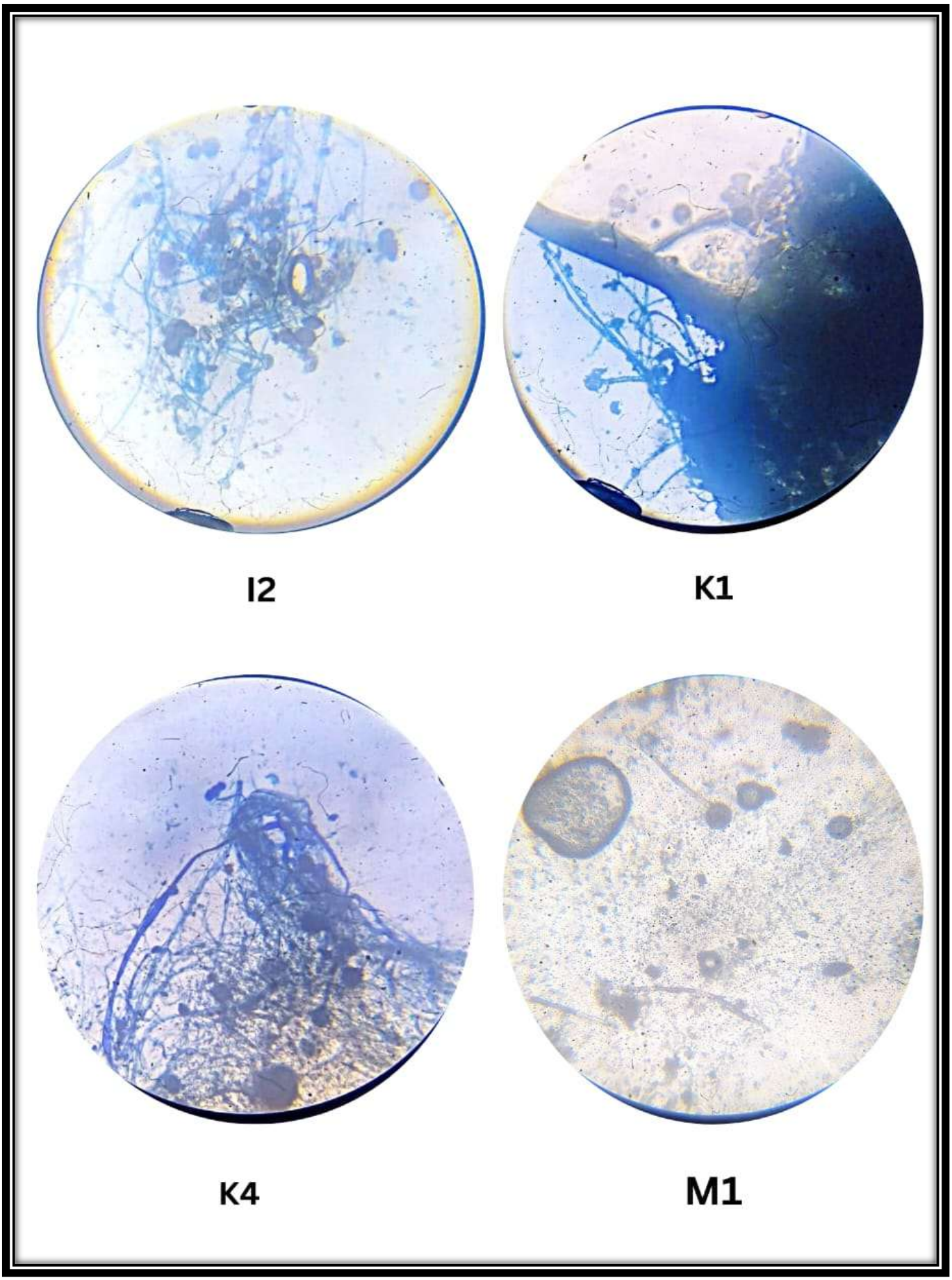


Figure 11: Microscopy of The A.niger Fungal Colonies- (I2, K1, K4, M1).



