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# Biodegradation of Low-Density Polyethylene (LDPE) Plastic by *Staphylococcus* spp. Isolated from Waste Disposal Sites in Urban Peshawar, Khyber Pakhtunkhwa, Pakistan

**Qaisar Shah[1], Saddam[2], Lalina Maroof[3], Nadeem Ullah[4], Khadija Rahman[5], Attiqa Raza[6], Ruqia Hayat[7], Faiza Noor[8], Bilal Musa[9], Hilal Saeed[10]**

[1-10] Department of Health Sciences, City University of Science and Information Technology (CUSIT), Peshawar, KP, Pakistan.

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**Correspondence to:** Lalina Maroof, Department of Health Sciences, City University of Science and Information Technology (CUSIT), Peshawar, KP, Pakistan.
**Email:** mlaleena@gmail.com

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## ABSTRACT

**Background:** Polyethylene (PE), a polymer of ethylene, comprises of long chain backbone of carbon atom and hydrogen, which are linked covalently to each other and are derived from petrochemicals. Polyethylene is widely used due to its low cost, ease of production, versatility and durability. **Aims:** The current research study explores biodegradation of low density polyethylene by Staphylococcus species, isolated from various site of waste disposal in District Peshawar. **Methodology:** In this Experimental study, a total of 20 soil samples were collected using a sterile forceps from 5-15 cm depth at various trash disposal sites in Peshawar and transported in sterile zipper bags to Department of health sciences, City University Peshawar. A stock solution was made by dissolving 1 gram of soil samples in 9 mL of sterile distilled water. To get pure bacterial isolates, serial dilutions was carried out and inoculated on mannitol salt agar (MSA) media, followed by sub-culturing. Films were heat treated at 70oC for 10 days and were further irradiated with Ultra Violet rays (365nm). Using a sharp blade these films were sliced into 2/2cm 2 pieces. Each film was further treated with heat at 70°C for 250 hours. **Results:** The initial weight of plastic was 0.00745, while weight of these plastic after 90 days was recorded 0.08577 after loss with carbonyl index of 0.8686. **Conclusion:** It was found that various species of Staphylococcus could attach to and helps to partially degrade plastic films, as confirmed through weight loss and various analytical techniques like XRD, FE-SEM, FTIR and total carbon analysis. The study observed surface damage to plastic films and a slight reduction in total carbon, which indicates slow and surface-confined biodegradation.

## 1. INTRODUCTION

Plastics are complex materials made up of large molecular chains and have significant characteristics that have greatly changed daily life. Plastic is different from metals due to their unique molecular and structural properties [1]. Common types of plastics include polystyrene (PS), polyethylene terephthalate (PET), polyurethanes (PU), polyvinyl chloride (PVC), propylene (PP) and polyethylene [2]. Polyurethanes are used in various industries, including automotive and medical, for products like fibers and adhesives [3]. The productions of synthetic polymers have grown rapidly over the last twenty years, with around 140 million tons produced annually. The use of plastics for packaging in cosmetics, medications and cleaning agents is significantly increasing, with an annual growth rate of 12% [4]. Plastics are favored due to their water resistance and various other qualities such as strength and low weight. However, the durability of plastic also leads to serious environmental problems, as plastics do not degrade easily in soil and constitute a large portion of waste in landfills [5]. These plastic materials are widely used for packaging, furniture and components of automotive and aviation due to their durability, but they simultaneously create a significant environmental challenge. Plastics possess a non-biodegradable nature and constitute approximately 80% solid waste found on landfills, municipal refuse, coastal areas and various other place on land [6]. Annually, global plastic production has currently reached over 300 million tons. It was assumed that there could be more plastic in the oceans than at the end of 2025. In United States, synthetic polymers make up about 20% of municipal solid waste. Pakistan generates over 30 million tons of solid waste annually, with plastics accounting for a significant amount [7]. There are various methods for plastic degradation such as thermal degradation, which can affect their chemical properties and lead to environmental pollution from harmful by-products. Thermal degradation changes the physical and chemical traits of plastics when exposed to heat, which also generate secondary pollutants. Similarly, photo

oxidative degradation from UV light also damages the surface of plastics, weakening and their structure over time. Various types of plastics degrade at different wavelengths of light, which further complicating impact of plastic waste on natural environment.[8].

Numerous contaminants pose ecological and health risks, including fertilizers, pesticides, plastics and various chemicals. Polyethylene (PE) plastic is a major pollutant and poses significant threats. This current study aims to isolate various types of bacteria from soil samples that can degrade industrial-grade polyethylene (DPE) from soil, and to examine their biodegradation abilities.

