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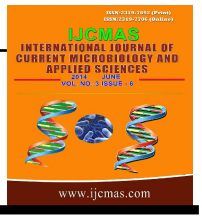


**Original Research Article**

# Screening and Identification of Low density Polyethylene (LDPE) Degrading Soil Fungi Isolated from Polythene Polluted Sites around Gwalior city (M.P.)

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LDPE, Biodegradation, Bioremediation, Degradation ability, *Aspergillus* sp, *Fusarium* sp, *Mucor* sp, *Penicillium* sp

**A B S T R A C T**

Plastic and polythene waste, including low and high density polyethylene, is accumulating continuously in the environment. It is posing an ever increasing ecological threat. The low density polyethylene is comparatively more vital environmental pollutant and its biodegradation was the focus of the present of study. This study reveals the biodegradation with the help of fungal sps. isolated from polythene polluted soil around Gwalior city,(M.P) India. The fungal sps. were identified by plating and staining techniques. 6 fungal sps. were isolated,3 were identified as *Aspergillus* sps. and other 3 were *Fusarium* sp, *Mucor* sp and *Penicillium* sp. The screening of isolates was based on their ability to utilize LDPE as a primary carbon source. Fungal sps. were cultured in synthetic media, containing LDPE, as the sole carbon source. The increase in fungal weight and weight loss of LDPE in the medium,were recorded at regular time intervals. The degradation ability was analyzed by clear zone technique, weight reduction of LDPE along with the increase in weight of fungal isolates. These degrading fungal sps. can be used for bioremediation of polyethylene.

## Introduction

The polyethylene is one of the major sources of environmental pollution, which is a polymer made of long chain monomers of ethylene with different densities. The worldwide utility of polyethylene is expanding at a rate of 12% per annum and approximately 140 million tones of synthetic polymers are produced each year at international level (Shimao, 2001) With such huge amount of polyethylene getting accumulated in the environment, their disposal evokes a big ecological issue.

Because of its very slow natural degradation, thus accumulates in environment in huge amount. It posses the main environmental pollution problems. The biodegradation is a promising method of solving this environmental issue among other physical and chemical degradation method. Our study illustrated the bio degradation of LDPE with the help of certain fungal sps. isolated from polythene polluted sites.

Microorganisms utilizing this organic complex polymer as carbon and biologically transforming to simpler one. The microorganisms secrete several LDPE degrading enzymes in different quantities, which expressed its degradation efficiency of the microorganism. (Bhardwaj *et al*, 2012). In several studies, fungi were considered favorable for the degradation of LDPE due to their higher ability to form hydrophobic enzyme proteins, which helped the fungal sps. in attachment to the polymer surface (Seneviratne, *et al.,* 2006 and Kershaw M.L. ,1998). Kim and Rhee, 2003) also recorded several bacterial sps. as biodegrading agents but the faster growth of fungal biomass was observed when compared to the bacterial sps. (Shah A.A. 2008). (Frazer A.C. 1994) concluded that the extra cellular enzymes were responsible for such degradation process. He also recorded that these microbes attached to the inert surface of polyethylene with the help of enzymes secreted by them and grow on film by utilizing the LDPE and the polymers are depolymerized and are degraded by the process of mineralization into the carbon dioxide ($CO_2$), water ($H_2O$) or methane ($CH_4$). The aim of this study was to isolate the soil fungi which were native to the site of polyethylene disposal sites and showing degradability in natural conditions. The LDPE degradation efficiency of isolates in laboratory condition was determined.

## Materials and Methods

### Collection of fresh samples

Low density polyethylene (LDPE) sheets were obtained from Gwalior Plastic Industry (Gwalior).

### Preparation of LDPE Powder:

The process of Pramila and Ramesh (2011) was followed to prepare the LDPE powder from sheets. The LDPE sheets were cut into small pieces and immersed in xylene and boiled for 15 minutes. To remove the xylene from the solution LDPE solution was treated with ethyl alcohol. The xylene-ethyl alcohol was evaporated and thus obtained LDPE Powder was washed with ethanol to remove the residue of xylene and again it was allowed to evaporate. The powder was dried in hot air oven at 40-50$^{\circ}$C for overnight.