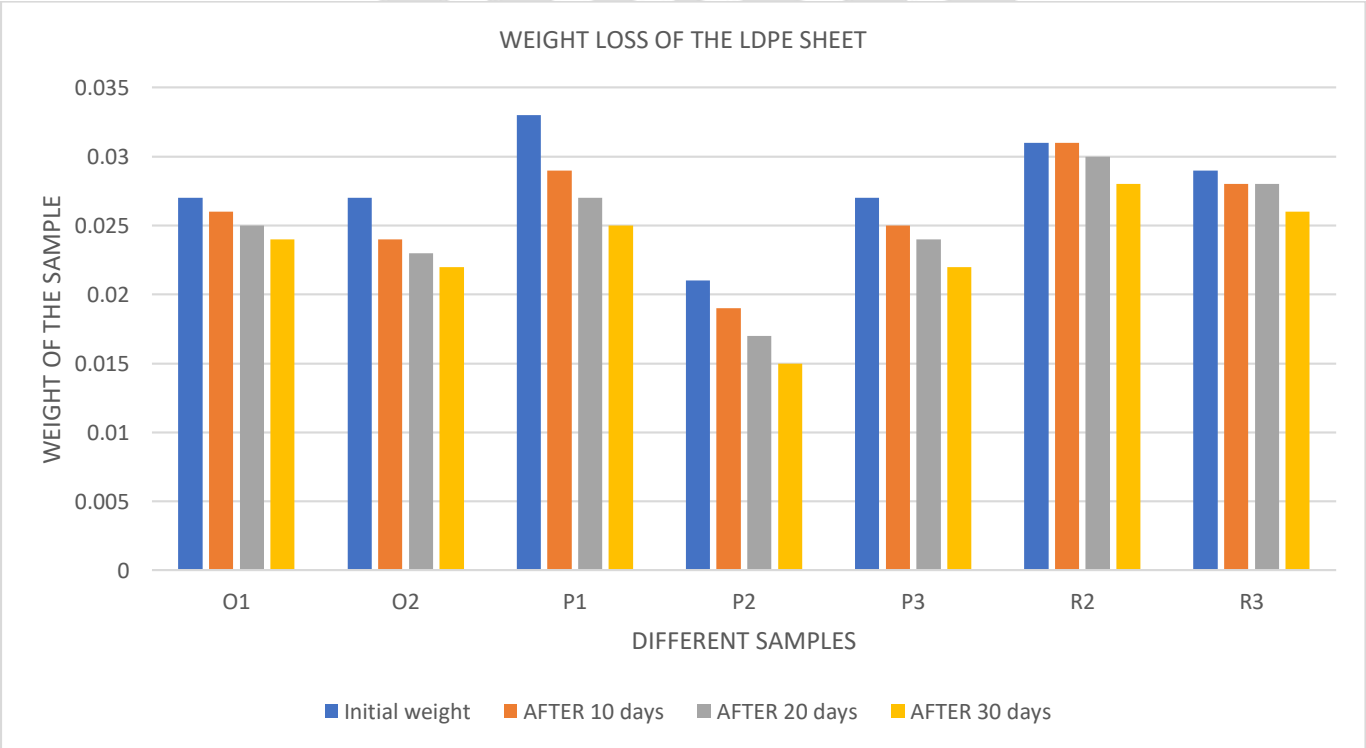
4.5 Evaluation for Loss of Weight In L.D.P.E Sheets

The observed weight loss in LDPE sheets, exposed to *Aspergillus niger* isolates paints a compelling, albeit nuanced, picture of potential fungal biodegradation as shown in (table 6). Over the 30day incubation, witnessing samples like P2 shed nearly 29% of its mass (0.021g to 0.015g) was particularly striking, suggesting certain fungal strains or conditions can drive significant breakdown. This wasn't an isolated event a consistent downward trend was evident across most samples O1, O2, P1, P3, R3 all showed progressive weight reduction. Calculating the average loss reveals a clear pattern plastic is disappearing from these inoculated systems over time. It feels like we're observing nature's subtle workforce, the fungi, slowly dismantling a persistent pollutant through enzymatic activity, likely breaking down the polymer chains piece by piece. However, the process wasn't uniform. The resilience of sample R2, showing minimal initial change (0.031g to 0.031g at 10 days), serves as an important reminder of the inherent variability in biological systems. Differences in fungal colonization efficiency, biofilm formation, local microenvironments within the water, or even slight variations in the plastic sheets themselves could all contribute to this patchwork effect. While the lack of sterile abiotic controls means we must cautiously acknowledge potential contributions from physical factors like adsorption or leaching, the progressive and substantial weight loss specifically in the *A.niger* exposed samples strongly points towards active biological degradation as the primary driver. This data offers tangible hope. It suggests that fungi naturally inhibiting our water bodies, like this characterized *A.niger* , possess an inherent capability to attack plastic waste. While far from an instant solution, understanding and potentially enhancing this slow, microbial digestion could become a valuable tool in our long-term strategy to mitigate the pervasive plastic burden accumulating in aquatic ecosystems worldwide.

