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*Research Article*

# Biodegradation of Low-Density Polyethylene (LDPE) Bags by Fungi Isolated from Waste Disposal Soil

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Plastics are available in different shapes nowadays in order to enhance the living standard. But unfortunately, most of these plastics are synthetic in nature that is why they show resistance to physical and chemical degradation processes and enhance environmental hazards. The aim of the present research study was to isolate and identify beneficial fungal species from soil that have the capability to degrade plastic. Soil samples from a waste disposal site at Peshawar district were diluted and inoculated on sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) for fungus isolation. After isolation, the identifications of fungal species were done using standard identification techniques such as colony morphology and microscopic examination. The isolated fungal species that were identified were *Aspergillus Niger*, *Aspergillus flavus*, *Penicillium*, white rot, and brown rot fungi. After isolation, a degradation experiment was conducted to evaluate the capability of fungal isolates towards degradation of plastic. For this purpose, a 2 $cm^2$ plastic piece was treated with fungal isolates for one month in a liquid culture system. The weight loss percentage was estimated at 22.9%, 16.1%, 18.4%, and 22.7% by *Aspergillus Niger*, *Aspergillus flavus*, brown rot, and white rot, respectively, which was confirmed by the Fourier transform analysis. The obtained FTIR peaks revealed the C–H bond deformation in alkenes, ketones, and esters. It has been concluded from the study that fungal species play a significant role in the degradation of synthetic plastic which can be used in bioreactors in future studies for the degradation of complex plastic materials.

## 1. Introduction

Plastics are durable synthetic manmade materials and moldable compounds, and the materials made from them can be pushed into almost any desired shape and retain that shape for a long period of time [1, 2]. In the past 25 years, plastic products have been widely used in the food, clothes, shelter, shipping, building, medical, and leisure sectors [2, 3]. Plastics (such as polythene and polypropylene) are resistant to degradation and, because of this resistance, plastics are disposed in waste dumps that remain perpetual in their initial state [4, 5]. Improper disposal of plastics plays a significant role in potentially harming life through causing environmental pollution. In addition to this, the burning process of polyvinylchloride (PVC) plastics produces persistent organic pollutants (POPs) known as furans and dioxins [6, 7].

Plastics are proven to be harmful to human, animal, and plant health [8, 9]. Mainly, two broad classes of plastic-related chemicals are of critical concern for human health: bisphenol-A (BPA) and phthalates [10, 11]. In the late 1980s, scientists started to wonder if plastics could be designed to become susceptible to different microbial attacks, making them degradable in a microbial active

environment [12]. Biodegradable plastics opened the way for new considerations of waste management strategies since these materials are designed to degrade under environmental conditions or in municipal and industrial biological waste treatment facilities [13].

Microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics [14]. The biodegradation process of plastics proceeds actively under different soil conditions according to their properties because the micro-organisms responsible for the degradation process are different from each other and they have their own optimal growth conditions in the soil [15]. Polymers especially plastics are potential substrates for heterotrophic micro-organisms [16].

The biodegradation process is governed by different factors that include polymer characteristics, type of organism, and nature of pre-treatment [17]. The polymer characteristics such as its mobility, tacticity, crystallinity, molecular weight, the type of functional groups and substituents present in its structure, and plasticizers or additives added to the polymer all play an important role in its degradation [18]. Different bacteria and fungi are involved in the process of biodegradation, and these fungi are also involved in utilizing polyethylene [19]. Keeping in view the biodegradable potential of microorganisms, the current study aims to isolate and identify plastic degrading fungi from waste disposal sites and to evaluate the biodegradable ability of these isolated fungi against polythene bags.

## 2. Methods and Materials

*2.1. Sample Collection.* The soil samples were collected from different waste disposal sites of Peshawar such as Gunj Gate, Gulbahar, Jamil Chowk, Nishtarabad, Ring Road, and Agha Mir Jani Road. A total of 15 soil samples were collected from a depth of 3–5 cm and sealed in sterilized bags immediately after sampling and transported to the microbiology lab of Abasyn University Peshawar for isolation of fungi.

*2.1.1. Pretreatment of LDPE Plastic.* For making the plastics hydrophobic, pretreatment was performed by using UV (360 nm) and heat treatment (70°C) [26].

*2.2. Isolation and Screening of Low-Density Polyethylene Degrading Fungi.* One gram of soil and a plastic waste sample were suspended in 50 ml of sterile distilled water and the suspension was incubated in the shaking incubator at 28°C for 30 minutes at 150 rpm, then 0.1 ml from different suspensions were spread directly on the surface of potato dextrose agar plates and the plates were incubated at the temperature of 28°C for 24 hours [27].

