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Isolation and Identification of Plastic Degrading Fungi from Bangalore, Karnataka, India

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ABSTRACT

Plastic is one of the most widely used raw materials across industries worldwide. However, it poses a significant environmental threat due to its slow decomposition rate, taking 100 to 1000 years to break down. Plastic waste contributes to climate change, fills landfills, and releases harmful pollutants such as CO_2 and dioxins, worsening environmental degradation. To address this crisis, scientists are exploring biodegradation methods, particularly using microbes, which have the ability to break down both organic and inorganic materials. Fungi, known for their enzymatic capabilities, have shown promise in degrading plastic components. This study focuses on identifying fungal species from plastics dump yards and waste sites to analyze their potential in plastic degradation. The research utilized serial dilution, spread plating, cotton blue staining, and light microscopy for fungal identification. A total of 12 fungal species were isolated from soil samples, including *Penicillium, Aspergillus niger, Trichoderma, Verticillium, Cladophialophora, Chaetomium, Epicoccum, Colletotrichum,* and *Aspergillus terreus*. Among these, *Penicillium, Aspergillus niger,* and *Trichoderma* were the most dominant species. These fungi have demonstrated the potential to degrade plastic waste in landfills and dump sites across India, offering an eco-friendly alternative to plastic disposal and contributing to sustainable waste management.

Keywords: Plastic degradation, Aspergillus, Trichoderma, dump sites, Eco-friendly method, Karnataka, India

INTRODUCTION

Plastic pollution has become a pressing environmental concern; with around 400 million metric tons of plastics produced per annual from which an estimated amount of 20 million metric tons of plastic littering in to the different ecosystems each year worldwide If current trends continue, plastic production could reach 1.1 billion tonnes by 2050 (Jambeck et al., 2015). Karnataka state generated 3,60,780.6 tonnes of plastic waste, making it one of the top five plastic waste generators in the country in 2022 to 2023. Among different places of Karnataka the Bengaluru city generates a 2.96 lakh tonnes of plastic waste, annually. From these only 57% of plastic waste was recycled in 143 recycling units and remaining was converted to residue derived fuel through cement factories. So to adopt eco-friendly methods for management of plastics the microbes like fungi, play a crucial role in breaking down plastic polymers (Sutherland et al., 2012). Recent studies have identified various fungal species capable of degrading different types of plastics (Shah et al., 2018; Kumar et al., 2020) The isolation and identification of plastic-degrading fungi are essential steps towards understanding their potential applications in plastic waste management and bioremediation (Singh et al., 2020). Recent research efforts have focused on exploring and utilizing microbial degradation of plastics as a cost-effective and environmentally friendly approach to address the current challenges posed by plastic waste (Okal et al., 2023; Viel et al., 2023), While the potential of fungi in plastic degradation was recognized, there is a limited understanding of the specific fungal communities present in plastic-contaminated environments within Bengaluru, Karnataka, and their potential contribution to bioremediation in this region. This study aims to isolate and identify fungal species from plastic-contaminated soil samples in Bengaluru that exhibit potential for plastic degradation, providing a baseline understanding of the local fungal resources for sustainable plastic waste management.

MATERIALS AND METHODS

Study Area

The study area comprised four distinct locations within Bengaluru, Karnataka, identified as sites with significant plastic waste accumulation due to their use as informal dump yards by local communities and street vendors. These places are heaps of organic and inorganic waste along with visible plastic accumulation and moderate moist soil. So, there are a huge chance of having variety of microorganisms mainly fungi because most of them are saprophytic in nature. The soil samples are collected from the KG colony gm playa 12°58'22.04"N, 77°39'45.18"E, Near New Florence English High School (Bhadrappa layout), 13°02'49.4"N 77°34'33.4"E, Home soil

(Nagashettyhalli), 13°02'36.4"N 77°34'26.4"E, Gm palya main road 13°02'49.4"N 77°34'33.4"E accumulated with single used plastic, milk packets, food wrappers, and various plastic bottles for isolation of fungi. The microbial communities of these particular plastic-contaminated sites in Bengaluru are being reported for the first time in this study.