Table 6: Loss Of Weight In 10 Days Interval of LDPE Sheet.

Sample Code	Initial Weight(g)	After 10 Days (g)	After 20 Days (g)	After 30 Days (g)
O1	0.027	0.026	0.025	0.024
O2	0.027	0.024	0.023	0.022
P1	0.033	0.029	0.027	0.025
P2	0.021	0.019	0.017	0.015
P3	0.027	0.025	0.024	0.022
R2	0.031	0.031	0.03	0.028
R3	0.029	0.028	0.028	0.026

Graph 2: Weight Loss Of LDPE Sheet at Different Time Interval.



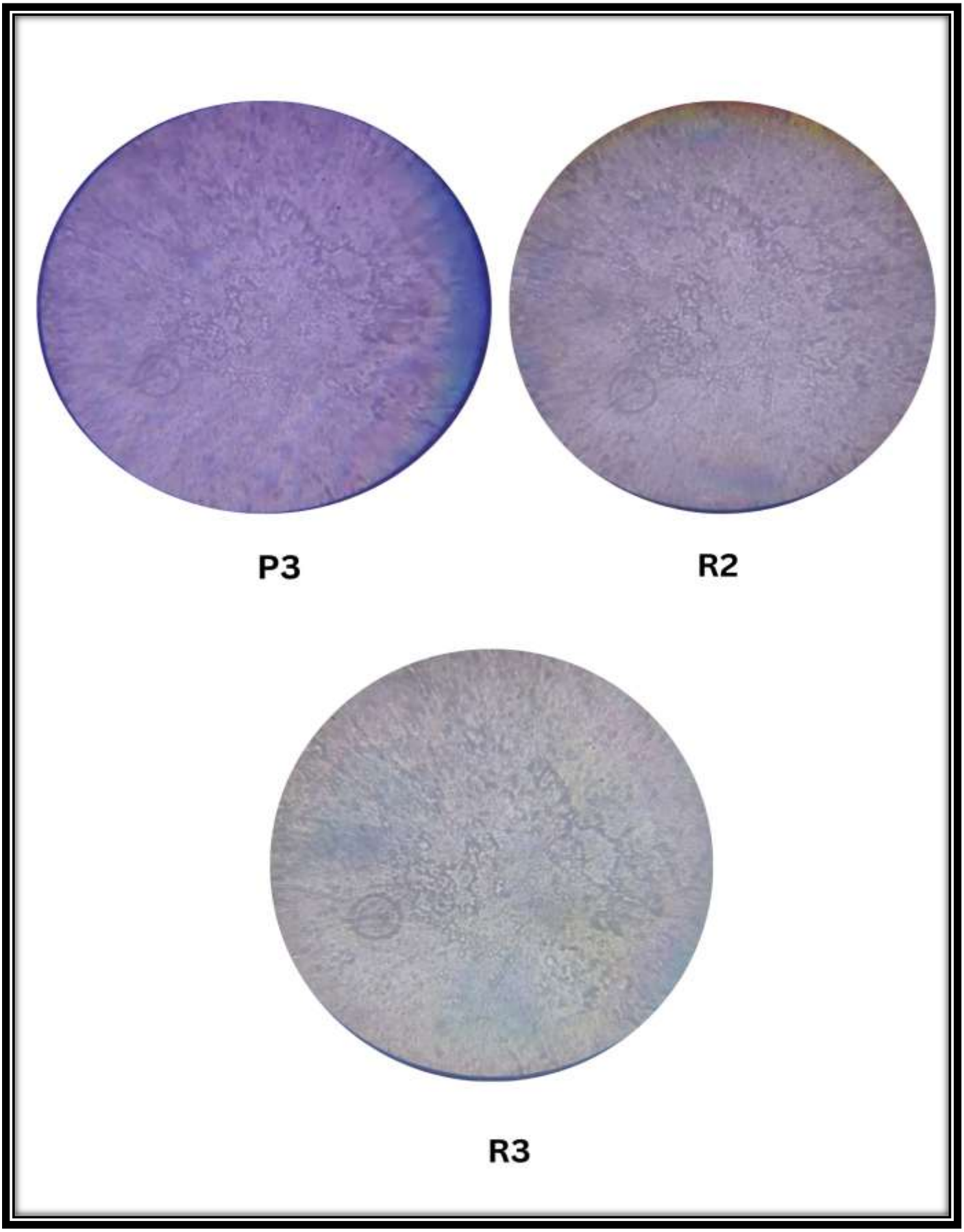
5. RESULT

Our exploration into *Aspergillus niger*'s plastic-degrading abilities revealed a story of microbial resilience and tangible progress. By exposing LDPE sheets to *A.niger* contaminated water from diverse environments, we witnessed a silent but powerful battle against plastic pollution. We had discovered that Over 30 days, *A.niger* consistently chipped away at the LDPE sheets, proving its potential as a natural plastic degrader. The average weight loss of LDPE sheet escalated steadily as follow in (Table 7, Graph 3).

- Aftar 10 days: Plastic sheets shed 0.00186 g on average a subtle but promising start.
- Aftar 20 days: Loss nearly doubled to 0.003 g, showing accelerated breakdown.
- Aftar 30 days: A striking 0.00471g average loss equivalent to ~15% of the plastic's initial mass vanishing.
- Pond Sample (P2): Stole the spotlight with 29% weight loss the plastic shrank from 0.021 g to 0.015 g. This suggests certain aquatic environments (like nutrient-rich ponds) turbocharge *A.niger's* appetite for plastic.
- Ocean Samples (O1,O2): Defied expectations Despite seawater's salinity, they achieved 11–18% degradation.
- River Sample (R2): Started slow (no change at Day 10) but caught up, ending at 10% loss. Even "slower" fungi made progress.
- Every environment hosted plastic-eating fungi: *A.niger* thrived in oceans, ponds, and rivers proving its adaptability. Degradation is progressive: The longer the exposure, the greater the breakdown. This wasn't luck, it was biology in action. Real-world relevance: If *A.niger* can achieve 15% average plastic loss in just 30 days without optimization, imagine its potential in engineered systems.

From fig-12 and 13 microscopic picture of LDPE sheets shows potential bio-degradation on the LDPE surface by the action of *A.niger.*

Figure 12: Microscopic Image L.D.P.E sheet to immersed in to samples (P3, R2, R3).