### Media used

SM contains the following constitutions in 1000ml distilled water($K_2HPO_4$, 1 g;$KH_2PO_4$, 0.2 g; NaCl, 1g; $CaCl_2.2H_2O$, 0.002 g; $(NH_4)_2SO$, 1 g;$MgSO_4.7H_2O$, 0.5 g;$CuSO_4.5H_2O$, 0.001 g; $ZnSO_4.7H_2O$,0.001 g; $MnSO_4.H_2O$, 0.001g and $FeSO_4.7H_2O$, 0.01g.) (Pramila and Ramesh 2011).

### Sample collection for Isolation of Fungus

Soil of different waste disposal sites, dumped with polythene bag and plastic waste, were collected from Gwalior city. The soil samples were collected from the depth of 5-6 cm in sterile container and it was air dried at room temperature. These soil samples were placed at 4$^{\circ}$C for further studies.

### Isolation of soil fungi, associated with material (polyethylene bags and plastic bags).

1g of dried soil sample was transferred into a conical flask containing 99ml of sterile distilled water. The contents were shaken and serially diluted. Fungi, associated with materials (polyethylene bags and plastic bags**)** were isolated by pour plate method using Czapek's dox agar. These plates were incubated at 28$^{\circ}$C for 7 days. The fungal growth was isolated and sub-cultured to get pure colonies and then preserved in slant at $5^0$ C in refrigerator (Nigam,S.S.,1965 and Warcup, J.H.,1950).

**Screening of polyethylene degrading fungal sps. by clear zone method**

LDPE powder was added to Synthetic medium at a concentration of 0.1% (w/v) and the culture was kept in shaker oven for one month at $28^0$ C. The fungal colonies thus obtained, were isolated. These isolated sps. were inoculated on LDPE powder containing Czapek dox agar plates and incubated at $28^0$ C for 7 days. The development of zone of clearance around the fungal colonies was recorded. These organisms were selected for further analysis.

**Colonization study**

The colonizing capacity of the fungi on LDPE film was studied by growing the fungi in Petriplates. Synthetic medium was aseptically poured into Petriplates. LDPE sheets were cut into small pieces 2cm x 2cm of similar weight, disinfected with 70% ethanol for 30 min. and transferred to sterile distilled water for 20 min. Six LDPE sheets of similar weight were placed in Petriplates containing the Synthetic medium (without yeast extract). These sheets were inoculated with screened colonies of similar sized fungi using the cork borer. The Petriplates were incubated at $28^0$ C temperature and results were determined after 1 to 4 weeks on the basis of increased weight of fungi (Fresh weight).

**Identification of Polyethylene degrading fungi**

On the basis of macroscopic and microscopic examination, the fungal sps were identified. The Lactophenol cotton blue staining was used (Raper and Fennell 1987).

Identified fungal sp were individually inoculated in Synthetic medium containing LDPE sample. All incubated at $28^{\circ}$C temperature for 4 weeks and blank was also incubated along with experimentals.

**Dry weight determination**

The exposed LDPE polyethylene pieces were recovered after 30 days of incubation from culture medium and washed with methanol and followed by washing with distilled water and dried it at room temperature for 12 hours (Weight).

## Results and Discussion

The fungi screened and identified from polluted sites which can utilize the LDPE as carbon source were noticed as *Aspergillus flavus* (F1), *Aspergillus niger* (F2) *Aspergillus japonicus* (F3) , *Mucor* sp (F4), *Penicillium* sp (F5) and *Fusarium* sp(F6),based on the microscopic examination and morphological characteristics. The increase in fresh weight of the fungal isolates observed in the colonization observation after 1 to 4 week of the experiment (Table-1).

Among the fungi strains, F3(36%) , F6(32%) and F1(30%) showed maximum weight reduction, whereas moderate weight reduction of LDPE was exhibited by F5(24%) and F2(20%). The minimum degradation in the present study was recorded by F4 (16%) after 4 week of experiment (Table -2).

Raaman et al (2012) focused the degradation potential of *Aspergillus japonicus* and noticed the degradation rate as 12% but this rate was only 8% by *A. niger* in one month. Kathiresan and Bingham (2003) reported the *Aspergillus glaucus* and *A. niger*, as potent polythene degrading fungal sps. The *Aspergillus glaucus* was more efficient biodegrading agent in comparison to the *A. niger*. The rates were 28.8% and 7.26% respectively after one month of exposure.