*2.3. Media and Chemicals*

*2.3.1. Potato Dextrose Agar.* Potato dextrose agar (PDA) and potato dextrose broth were used for the isolation of fungal species. Sabouraud agar or sabouraud dextrose agar (SDA) is used to isolate dermatophytes and other types of fungi, and can also grow filamentous bacteria such as *Nocardia*. Each medium was prepared using standard microbiological procedures [28].

*2.4. Identification of Fungal Isolates.* Identification of fungal isolates was performed according to their morphological and microscopic characteristics [29].

*2.5. Plate Morphology and Microscopic Identification.* Plate morphology was conducted to determine the fungi on the basis of their color and edges. For microscopic identification, one drop of lactophenol blue solution was added to the slide. Fungal culture was emulsified on it. Then, the slide was fixed with a cover slip. The slide was observed under a microscope and fungal isolates were identified on the basis of their hyphae and spores [30].

*2.6. Determination of Weight Loss.* Preweighed discs of $2 \times 2\ \text{cm}^2$ LDPE were taken and weighed using an analytical balance. The plastic discs were sterilized using a universal disinfectant. Fungal isolates were subsequently subcultured in potato dextrose broth to get 24 hours fresh culture. The plastic disc was aseptically transferred to the conical flask containing 50 ml of minimal salt media (MSM). Each flask was inoculated with fungal isolates. Control was maintained with plastic discs in the microbe-free medium. The flasks were incubated in a shaking incubator for 30 days at 28°C and 150 rpm [31]. After one month, the plastic discs were collected, washed thoroughly using distilled water and 1 molar sodium chloride, and centrifuged to remove all the impurities and fungal biofilm from the plastic discs. The plastics were then shade-dried and the weight loss percentage of the plastic samples was calculated using the following formula [26]:

$$\%\ \text{decrease of plastic weight} = \frac{R_1 - R_2}{R_1} \times 100, \quad (1)$$

($R_1$) = initial weight of plastic film and ($R_2$) = final weight of plastic film.

*2.6.1. FTIR Analysis.* The changes in functional groups and the deformation of bonds after treating polyethylene bags with fungi for 30 days were detected by FTIR in the spectral range of 4000–1000 $\text{cm}^{-1}$ [19].

*2.7. Statistical Analysis.* All the experiments were repeated and performed three times, and to find out the mean and standard deviation, Microsoft Excel was used.

## 3. Results

*3.1. Isolation of Fungal Isolates.* A total of 15 soil samples were collected from different waste disposable sites of Peshawar, KPK, Pakistan. Different fungal isolates were identified, out of which 5 isolates were further studied. The

Table 1: Microscopic and macroscopic identification of the fungal isolates from waste disposal sites.

| Sr. no. | Code no. | Growth media | Growth morphology | Fungi | Hyphae | Spores | Sporangium |
|---|---|---|---|---|---|---|---|
| 1 | BS001 | Sabouraud dextrose agar | Dark greenish color with white edge | *Aspergillus niger* | Septate | Round spores | Clear sporangium |
| 2 | BS002 | Sabouraud dextrose agar | Light greenish yellow color with white edge | *Aspergillus flavus* | Septate | Clear round spores | Clear sporangium |
| 3 | BS003 | Potato dextrose agar | Full white color with full white edge | White rot fungi | Septate having holes in hyphae | No spores | No sporangium |
| 4 | BS004 | Potato dextrose agar | Light brown color with white edge | Brown rot fungi | Septate having holes in hyphae | No spores | No sporangium |
| 5 | BS005 | Potato dextrose agar | Green with blackish shades with white edge | *Penicilllium* | Septate hyphae | Round spores | Clear sporangium |

five fungal isolates were identified on the basis of their morphology, color, and microscopic study. After this fungal consortium was used for checking the plastic degrading activity of isolated fungi.

*3.2. Identification of Fungal Isolates.* *Aspergillus niger* showed a dark greenish color with white edges on plate morphology, while *Aspergillus flavus* showed a light greenish yellow color with white edges. Different shades of color depend on the substrate pH and the substrate itself. A light shady color was observed for white rot fungi, and a light brown color with white edges was observed for brown rot fungi. A green color with blackish shades with white edges was observed for *Penicillium*, as shown in Table 1 and Figure 1.