## 2. MATERIAL AND METHODS

### 2.1 Study Area

A total of 20 soil samples were collected using a sterile forceps from 5-15 cm depth at various trash disposal sites in in urban regions of Peshawar and transported in sterile zipper bags to Department of health sciences, City University Peshawar.

### 2.2 Sample Processing

A stock solution was made by dissolving 1 gram of soil samples in 9 mL of sterile distilled water. To get pure bacterial isolates, serial dilution was carried out and inoculated on mannitol salt agar (MSA) media, which was followed by sub-culturing as described by [9].

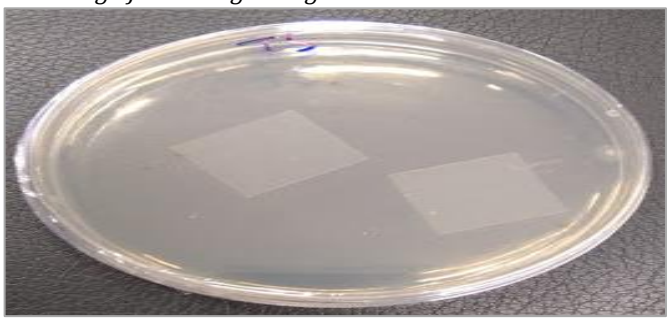
### 2.3 Pre-treatment of Plastic Films

Polythene plastic bags were cut into two (2/2 $cm^{2)}$ and were heat treated at 70°C for 10 days and was then irradiated with ultra violet rays (365nm). Tween 80 was then used to disinfect treated plastic films. Following these procedures, plastic films were washed with ethanol and then rinsed with sterile distilled water, allowed to air dried and was used in further study as per recommended protocol mentioned by [10].

Using a sharp blade, plastic films were sliced into 2/2cm2 pieces. Each film was treated with heat at 70°C for 250 hours in a hot air oven before exposer to UV (365nm) radiations for 500 hours with an equal exposure on all sides of plastic film [11]).

**Figure 1**

*Screening of IDPE Degrading Bacteria in Mineral Salt Media*



## RESULTS

Identified *staphylococcus* species were checked for IDPE degradation. Plastic bags were cut into 2/2 $cm^2$ films and put into mineral salt medium containing IDPE film and no carbon source. Inoculated plates were incubated at 37°c for 30 days. Bacterial species capable of decomposing IDPE were only survive on this media and were thus further processed as per protocol mentioned by [12]).

**Table 1**

*Composition of mineral salt media used in screening of polyethylene degrading bacteria.*

| Media Component | G/1000ML |
|---|---|
| Dipotassium Hydrogen Phosphate (K2HPO4) | 0.5 |
| Potassium Dihydrogen Phosphate (KH2PO4) | 0.04 |
| Sodium Chloride (NACL) | 0.1 |
| Calcium Chloride Dihydrate (CACL2.2H2O) | 0.002 |
| Ammonium Sulfate ((NH4)2SO4 ) | 0.2 |
| Magnesium Sulfate Heptahydrate (MGSO4.7H2O) | 0.02 |
| Ferrous Sulfate (FESO4 ) | 0.001 |
| Manganese Sulfate (MNSO4) | 0.01 |
| AGAR | 1.5 |
| Double Deionized Distilled Water | 1000ML |

### 3.1. Identification of isolates

Identification in the isolates were done using morphological and biochemical tests.

### 3.2. Gram staining

In order to differentiate between gram positive and gram-negative bacteria the isolates will be subjected to gram reaction using standard gram staining techniques. Gram-Gram positive bacteria were appeared as purple colonies, while gram negative as pink colonies based on differences in their cell wall composition.

Gram staining was performed in order to classify bacteria as either gram positive (purple or blue staining) or gram negative (pink or red staining) that was based on differences in composition of cell walls of gram positive and gram negative [13].

### 3.3. Biochemical tests

Pure bacterial isolates were further identified using different biochemical tests as per protocol mentioned by [14]).

### 3.4. Culturing and harvesting of plastic films

Each plastic film was placed in a sterile mineral salt medium Erlenmeyer flask. Each flask was inoculated with a pure culture of IDPE degrading staphylococcus bacteria, while one flask was left as a control. For a duration of 90 days, all these flasks were placed in incubator and incubated at 37 C in a shaking incubator at 150 rpm [9].