Isolation of Fungi from Soil Samples

Approximately one kilogram of soil sample was collected from the study area. The samples were then transported to the laboratory for further analysis. The collected soil was air dried, powdered, and sieved to obtain a fine-textured sample suitable for microbial isolation. To isolate fungal species, the soil dilution method was employed (Waksman, 1927). One gram of the air dried powdered soil sample was weighed and dissolved in 10 mL of distilled water in a test tube labeled as 10^{-1}. Serial dilutions were then prepared up to 10^{-5}. Approximately 15 mL of Potato Dextrose Agar (PDA) medium was poured into sterile petri dishes and allowed to solidify. Each plate was labeled according to the dilution levels (10^{-2} to 10^{-5}). 1 mL of the diluted inoculum were spread onto the PDA plates using an 'L'-shaped glass rod to ensure uniform distribution. Each dilution was plated in triplicate and incubated inverted at 27°C for 3 to 7 days to allow fungal growth (Seifert, 1992).

Identification of fungi

The fungal species were identified using micro-preparation slide techniques, where the isolates were stained with cotton blue dye and observed under a light microscope. Various morphological characteristics such as spore shape, size, color, (Atlas 2010) conidial structure, mycelium, and branching patterns were analyzed for preliminary identification. The observed features were then compared with established taxonomic references, including the Manual of Soil Fungi, Manual of *Aspergillus* (Raper & Fennell, 1965), A Manual of *Penicillium* (Raper & Thom, 1949), Soil Fungi (Domsch et al., 1980), and Hyphomycetes (Subramanian, 1971). This detailed comparison ensured accurate identification of the fungal species based on their distinct morphological traits. Morphological identification can have limitations so the molecular methods could provide more definitive identification in future studies. Total no. of organisms per gram of soil, the average of isolate is calculated as per the standard formula. Organism per gram of soil = no. of colonies X dilution factor/weight of air dried soil.

RESULT

In the present paper the KG Colony and Gm playa plastic dump site exhibited the highest number of *Aspergillus niger* isolates, Bhadrappa layout plastic deposit sites showed the highest counts of *Trichoderma viride*, Chaetomium sp, Nagashettyhalli plastic deposit sites showed the highest counts of *Trichoderma* sp. The highest concentration of fungal organisms shown in all study areas was *Penicillium chrysogenum, Aspergillus niger*, and *Trichoderma harxianum* respectively (Table 1 and 2). There were 14 different fungal isolates includes 9 general and 12 species from the soil sample collected from four locations. *Aspergillus flavus, A. niger, A. terreus, Chaetomium, Cladophialophora, Coletotrichum, Epicoccum, Penicillium chrysogenum, Pemicillium sp R. stolonifer, T. harzianum T. viride, Trrichoderma* sp *Verticillium* (Figure 1 A - G). Based on the number of colonies observed, *A. niger, T. viride* and *Trichoderma* sp. were the most frequently isolated species (Table 1 amd 2). In contrast, *A. terreus*, *Cladophialophora*, and *Colletotrichum*, were isolated less frequently (Table 1 and 2).

Table 1: The concentration of different plastic degrading fungi from different dump sites of Bangalore

Sl. No	Area	Dilution Factor	No. of fungal Isolates	Average	Organism per gram of soil
1.	KG Colony	10^-2	*Verticillium (10)*	3	300
			Aspergillus niger (15)	5	500
			Aspergillus flavus (10)	3	300
			Rhizopus stolonifer(7)	2	200
2.	KG Colony	10^-3	*Verticillium (3)*	1	1000
			Penicillium (8)	3	3000
			Aspergillus niger (6)	2	2000

Sl. No	Area	Dilution Factor	No. of fungal Isolates	Average	Organism per gram of soil
			Aspergillus flavus (5)	2	20000
3.	KG Colony	10^-4	*Rhizopus stolonifer (3)*	1	10000
			Aspergillus niger (5)	3	30000
			Verticillium (4)	1	100000
4.	KG Colony	10^-5	*Aspergillus terreus (4)*	1	100000
			Rhizopus stolonifer(6)	2	200000
			Trichoderma (11)	5	500
5.	Bhadrappa layout	10^-2	*Chaetomium(10)*	5	500
			Penicillium (8)	4	800
			Trichoderma harzianum (9)	5	5000
6.	Bhadrappa layout	10^-3	*Chaetomium (4)*	2	2000
			Penicillium chrysogenum (6)	3	6000
			Epicoccum (9)	5	50000
7.	Bhadrappa layout	10^-4	*Chaetomium(11)*	6	60000
			Trichoderma viride (13)	6	60000
			Trichoderma viride(30)	15	1500000
8.	Bhadrappa layout	10^-5	*Chaetomium(18)*	9	900000
			Penicillium (8)	4	400000
			Aspergillus niger (10)	5	500
9.	Nagashettyhalli	10^-2	*Epicoccum (4)*	2	200
			Penicillium chrysogenum(16)	8	800
			Trichoderma (35)	17	3500
			Epicoccum (1)	1	1000
10.	Nagashettyhalli	10^-3	*Aspergillus niger (5)*	3	3000
			Trichoderma (4)	2	2000
			Penicillium (1)	1	1000
			Aspergillus niger (15)	7	70000
11.	*Nagashettyhalli*	*10^-4*	*Penicillium chrysogenum(10)*	5	50000
			Trichoderma (4)	2	20000
			Col;etotrichum (3)	2	20000
12.	*Nagashettyhalli*	*10^-5*	*Trichoderma viride (27)*	13	1300000
			Epicoccum (2)	1	100000
			Penicillium chrysogenum(2)	1	100000
			Trichoderma (23)	13	1300
13.	*GM Palya*	*10^-2*	*Aspergillus niger (12)*	6	600
			Aspergillus flavus (1)	1	100
			Cladophialophora (5)	3	3000
14.	*GM Palya*	*10^-3*	*Trichoderma (18)*	9	9000
			Penicillium chrysogenum(9)	5	5000
			Trichoderma (7)	4	40000
15.	*GM Palya*	*10^-4*	*Chaetomium (2)*	1	10000
			Aspergillus niger (11)	6	60000
			Aspergillus niger (8)	4	400000
16.	*GM Palya*	*10^-5*	*Penicillium (5)*	3	300000
			Trichoderma harizianum (10)	5	500000