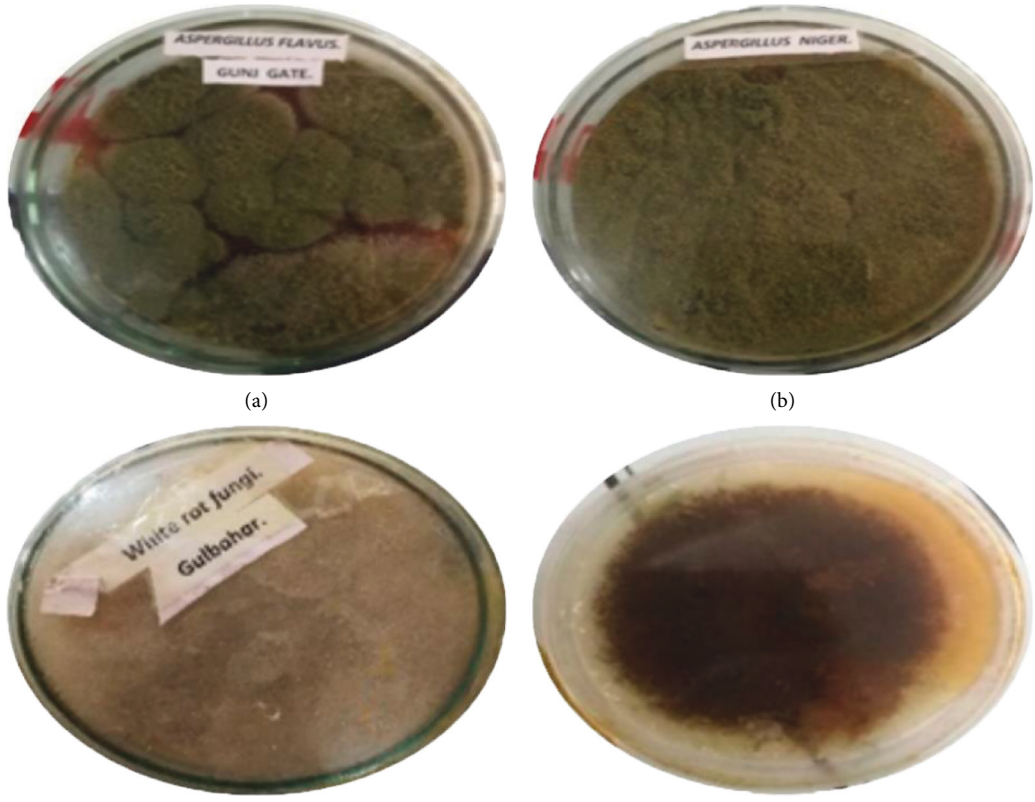
*Aspergillus niger* was observed to have septate hyphae, round spores, and a clear sporangium on microscopy. *Aspergillus flavus* have septate hyphae, clear round spore, and clear sporangium. White rot and brown rot fungi showed septate hyphae having holes and no spores and sporangium were observed for white and brown rot fungi, while a septate body, round spore, and a clear sporangium were observed for *Penicillium*, as shown in Table 1 and Figure 2.

*3.3. Weight Loss Estimation.* In the biodegradation experiment, *Aspergillus niger, Aspergillus flavus*, and brown rot and white rot fungi showed 22.9%, 16.1%, 18.4%, and 22.7% reductions in the polyethylene discs after incubation with the respective isolate for 30 days, while there was no weight reduction observed in the case of *Penicillium*. Control showed no reduction in weight loss. The initial weight $R_1$ of plastic was 17.4 mg before treated with *Aspergillus niger*. After treating and incubation of 30 days in a shaking incubator, the final weight $R_2$ of 13.4 mg was observed to have a significant weight loss of 4 mg and biodegradation of LDPE up to 22.9%, as shown in Table 2 and Figure 3.

*3.4. FTIR Analysis.* In the process of polyethylene bag biodegradation, the isolated fungal specie secretes some sort of enzyme that initiates red-ox reaction, lysis of bonds, and more specifically, esterification and some geometrical changes to plastic bags that possibly result in the change of the inner molecular structure of low-density polyethylene bags. The change in inner molecular makeup was detected by using Fourier transform infrared spectroscopic analysis. The result revealed alteration in functional groups and deformation of C–H bonds, ketones, and double bonds in alkenes for which FTIR peaks were observed at 3440 $cm^{-1}$, 1702 $cm^{-1}$, and 1610 $cm^{-1}$, respectively, as shown in Figure 4. Nontreated polyethylene discs showed no deformation as compared to treated polyethylene bags with fungal species. The deformation in the bond is due to the microbial action on LDPE.