### 3.5. Monitoring bio-degradation by percent weight loss

All plastic films were collected and washed for 4 hours at 50°c with a 2% sodium dodecyl sulphate (SDS) solution after 90 day incubation period. The films were then washed twice with double-deionized sterile distilled water and once more with 70% ethanol. To ensure that all bacterial cells and debris were eliminated, this step was performed twice. Prior to being weighed and percentage weight loss being calculated, all of these plastic films were laid out on filter paper and allowed to dry overnight at a temperature of 45 to 50°c as mentioned by [15].

The percentage of weight loss was used to calculate bacterial isolates' capable for biodegradation using the following formula:

$$Percent\ weigth\ loss = \frac{Difference\ in\ initial\&\ final\ weigth\ of\ sample}{Initial\ weigth\ of\ the\ sample} \times 100$$

### 3.6. Analytical procedures

To confirm level of IDPE film biodegradation by IDPE degrading bacteria, the following analytical techniques were applied.

### 3.7. Confirmation of bio-degradation using Emission Scanning Electron Microscopy:

After 90 days treatment with low-density polyethene IDPE degrading bacteria, plastic films were examined for slightly changes in superficial morphology that can be minor holes, cracks or pits. All tested samples were coated with platinum using platinum sputter coater (lica em ace 600) before being analyzed using Field Emission Scanning Electron Microscopy [16].

### A. The fourier transform infrared spectroscopy (FTIR) analysis

After UV exposure, structural integrity of IDPE films was examined and bacteria incubation was assessed using an FTIR spectrophotometer (Nicolet 6700, USA). Each sample was examined using a spectrum from 4000-650 cm-1. To validate production of carbonyl residues, FTIR measurements were performed before and after pretreatments. It is necessary to understand production and decay of carbonyl peaks to follow process of biodegradation. When plastic film was exposed to isolated bacteria, the carbonyl index (ci) was created to measure level of biodegradation. The ci was calculated using relative intensities of carbonyl and ch-2 groups at 1712 cm-1 and 1462 cm-1 respectively.

Carbonyl Index (CI) was obtained through the following formula:

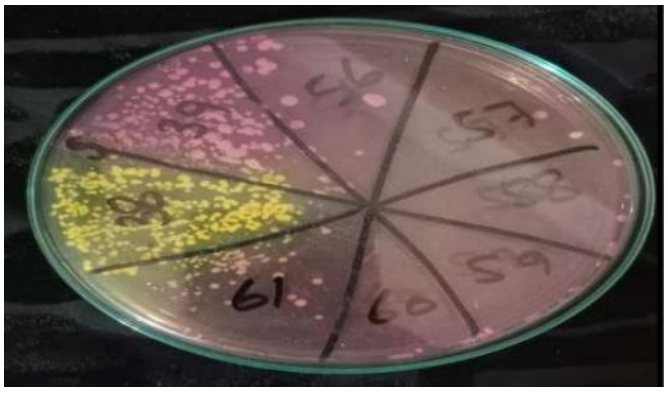
$$\boldsymbol{Carbonyl\ index\ (ci)} = \boldsymbol{absorption\ at\ 1712cm} - \boldsymbol{1\ (maximum\ of\ carbonyl\ peak)}$$

Absorption at 1462 $cm^{-1}$ (maximum of carbonyl peak)

From 20 soil samples, several isolates were inoculated on selective media. These soil samples were first cultured on nutrient agar media. After that, individual colonies were grown on mannitol salt agar (MSA) media plates. The colonies were then subculture on mannitol salt agar (MSA) media plates to produce pure isolated bacterial colonies, which were subsequently identified through various recommended biochemical tests. Among these isolates, 55 isolates of *Streptococcus aurous* were selected on the basis of sub culturing.

**Figure 1**

*Screening result showing isolate's growth on IPEP films placed on MSM media. A (control 1), s007, s0010, s0032, s0034*



### Isolation of Bacteria

On sterile Trypticase Soya Agar (TSA) plates, successively diluted soil samples were inoculated from various bases. Several different colonies were found on media plates after 3-4 days of incubation at 37 oC. All unique colonies were selected based on colour and morphology and were sub cultured to obtain pure bacterial isolates (Figure: 2).These selected soil samples yielded 11 isolates of bacteria, which were then processed for examination (Table: 2).