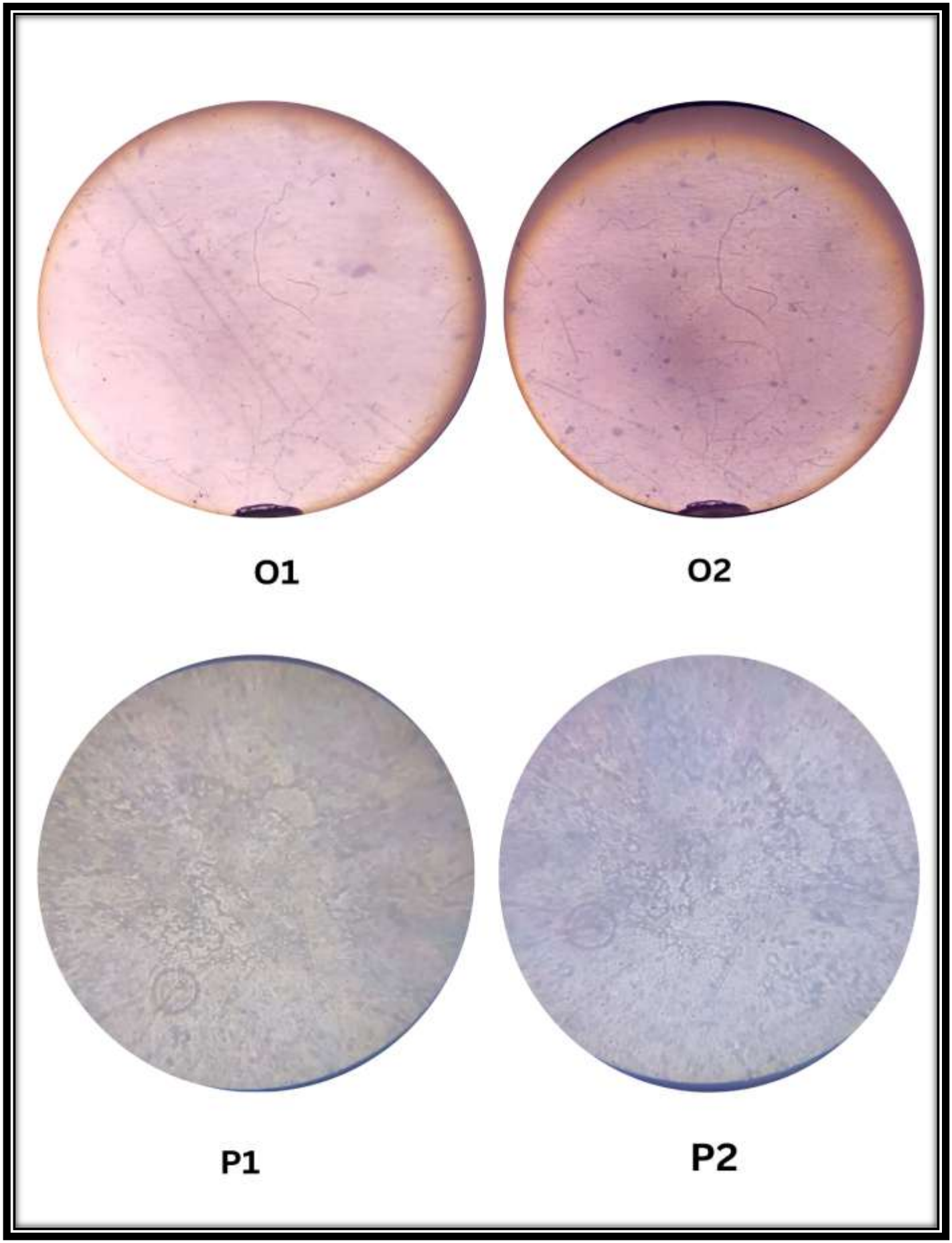
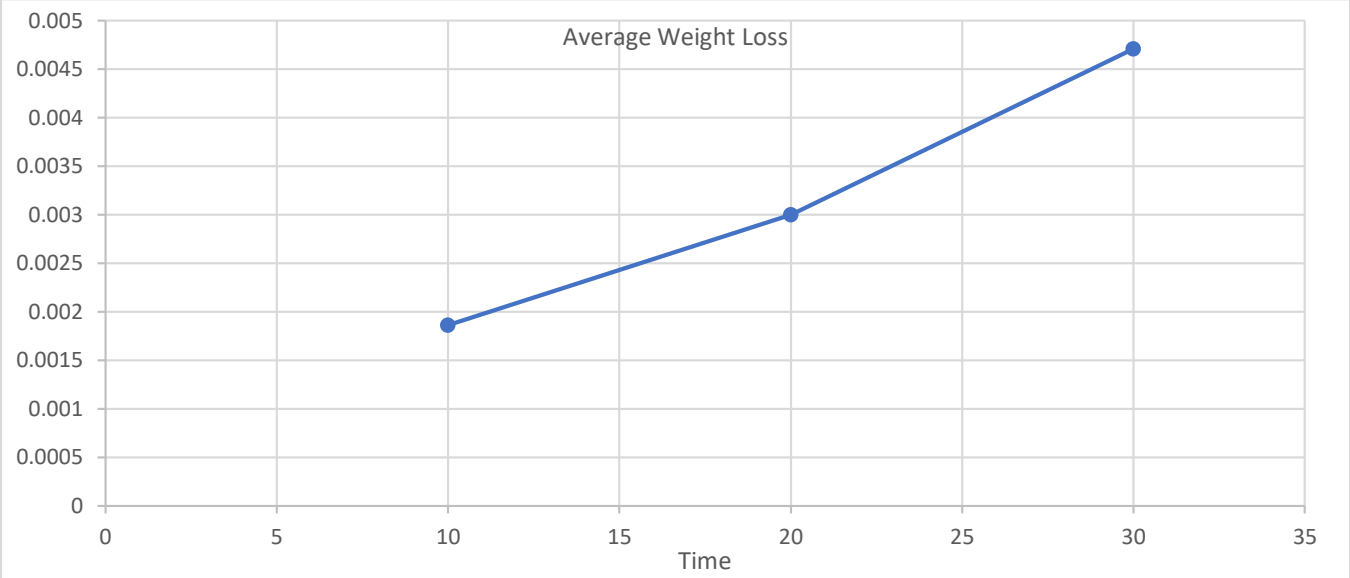

O1
O2
P1
P2

Table 7: Average weight loss of LDPE sheet.

Time(day)	Average Weight Loss(g)
10	0.00186
20	0.003
30	0.00471

Graph 3: Potential weight loss LDPE sheet in different time interval.