## 4. Discussion

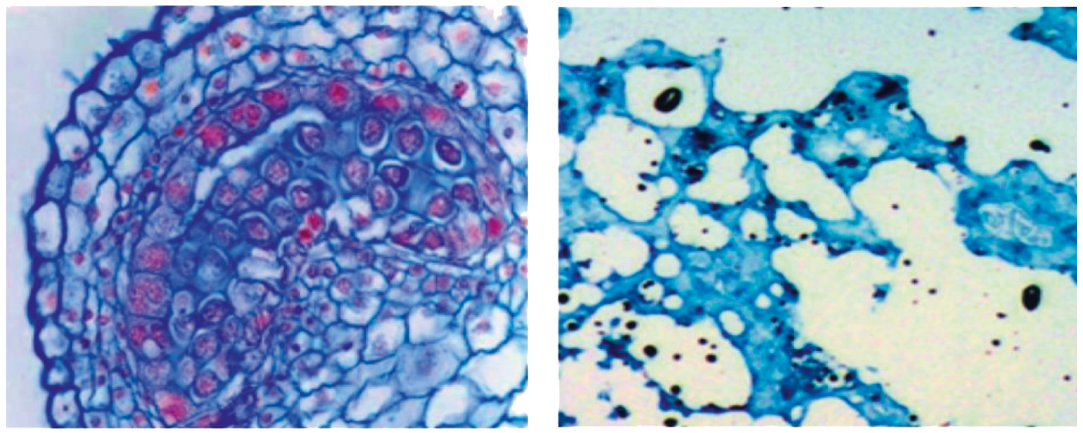
This study has covered the major concern of environmental hazards due to plastic accumulation. Plastics are polymers which cannot be degraded easily. In recent years, biodegradable plastics have been considered alternatives to nonbiodegradable plastics because biodegradable plastics can easily be degraded by microbes. Microorganisms play a vital role in the biological decomposition of materials, including synthetic polymers in the natural environment. Our results revealed the maximum biodegradation percentage of 22.9% for *Aspergillus niger* and the lowest for *Aspergillus flavus* that was 16.1%, while 0% biodegradation was observed for *Penecillium.* The *Penecillium* showed no biodegradation of plastic bags for the reason that it has no such type of biodegradable enzymes that other fungi have. In the current study, the biodegradability was analyzed thoroughly only in MSM liquid medium according to previously published data [20, 21]. In the depolymerization process, two categories of enzymes are actively involved in the biological degradation of polymers: extracellular and intracellular depolymerizes [22]. During degradation, exoenzymes from microorganisms break down complex polymers yielding smaller molecules of short chains, such as oligomers, dimmers, and monomers, which are smaller and can pass through the semipermeable outer membranes of the microbes and then utilize as carbon and energy sources [23]. When plastics are used as substrates for fungi, evaluation of their biodegradability should not only be based on their chemical structure, but also on their physical properties (such as melting point, glass transition temperature, crystallinity, and storage modulus). Our results are in correspondence with [24]. The biodegradation was confirmed by FTIR analysis, which showed specific bond deformation peaks. These peaks




(a) (b)

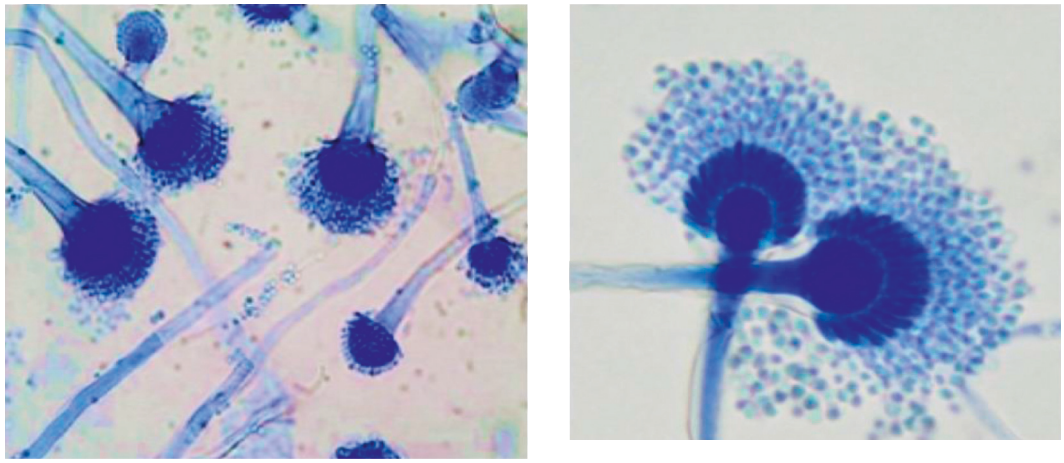
(c) (d)

Figure 1: Different isolated colonies of fungi on (a, b) sabouraud dextrose agar medium and (c, d) on potato dextrose agar medium. (a) *Aspergillus flavus*. (b) *Aspergillus niger*. (c) White rot. (d) Brown rot.