**Table 2**

*Growth of Staphylococcal isolates on MSA plates with their respective colony color. Morphological tests of 53 Staphylococcus Isolates.*

| S. NO | Sample Code | Growth | Color |
|---|---|---|---|
| 1. | S001 | + | YELLOW |
| 2. | S002 | + | YELLOW |
| 3. | S003 | + | YELLOW |
| 4. | S004 | + | YELLOW |
| 5. | S005 | + | YELLOW |
| 6. | S006 | + | YELLOW |
| 7. | S007 | + | YELLOW |
| 8. | S008 | + | YELLOW |
| 9. | S009 | + | YELLOW |
| 10. | S010 | + | YELLOW |
| 11. | S011 | + | YELLOW |
| 12. | S012 | _ | PINK |
| 13. | S013 | + | YELLOW |
| 14. | S014 | _ | PINK |
| 15. | S015 | + | YELLOW |
| 16. | S016 | + | YELLOW |
| 17. | S017 | _ | PINK |
| 18. | S018 | _ | PINK |
| 19. | S019 | + | YELLOW |
| 20. | S020 | + | YELLOW |
| 21. | S021 | _ | PINK |
| 22. | S022 | _ | PINK |
| 23. | S023 | _ | PINK |
| 24. | S024 | _ | PINK |
| 25. | S025 | _ | PINK |
| 26. | S026 | _ | PINK |
| 27. | S027 | _ | PINK |
| 28. | S028 | _ | PINK |
| 29. | S029 | _ | PINK |
| 30. | S030 | _ | PINK |
| 31. | S031 | _ | PINK |
| 32. | S032 | _ | PINK |
| 33. | S033 | + | YELLOW |
| 34. | S034 | _ | PINK |
| 35. | S035 | _ | PINK |
| 36. | S036 | _ | PINK |
| 37. | S037 | _ | PINK |
| 38. | S038 | + | YELLOW |
| 39. | S039 | _ | PINK |
| 40. | S040 | + | YELLOW |
| 41. | S041 | _ | PINK |
| 42. | S042 | _ | PINK |
| 43. | S043 | _ | PINK |
| 44. | S044 | _ | PINK |
| 45. | S045 | _ | PINK |
| 46. | S046 | _ | PINK |
| 47. | S047 | _ | PINK |

| | | | |
|---|---|---|---|
| **48.** | S048 | _ | PINK |
| **49.** | S049 | _ | PINK |
| **50.** | S050 | _ | PINK |
| **51.** | S051 | _ | PINK |
| **52.** | S052 | _ | PINK |
| **53.** | S053 | + | YELLOW |

### 4.1: Gram Straining

In order to differentiate between gram positive and negative bacteria, all bacterial isolates were subjected to gram staining reaction using recommended gram staining techniques. Gram-positive bacteria appeared as purple colonies, while gram negative as pink colonies due to difference chemical composition of their cell wall.

**Figure 2**

*Clusters of gram-positive cocci observed under microscope. Gram staining was performed on all 53 isolates. Gram-positive cocci bacteria showed purple colonies under the microscope, and had pink colonies.*

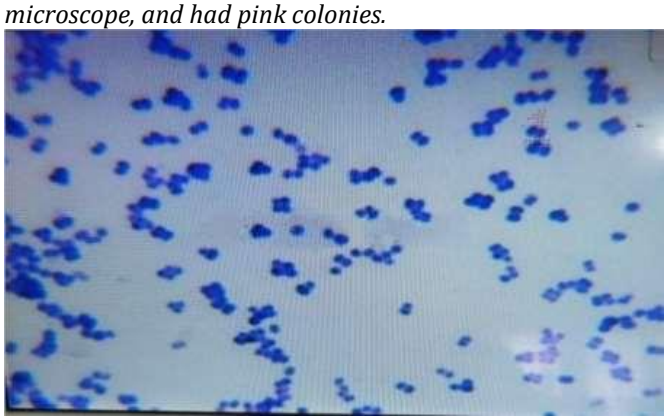


**Table 3**

Demonstrates bacterial isolates from landfill and recycling plant soils.

| Sample location | Dilution Factor | Bacterial Isolates | | | | | |
|---|---|---|---|---|---|---|---|
| | $10^{-3}$ | S001 | S002 | S003 | S004 | S005 | S010 |
| | $10^{-4}$ | S0030 | S0031 | S0050 | S0036 | S0027 | S0018 |
| | $10^{-5}$ | S0024 | S0023 | S0044 | S0046 | S0032 | - |

### 4.2. Catalase test

Catalase is an enzymatic catalyst, which speed up decomposition of hydrogen peroxide (H2O2) into water and oxygen gas. This enzymatic reaction serves to efficiently reduce accumulation of potentially harmful byproducts. This enzyme play protective role through neutralizing bactericidal effects of hydrogen peroxide. However, catalase is not typically present in anaerobic bacteria. The primary function of this enzyme is to convert hydrogen peroxide into oxygen and water through its four hemecatagories, which contain iron and allow the enzyme to react with hydrogen peroxide.