6.CONCLUSION

This study successfully isolated and characterized *Aspergillus niger* as a potential plastic-degrading fungus from a variety of aquatic environments, including oceans, lakes, ponds, and rivers. Environmental conditions appeared to play a crucial role in the degradation efficiency. Ponds provided relatively optimal conditions for fungal activity, with moderate levels of total dissolved solids (TDS: 328–682 ppm) and slightly acidic pH (6.45–6.79). Surprisingly, even ocean samples despite their high salinity (TDS: 862–911 ppm) and alkaline pH (7.82–8.43) up ported degradation levels ranging from 11% to 18%, showcasing *A.niger's* remarkable adaptability.

The natural occurrence of *Aspergillus niger* in diverse aquatic ecosystems, coupled with its ability to break down LDPE without any artificial optimization, positions it as a promising agent for plastic bioremediation. This study lays an important foundation for developing eco-friendly and sustainable strategies to combat plastic pollution using native fungal species. Future research should focus on optimizing degradation conditions, investigating the enzymatic pathways involved in plastic breakdown, and scaling up the process for practical applications such as wastewater treatment and marine plastic cleanup. Overall, this work highlights the potential of *A.niger* as a naturally in addressing one of the most pressing environmental challenges of our time. This sparks further curiosity and action uncovering the specific enzymes at work, improving how we apply them in the real world, and eventually turning this knowledge into powerful tools for cleaning our wastewater, our shorelines, and even our oceans polluted with plastic wests.

7. REFERENCES

[1] Charnock C. Norwegian Soils and Waters Contain Mesophilic, Plastic-Degrading Bacteria. Microorganisms. 2021 Jan 3;9(1):94.

[2] Wang H, Neal B, White B, Nelson B, Lai J, Long B, et al. Microplastics removal in the aquatic environment via fungal pelletization. Bioresource Technology Reports. 2023 Sep;23:101545.

[3] Vaksmaa A, Guerrero-Cruz S, Ghosh P, Zeghal E, Hernando-Morales V, Niemann H. Role of fungi in bioremediation of emerging pollutants. Front Mar Sci. 2023 Mar 6;10:1070905.

[4] Kim SH, Lee JW, Kim JS, Lee W, Park MS, Lim YW. Plastic-inhabiting fungi in marine environments and PCL degradation activity. Antonie van Leeuwenhoek. 2022 Dec;115(12):1379–92.

[5] Benavides Fernández CD, Guzmán Castillo MP, Quijano Pérez SA, Carvajal Rodríguez LV. Microbial degradation of polyethylene terephthalate: a systematic review. SN Appl Sci. 2022 Oct;4(10):263.

[6] Overy DP, Rämä T, Oosterhuis R, Walker AK, Pang KL. The Neglected Marine Fungi, Sensu stricto, and Their Isolation for Natural Products' Discovery. Marine Drugs. 2019 Jan 10;17(1):42.

[7] Pan D, Su F, Liu C, Guo Z. Research progress for plastic waste management and manufacture of value-added products. Adv Compos Hybrid Mater. 2020 Dec;3(4):443–61.

[8] Ibor OR, Mpama NOL, Okoli CP, Ogarekpe DM, Edet UO, Ajang RO, et al. Occurrence, identification and characterization of plastic pollution from an open solid waste dumpsite in Calabar, Southern Nigeria. Environmental Advances. 2023 Apr;11:100338.

[9] Malik S, Maurya A, Khare SK, Srivastava KR. Computational Exploration of Bio-Degradation Patterns of Various Plastic Types. Polymers. 2023 Mar 20;15(6):1540.

[10] Bergmann M, Collard F, Fabres J, Gabrielsen GW, Provencher JF, Rochman CM, et al. Plastic pollution in the Arctic. Nat Rev Earth Environ. 2022 Apr 5;3(5):323–37.

[11] Van Emmerik T, Schwarz A. Plastic debris in rivers. WIREs Water. 2020 Jan;7(1):e1398.

[12] Wang L, Nabi G, Yin L, Wang Y, Li S, Hao Z, et al. Birds and plastic pollution: recent advances. Avian Res. 2021 Dec;12(1):59.

[13] Zhao X, You F. Cascading Polymer Macro-Debris Upcycling and Microparticle Removal as an Effective Life Cycle Plastic Pollution Mitigation Strategy. Environ Sci Technol. 2023 Apr 25;57(16):6506–19.