Vasile, C (1993) concluded that the organisms get attached to the surface of polythene film, it starts growing by using its polymer as the carbon source. In the primary degradation, and by the cleavage of the main chain, led to the formation of low-molecular weight fragments (oligomers), dimers or monomers. The degradation was due to the extra cellular enzyme secreted by the organism (Narayan, R., 2006). He also observed that the low molecular weight compounds were further utilized by the microbes as carbon and energy sources. Das and Kumar (2014) studied the LDPE degradation ability of four *Aspergillus* and one *Fusarium* sps after the exposure for 60 days. They observed that the degradation potential of *Fusarium* sp was more when compared to the *Aspergillus sps*.

In the present study the screening and identification of LDPE degrading fungi from polythene polluted sites were focused. LDPE was degraded by the six potent fungal strains after the exposure for period of 30 days. Among the fungal sps, *Aspergillus japonicas* (36%), *Fusarium* sp. ( 32%) and *Aspergillus flavus* (30%) showed maximum but moderate degradation was noticed for *Penicillium* sp (24%) The *Aspergillus niger* and *Mucor sp.* exhibited 20% and 16% degradation of LDPE, which was minimum, among the fungal sps. recorded in the present study.

**Table.1** Fresh weight of the fungus

| Name of the isolates | Fresh weight of the fungus(g) | | | |
|---|---|---|---|---|
| | After 1 week | After 2 week | After 3 week | After 4 week |
| *Aspergillus flavus* (F1) | 0.015 | 0.020 | 0.029 | 0.034 |
| *Aspergillus niger* ( F2*)* | 0.010 | 0.018 | 0.021 | 0.029 |
| *Aspergillus japonicus* (F3 ) | 0.018 | 0.022 | 0.029 | 0.038 |
| *Mucor sp* ( F 4) | 0.006 | 0.009 | 0.013 | 0.026 |
| *Penicillium sp*( F 5) | 0.008 | 0.012 | 0.018 | 0.028 |
| *Fusarium sp (*F6) | 0.012 | 0.018 | 0.026 | 0.032 |

**Table.2** Weight loss comparison

| Treatment | Weight of P.E. (mg) | | Weight of P.E degraded (mg) | Percentage of degraded P.E. in month |
|---|---|---|---|---|
| | Intial weight | Final Weight | | |
| Control | 50 | 50 | 25.8 | 0 % |
| *Aspergillus flavus* (F1) | 50 | 35 | 15 | 30 % |
| *Aspergillus niger* ( F2*)* | 50 | 40 | 10 | 20 % |
| *Aspergillus japonicus* (F3 ) | 50 | 32 | 18 | 36 % |
| *Mucor sp* ( F 4) | 50 | 42 | 08 | 16 % |
| *Penicillium sp*( F 5) | 50 | 38 | 12 | 24 % |
| *Fusarium sp (*F6) | 50 | 34 | 16 | 32 % |