### 4.3. Coagulase test

The coagulase test was performed on all Streptococcus aurous bacteria. All bacterial isolates were coagulase negative as shown in figure 4.

**Figure 3**

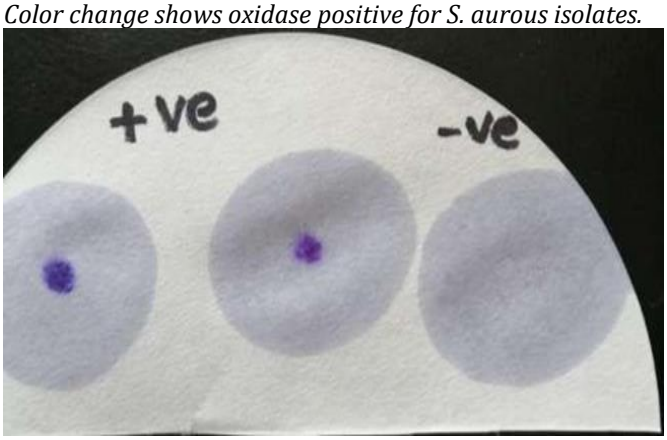
*Image shows coagulase test positive for Streptococcus aurous with a control.*


### 4.4. Oxidase test

All isolated bacteria from 53 samples were passed through oxidase test. Among these isolates, some were oxidase-positive, as proven by a change in color, when oxidase reagent was added, while some isolates were oxidase negative, as no color change occurred, when oxidase reagent was mixed with bacterial isolates, which is shown in figure 5.

**Figure 4**

*Color change shows oxidase positive for S. aurous isolates.*

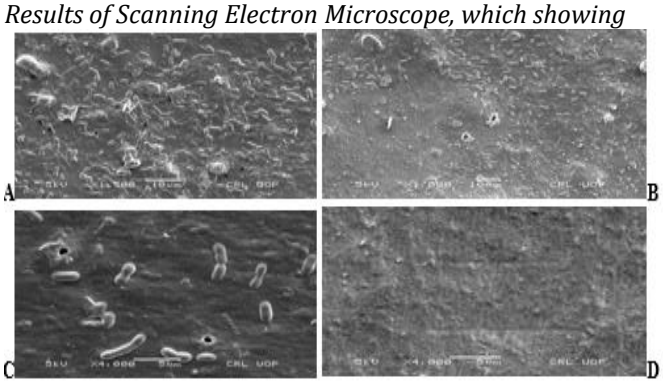



After 90 days of plastic film exposure to bacteria, IDPE films showed bacterial adhesion on their surface. Although no visible changes were noted as seen in figure 5, but microscopic analysis of these plastic films revealed minor cracks and disruptions. These bacterial isolates were able to break down IDPE films, as seen in comparisons with control films at high magnifications.

### 4.5. Fourier transforms infrared spectroscopy (FTIR)

IDPE films were analyzed through FTIR to study changes in their carbonyl groups, through which, biodegradation can be easily detected. The FTIR analysis, which covered a range of 4000-650 cm-1, showed a noticeable carbonyl peak at 1712 cm-1 that decreased after 90 days of bacterial treatment, except for untreated control, which indicates that biodegradation occurred. The carbonyl index, which compares the carbonyl peak to another peak, demonstrated that a drop in this index signifies an increased biodegradation, particularly with *Staphylococcus aurous*, which showed largest decline. All samples displayed carbonyl residues that gradually decreased, confirming microbial activity.