[14] Chakraborty P, Chandra S, Dimmen MV, Hurley R, Mohanty S, Bharat GK, et al. Interlinkage Between Persistent Organic Pollutants and Plastic in the Waste Management System of India: An Overview. Bull Environ Contam Toxicol. 2022 Dec;109(6):927–36.

[15] Chen J, Wang W, Liu H, Xu X, Xia J. A review on the occurrence, distribution, characteristics, and analysis methods of microplastic pollution in ecosystem s. Environmental Pollutants and Bioavailability. 2021 Jan 1;33(1):227–46.

[16] Zhang M, Haward M, McGee J. Marine plastic pollution in the polar south: Responses from Antarctic Treaty System. Polar Record. 2020;56:e36.

[17] Quero GM, Luna GM. Surfing and dining on the "plastisphere": Microbial life on plastic marine debris. Adv Ocean Limnol [Internet]. 2017 Dec 19 [cited 2025 Mar 18];8(2). Available from: https://www.pagepressjournals.org/index.php/aiol/article/view/7211.

[18] Gregory MR. The hazards of persistent marine pollution: drift plastics and conservation islands. Journal of the Royal Society of New Zealand. 1991 Jun;21(2):83–100.

[19] Ayala F, Zeta-Flores M, Ramos-Baldárrago S, Tume-Ruiz J, Rangel-Vega A, Reyes E, et al. Terrestrial mammals of the Americas and their interactions with plastic waste. Environ Sci Pollut Res. 2023 Mar 27;30(20):57759–70.

[20] Nava V, Dar JY, De Santis V, Fehlinger L, Pasqualini J, Adekolurejo OA, et al. Zooming in the plastisphere: the ecological interface for phytoplankton–plastic interactions in aquatic ecosystems. Biological Reviews. 2025 Apr;100(2):834–54.

[21] Gil MA, Pfaller JB. Oceanic barnacles act as foundation species on plastic debris: implications for marine dispersal. Sci Rep. 2016 Jan 27;6(1):19987.

[22] Wilcox C, Van Sebille E, Hardesty BD. Threat of plastic pollution to seabirds is global, pervasive, and increasing. Proc Natl Acad Sci USA. 2015 Sep 22;112(38):11899–904.

[23] Yu RS, Yang YF, Singh S. Global analysis of marine plastics and implications of control measure strategies. Front Mar Sci. 2023 Dec 11;10:1305091.

[24] Cózar A, Echevarría F, González-Gordillo JI, Irigoien X, Úbeda B, Hernández-León S, et al. Plastic debris in the open ocean. Proc Natl Acad Sci USA. 2014 Jul 15;111(28):10239–44.

[25] Lau WWY, Shiran Y, Bailey RM, Cook E, Stuchtey MR, Koskella J, et al. Evaluating scenarios toward zero plastic pollution. Science. 2020 Sep 18;369(6510):1455–61.

[26] Choi SY, Lee Y, Yu HE, Cho IJ, Kang M, Lee SY. Sustainable production and degradation of plastics using microbes. Nat Microbiol. 2023 Nov 29;8(12):2253–76.

[27] Temporiti MEE, Nicola L, Nielsen E, Tosi S. Fungal Enzymes Involved in Plastics Biodegradation. Microorganisms. 2022 Jun 8;10(6):1180.

[28] Srikanth M, Sandeep TSRS, Sucharitha K, Godi S. Biodegradation of plastic polymers by fungi: a brief review. Bioresour Bioprocess. 2022 Dec;9(1):42.

29] Mohamed H, Shah AM, Nazir Y, Naz T, Nosheen S, Song Y. Biodegradation of poly (vinyl alcohol) by an orychophragmus rhizosphere-associated fungus Penicillium brevicompactum OVR-5, and its proposed PVA biodegradation pathway. World J Microbiol Biotechnol. 2022 Jan;38(1):10.

[30] Rodrigo OA, Daynet SDC, Jaime NM, Nora F, JosÃ© ÃB, Alejandro A, et al. Analysis of the degradation of polyethylene, polystyrene and polyurethane mediated by three filamentous fungi isolated from the Antarctica. Afr J Biotechnol. 2021 Feb 28;20(2):66–76.

[31] Gilani IE, Sayadi S, Zouari N, Al-Ghouti MA. Plastic waste impact and biotechnology: Exploring polymer degradation, microbial role, and sustainable development implications. Bioresource Technology Reports. 2023 Dec;24:101606.