*P.E- polyethylene low density

**Figure.1** Weight of polymer before and after biodegradation

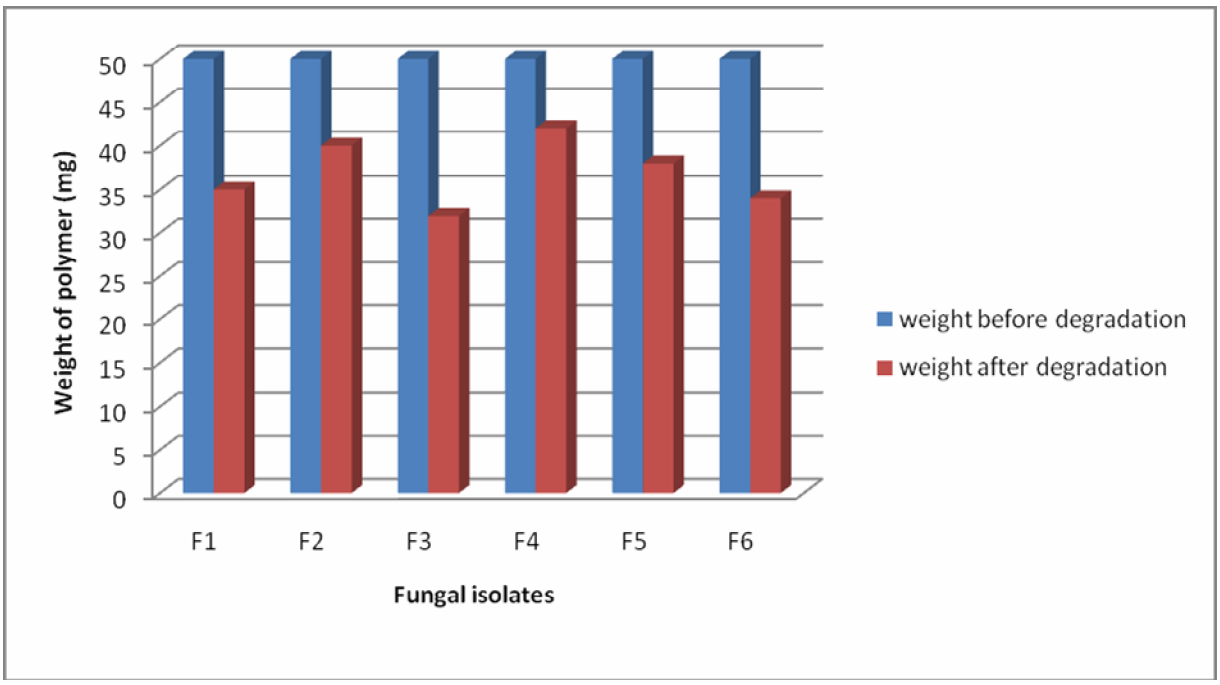


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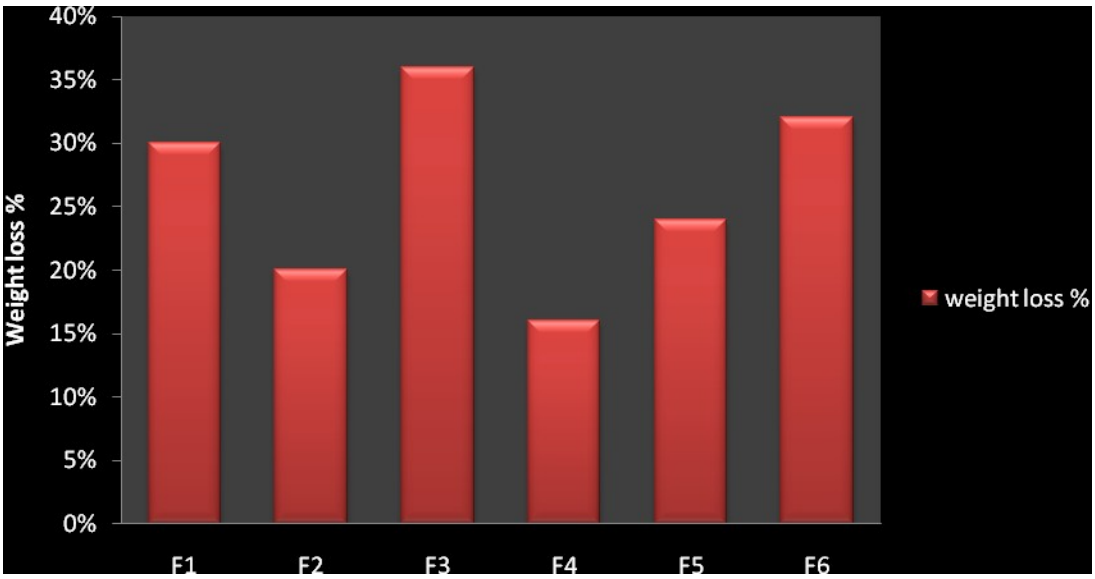
**Fig.2** Percentage weight loss of P.E films after treatment



The soil fungi were able to bring degradation of synthetic polymer in natural condition and isolated fungal sps were also efficient in biodegradation in laboratory condition. Fungal strains, *Aspergillus niger* and *A. japonicas Aspergillus flavus.*

*Fusarium* sp, *Penicillium* sp, *Mucor* sp, *were* screened for LDPE degradation under laboratory conditions. Their effectiveness for degradation was studied over a period of 1 to 4 weeks. Biodegradation was measured in terms of weight loss, which was nearly 16 to 36 % after a period of 4 weeks. Fungal strain *Aspergillus japonicas* F3(36%), *Fusarium* sp F6( 32%), *Aspergillus flavus* F1(30%) showed effective degradation results in 4 weeks as compare to *Penicillium* sp F5(24%), *Aspergillus niger* F2(20%), *Mucor* sp F4 (16%) . These fungal isolates were responsible for decreasing the weight of LDPE films by adhering on its inert surface. This study can be used as valuable microbial tool in the field of bioremediation to solve the inert polythene and plastic waste management

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