**Figure 6**
*Results of Scanning Electron Microscope, which showing*




*A: Staphylococcus specie, B: Staphylococcus specie, C: Staphylococcus specie, D: Staphylococcus aurous*

**Figure 6** ***FE-SEM Micrograph of IDPE films after 90 days of bacteria incubation***

UV radiations were bombarded on new plastic to confirmed points of bacterial attachment for degradation of bacteria. The plastic was placed in plates for 90 days incubation period, where plastic degradation was detected. The initial weight of plastic film and new weight after 90 days of treatment was recorded, which is shown in table 4. According to table 4, *Staphylococcus aurous* caused plastic degradation. The initial weight of plastic was 0.00745, while weight after 90 days was recorded 0.08577 after loss with carbonyl index of 0.8686, which was confirmed after scanning electron microscope (for morphological changes) and FTIR

**Table 4**
*Results of Bacteria identified, capable of degrading plastic films*

| Sample | I1 | I2 | Carbonyl Index CI CI= I1/I2* |
|---|---|---|---|
| **UV Treated Control A** | 0.01256 | 0.08387 | 0.149755 |
| ***Staphylococcus* spp** | 0.01166 | 0.0848 | 0.137513 |
| ***Staphylococcus* spp** | 0.01016 | 0.08499 | 0.119543 |
| ***Staphylococcus spp*** | 0.00766 | 0.08164 | 0.093827 |
| ***Staphylococcus auras*** | 0.00745 | 0.08577 | 0.08686 |

## DISCUSSION

The current study investigates biodegradation of Low-Density Polyethylene Plastic (LDPE) sheets by various types of bacteria isolated from soil samples, focusing on physical and chemical changes caused by plastic derivative enzymes of microorganisms. The soil from landfills and other plastic burial sites serves as a crucial reservoir for these bacteria [17] [18]. This study identifies three unidentified bacterial strains capable of degrading LDPE in soil contaminated with plastic waste. The first to isolate native bacteria from soil in Peshawar KP, Pakistan, revealed potential for degradation. Various bacterial species, including Bacillus species and Enterobacter asburiae, have been found to develop on plastic film sheets, demonstrating the potential of plastic-degrading bacteria [19] [20]. The biodegradation of plastic polymers requires microbe attachment to surface, leading to the creation of biofilm. The duration of microbial colonization affects the entire biodegradation period. Surface modification, such as UV and heat treatments, can promote microbial colonization [18] [21].

The biodegradation assay involved pretreated films incubated in mineral salt media for 90 days with LDPE films as the sole carbon source. The research aimed to optimize cultural conditions, but prior experiments showed LDPE biodegradation in liquid culture systems. The current investigation used ideal settings, including 5mL of inoculum in 100mL of MSM media with LDPE films at 37°C for 90 days at 100 rpm. The study found that biodegradation of LDPE films is a gradual process, with no visible signs of erosion after 100 days of incubation. This low degradation rate supports previous research showing minor surface holes and degrading eroded areas after 32 years of soil burial [19] [22] [23]. The study examined LDPE films after UV irradiation and bacteria in mineral salt solution. FTIR spectroscopy revealed increased bacterial adhesion and chemical changes. The synthesis of carbonyl groups decreased after 90 days of incubation [24]. found that carbonyl peak drops and double bond increases in LDPE films exposed to biotic environments, indicating carbonyl residues are reduced by –oxidation [25]. Microorganisms thrive better in UV-irradiated LDPE medium due to carbonyl peak production, which provides a site for attachment. FTIR spectra show carbonyl groups induce biodegradation, with B. siamensis having the greatest drop in CI. Incubating LDPE with thermophilic Bacillus borstelensis strain 707 reduced the CI by 70%. The creation and disappearance of carbonyl peaks is crucial for understanding biodegradation mechanisms [26] [27] [28]. The study confirms that LDPE has enzymatic activity, with SEM images revealing localized breakdown by bacterial cells. Extracellular enzymes like laccases, manganese peroxidase, alkane hydroxylase, and lignin peroxidase are implicated in the biodegradation of LDPE [29] [30]. Four bacteria, Staphaures and three staphylococcus species, were identified and treated with UV radiation for 90 days [31]. Staphaureus caused the most degradation, with an initial weight of 0.00745 and a new weight of 0.08577 after 90 days [32] [33].

## CONCLUSION

This study focused on the biodegradation of pure IDPE treated films using bacterial isolates collected from soil samples in various urban regions in Peshawar KP, Pakistan. It found that various species of *Staphylococcus* bacteria could attach to and partially degrade plastic films, which was confirmed through weight loss and various analytical techniques like XRD, FE-SEM, FTIR and total carbon analysis. Four bacterial species were identified, including *Staphylococcus auras* and three others from the same genus of Staphylococcus. After exposing plastic film to UV light and incubating it for 90 days, the initial weight decreased from 0. 00745 to 0. 08577. The study observed surface damage to the films and a slight reduction in total carbon, indicating slow and surface-confined biodegradation. Future research will require genetic techniques to enhance the breakdown of IDPE.

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