Table 2: The total number of different plastic degrading fungi in different dump sites of Bangalore

S. No	Name of the fungi	KG Colony	Bhadrappa layout	*Nagashettyhalli*	GM Palya	Total
1	*Aspergillus flavus*	15	--	--	1	16
2	*Aspergillus niger*	26	--	30	31	85
3	*Aspergillus terreus*	4	--	--	--	4
4	*Chaetomium*	--	43	--	2	45
5	*Colletotrichum*	--	--	3		3
6	*Cladophialophora*	--	--	--	5	5
7	*Epicoccum*	--	9	7	--	16
8	*Penicillium chrysogenum*	--	6	28	9	43
9	*Penicillium*	8	16	1	5	30
10	*Rhizopus stolonifer*	16	--	--		16
11	*Trichoderma harzianum*	--	9	--	10	19
12	*Trichoderma viride*	--	43	27	--	70
13	*Trichoderma*	--	11	43	38	92
14	*Verticillium*	17	--	--	--	17

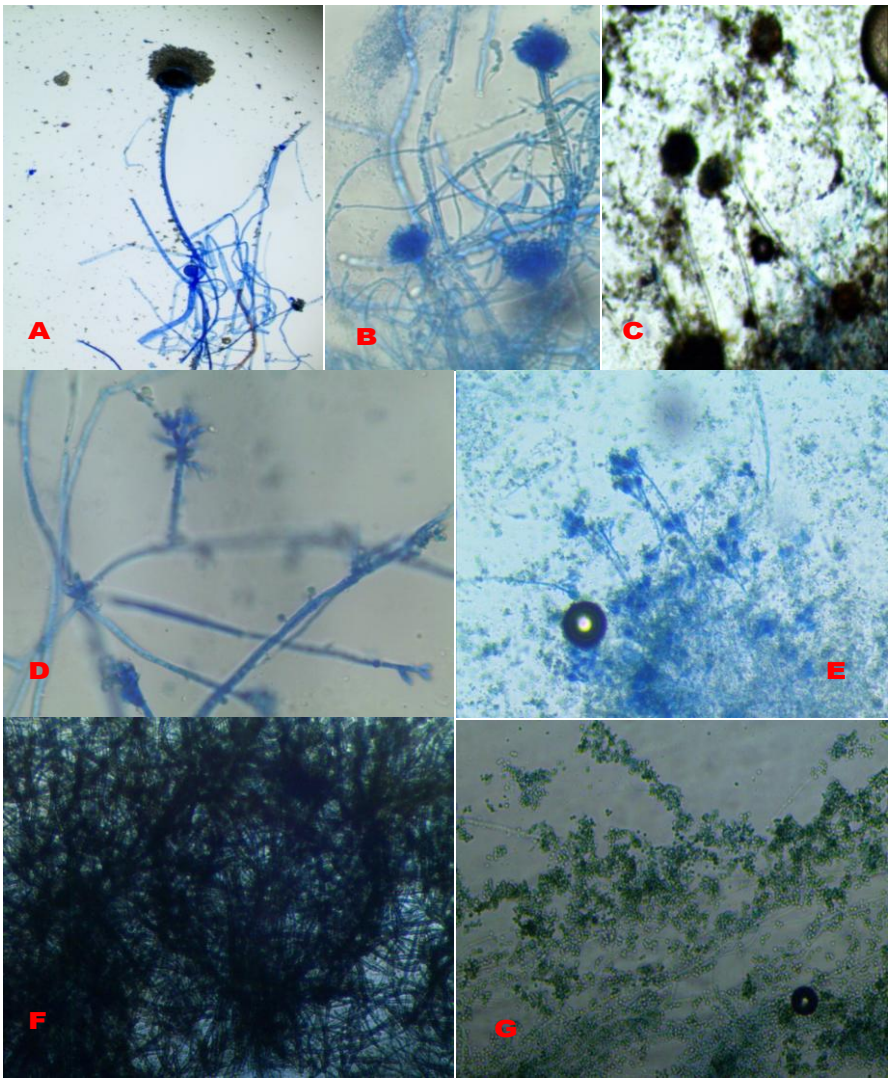

Figure 1: A, *Rhizopus stolonifera* 10X; B, *Aspergillus flavus* 10X; C, *Aspergillus niger* 10X; D, *Verticillium* sp. 40X; E, *Penicillium chrysogenum* 20X; F, *Chaetomium* sp. 10X; G, *Trichoderma harzianum* 10X.

DISCUSSION

Many terrestrial fungi, across the kingdom, degrade various types of plastic. Plastics are the fastest-growing habitat in the oceans, and we hypothesized that fungi isolated from the ocean would demonstrate high success rates in degrading polyurethane (PU) (Steinbach et al 2024). In the present study 12 different terrestrial soil fungi were isolated form plastic degradation environment. Ibrahim et al (2024) was able to screen the 18 selected fungal strains for their ability to degrade polyurethane (PU), and polyethylene (PE), The isolation of several fungal species from plastic-contaminated dump sites in Bengaluru, as demonstrated in this study, supports the established role of fungi in plastic degradation. The *P. chrysogenum*, have the capability to degrade polyethylene (PE) and polypropylene (PP) using enzymes like lipases, esterases, and proteases, which break down the plastic polymers (Kumar et al., 2020; Shah et al., 2018). In the present study also *P. chrysogenum* was isolated from different plastic dumpsites. The Plastic degradation, *Trichoderma* species, such as *T. harzianum*, have the capability to degrade polyethylene (PE), polypropylene (PP), and polyvinyl chloride (PVC) with the help of enzymes like cellulases, xylanases, and proteases (Kumar et al., 2020; Shah et al., 2018). In the present study also two different species of *Trichoderma* was isolated from different dumpsites of plastic, *A. niger* has been found to degrade PE, a common plastic used in packaging and plastic