(a) (b)

Figure 2: Continued.



(c) (d)

Figure 2: Microscopic results of different fungal isolates. (a) Brown rot fungi. (b) White rot fungi. (c) Aspergillus niger. (d) Aspergillus flavus.

Table 2: Illustrates the weight reduction of LDPE discs in terms of percent weight loss before and after incubation with fungal isolates.

| Sr. no. | Isolates | Initial weight (mg) | Final weight (mg) | Weight loss (mg) | Percentage (%) |
|---|---|---|---|---|---|
| 1 | *Aspergillus niger* | 17.4 | 13.4 | 4 | 22.9 |
| 2 | *Aspergillus flavus* | 18.6 | 15.6 | 3 | 16.1 |
| 3 | Brown rot | 9.2 | 7.5 | 7.2 | 18.4 |
| 4 | white rot | 4.4 | 3.4 | 1 | 22.7 |
| 5 | *Penecillium* | 5.0 | 5.0 | 0 | 0 |
| 6 | Control | 5.4 | 5.4 | 0 | 0 |

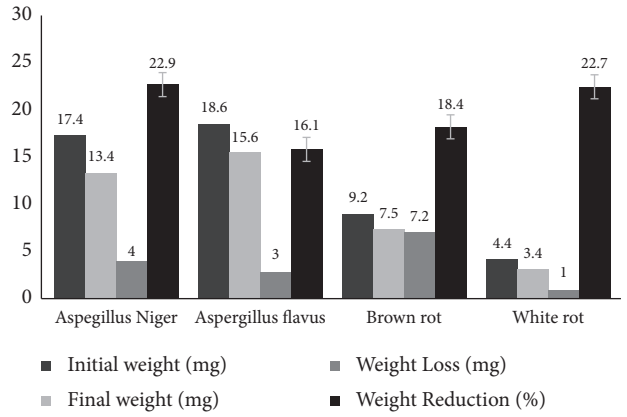



Figure 3: Graphical representation of percentage weight loss of plastic samples after treating with fungal isolates.

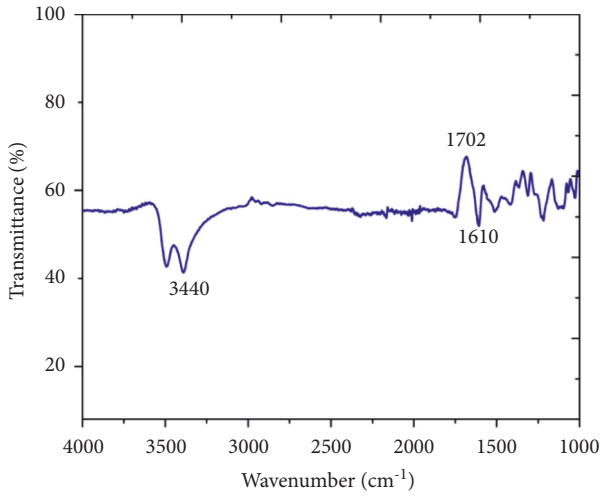



Figure 4: FTIR analysis of LDPE after 30 days of inoculating it with fungal isolates.

are due to the change in molecular geometry of LDPE by the enzymatic process of fungal isolates [23].

To understand the biodegradation mechanism of the fungi, the fungi use various mechanisms such as metabolic or enzymatic and microbial organization that convert the complex material into a simpler one with the release of carbon dioxide. It is based on two methods: growth and cometabolism. In this step, organic compounds are fully degraded (demineralized). The biodegradation mechanism involves a number of microorganisms, including fungi, bacteria, and yeasts [25]. The rate of degradation of contaminants is also based on the contaminant concentration and the quantity of "biocatalyst." In this regard, the number of cells that can metabolize the contaminant and the quantity of enzymes that are formed in each cell reflects the volume of the catalyst. In brief, fungi are able to degrade complex

plastics and thus prove themselves potent agents for bioremediation.

## 5. Conclusion

It has been concluded from the study that fungal species play a significant role in the degradation of synthetic plastic, which can be used in bioreactors in future studies for the degradation of complex plastic materials.

## Data Availability

All the data are available in the manuscript file. However, if some supplementary data are needed, they will be made available from the corresponding author upon reasonable request.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Authors' Contributions

S.G. contributed to the conceptualization, methodology, and investigation of the study; A. was responsible for writing and editing the manuscript; L.M. was responsible for software and figures; L. contributed to the revision and proofreading of the manuscript; S.F. was responsible for revision, proofreading, and editing.

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