[32] Priyanka N, Archana T. Biodegradability of Polythene and Plastic By The Help of Microorganism: A Way for Brighter Future. J Environment Analytic Toxicol [Internet]. 2011 [cited 2025 Mar 18];01(02). Available from: https://www.omicsonline.org/biodegradability-of-polythene-and-plastic-by-the-help-of-microorganism-a-way-for-brighter-future-2161-0525.1000111.php?aid=2984

[33] Khruengsai S, Sripahco T, Pripdeevech P. Low-Density Polyethylene Film Biodegradation Potential by Fungal Species from Thailand. JoF. 2021 Jul 23;7(8):594.

[34] Khruengsai S, Sripahco T, Pripdeevech P. Biodegradation of Polyester Polyurethane by Embarria clematidis. Front Microbiol. 2022 Jun 14;13:874842.

35] Abarca ML, Accensi F, Cano J, Cabañes FJ. Taxonomy and significance of black aspergilli. Antonie Van Leeuwenhoek. 2004 Aug;86(1):33–49.

[36] Silva JJD, Iamanaka BT, Ferranti LS, Massi FP, Taniwaki MH, Puel O, et al. Diversity within *Aspergillus niger* Clade and Description of a New Species: Aspergillus vinaceus sp. nov. JoF. 2020 Dec 17;6(4):371.

[37] Andersen MR, Lehmann L, Nielsen J. Systemic analysis of the response of *Aspergillus niger* to ambient pH. Genome Biol. 2009 May 1;10(5):R47.

[38] Gadd GM, editor. Fungi in Biogeochemical Cycles [Internet]. 1st ed. Cambridge University Press; 2006 [cited 2025 May 15]. Available from: https://www.cambridge.org/core/product/identifier/9780511550522/type/book

[39] Guedes G. Tuberculosis and immunosuppression. JMEN [Internet]. 2018 May 18 [cited 2025 May 15];6(3). Available from: https://medcraveonline.com/JMEN/tuberculosis-and-immunosuppression.html

[40] Šimonovičová A, Vojtková H, Nosalj S, Piecková E, Švehláková H, Kraková L, et al. *Aspergillus niger* Environmental Isolates and Their Specific Diversity Through Metabolite Profiling. Front Microbiol. 2021 Jun 23;12:658010.

[41] Máthé C, Bóka K, Kónya Z, Erdődi F, Vasas G, Freytag C, et al. Microcystin-LR, a cyanotoxin, modulates division of higher plant chloroplasts through protein phosphatase inhibition and affects cyanobacterial division. Chemosphere. 2024 Jun;358:142125.

[42] Toma MA, Nazir KHMNH, Mahmud MdM, Mishra P, Ali MdK, Kabir A, et al. Isolation and Identification of Natural Colorant Producing Soil-Borne *Aspergillus niger* from Bangladesh and Extraction of the Pigment. Foods. 2021 Jun 3;10(6):1280.

[43] Parija SC, Shivaprakash MR, Jayakeerthi SR. Evaluation of lacto-phenol cotton blue (LPCB) for detection of Cryptosporidium, Cyclospora and Isospora in the wet mount preparation of stool. Acta Tropica. 2003 Mar;85(3):349–54.

[44] Facklam R. What Happened to the Streptococci: Overview of Taxonomic and Nomenclature Changes. Clin Microbiol Rev. 2002 Oct;15(4):613–30.

[45] Rivas Plata E, Lücking R, Lumbsch HT. A new classification for the family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). Fungal Diversity. 2012 Jan;52(1):107–21.

[46] Lam WHK, Wong SC, Lo HK, editors. Transportation and Traffic Theory 2009: Golden Jubilee: Papers selected for presentation at ISTTT18, a peer reviewed series since 1959 [Internet]. Boston, MA: Springer US; 2009 [cited 2025 May 19]. Available from: https://link.springer.com/10.1007/978-1-4419-0820-9.

[47] Restrepo-Flórez JM, Bassi A, Thompson MR. Microbial degradation and deterioration of polyethylene – A review. International Biodeterioration & Biodegradation. 2014 Mar;88:83–90.

[48] Jain D, Kaur G, Bhojiya AA, Chauhan S, Khandelwal SK, Meena RH, et al. Phenetic Characterization of Nitrogen Fixing Azotobacter from Rhizospheric Soil of Southern Rajasthan. J Pure Appl Microbiol. 2021 Mar 1;15(1):428–36.