bags (Shah et al., 2018). *A. niger* as reportedly shown it degrades different types of plastic namely Polypropylene (Kumar et al., 2020) PVC, a plastic used in pipes, vinyl records using enzymes lipases, esterases, protease (Sutherland et al., 2012). In the present study also three different *Asperillus* sp was isolated from different plastic dumpsite; so isolation of these species from plastic-rich environments in Bengaluru suggests their potential role in local plastic degradation. Our study identified potential candidates of fungi for plastic degradation; further research like in vitro degradation assays is needed to confirm their plastic-degrading activity and efficiency. The dominant species observed in KG Colony is *A. niger,* Bhadrappa layout is *T. viride*, *Chaetomium* sp, Nagashettyhalli is *Trichoderma* sp. Gm playa is *A. niger* respectively; which indicates that these fungi were able to degrade the different kinds of plastic Bangalore. The morphological identification provides a preliminary assessment, so recent studies in molecular systematic could offer more precise identification and potentially reveal additional, less morphologically distinct species.

CONCLUSION

Plastic degrading fungi isolated from different local dumpsites with visible plastic materials in Bangalore, Karnataka were 14 different fungi, out of which 9 belong to different genera and 12 different species was observed. The highest concentration of fungal organisms shown in all study areas was *P. chrysogenum, A. niger*, and *T. harxianum* respectively. The soil samples from these locations shown potential plastic degrading fungi which may help in degrading plastic with various enzymes present in it. Moreover, different mycologists were able to prove that *P. chrysogenum, A. niger*, and *T. harxianum* were potential plastic degraders. So we have seen these fungiwere dominant and frequently isolated from local dumpsites of Bangalore. The prevalence of these potentially plastic-degrading fungi in Bengaluru's dump sites suggests a natural bioremediation potential that warrants further investigation for developing sustainable plastic waste management strategies in the region. There is a need for future studies focusing on in vitro degradation assays, enzyme characterization, and optimization of conditions for bioremediation.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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