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Microbial Degradation of Low-Density Polyethylene Using a Synergistic Consortium from Landfill Soil

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ABSTRACT

Background. Low-density polyethylene (LDPE) is a widely used plastic. A 4-5% annual increase in plastic usage has been observed since the 1960s. Once coined as a "magic material" for its resilience, flexibility, and affordability, plastic has now become an environmental threat due to its severe ecological burden, persistent nature, and non-degradability. The non-degradation of plastic is a major concern in this growing plastic world. The current study investigates the bacterial growth dynamics to degrade LDPE using it as the only carbon source. The bacteria's potential to grow in stressed environments enhances their bioremediation ability.

Methods. Four different types of Gram-positive and Gram-negative bacteria were isolated from the landfill soil sample. After initial screening using the Sherman Manual, bacterial strains were grown in an enriched medium and LDPE was added after pretreatment with UV and ethanol.

Results. At the start, no degradation was observed; gradually, the plastic started to degrade. Ultimately, almost 35% of degradation was observed after 60 days of incubation. Various parameters were also studied, including the light microscopic analysis, pH measurement, optical density, and FTIR analysis. During the experiment, the pH decreased, which caused an increase in the metabolic activity of bacteria. As a result of this high metabolic activity, an increase in the optical density of bacteria was observed. Holes were observed in the plastic sheet under the microscope after incubation. Peaks of 1150 cm^{-1} and 1870 cm^{-1} were observed in LDPE in the FTIR analysis after incubation in the bacterial consortia.

Conclusion. This study reveals the desired/positive effect of bacterial consortia on plastic degradation. Hence, this method can be used to reduce environmental pollution.

Keywords: bacterial consortia, degradation, FTIR, LDPE, SEM

Highlights

- Biodegradation of LDPE by landfill soil bacteria.
- Bacterial consortia were used for the degradation of LDPE.
- SEM analysis and FTIR analysis confirmed the degradation of LDPE by different functional groups.

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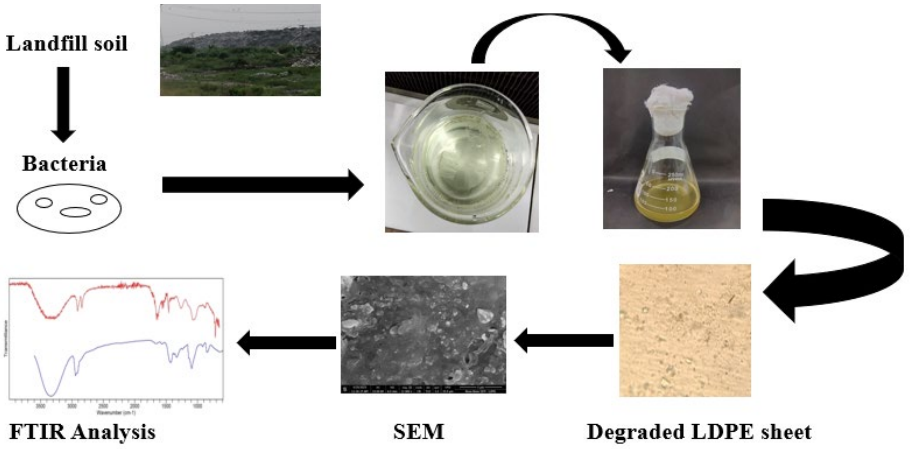
GRAPHICAL ABSTRACT



1. INTRODUCTION

The demand for plastics has increased due to their attractive and versatile applications in various industries and households. The increased use of plastic has led to plastic waste production, which has become a global threat [1]. Low-density polyethylene (LDPE) is a thermoplastic made up of ethylene monomers. It is highly branched, resulting in its low density. LDPE is one of the widely used plastics due to its versatility and effectiveness. It is used in plastic bags for groceries and food commodities. 400 billion of plastic bags are used around the globe before they are discarded [2]. The discarded plastic gets contaminated and releases harmful chemicals, including biphenols and other types of plasticizers. These compounds get released into the environment, soil, and different water reservoirs due to changes in the temperature, pH, and oxygen levels. Further, these compounds cause harmful effects in human beings who use the water from these water reservoirs [3].

The traditional methods used for the degradation of plastics include incineration, landfilling, and recycling. All these methods are expensive and release toxic, potentially carcinogenic compounds [4]. The biodegradation of plastics by microorganisms has gained traction in the bioremediation of plastics due to its eco-friendly nature and low cost [5]. Different bacterial genera including *Klebsiella* sp., *Pseudomonas* sp., *Bacillus* sp., and *Streptomyces* sp. have been reported to degrade LDPE [6]. LDPE is a highly complex compound that demands the combined activity of different bacterial species for its degradation. The use of bacterial consortia results in the increased efficiency of plastic degradation due to the ability of bacteria to break down complex substances into simpler ones [7]. These simpler compounds are then converted to oxygen and this oxygen is released into the environment. The dynamics and stability of bacterial consortia are essential for the efficacy, success, and sustainability of the bioremediation of plastics that results in the mitigation of plastic waste [8].

There are many limitations in the biodegradation of LDPE. A major limitation is the hydrophobicity of the LDPE surface [9]. Pretreatment with UV

and ethanol is the best way to make the surface of LDPE susceptible to degradation. Moreover, bacteria secrete various types of extracellular compounds that help them to colonize /them to colonize the plastic surface. Extracellular substances get attached to LDPE surface and help in the degradation of plastics [10]. Plastics dumped in the landfills are a source of pollution. However, these landfills are also the reservoirs of bacteria that degrade them. These bacteria thrive in stressed environments and their potential to overcome stress is comparatively higher than the bacteria living in favourable conditions [11].

For the biodegradation of LDPE, different strategies and methods are being considered. Different types of bacteria are currently used in consortia to achieve higher rates of plastic degradation. The current study deals with the isolation of bacteria from landfill soil and their potential to degrade plastic by observing different parameters, such as optical density and pH.

2. MATERIALS AND METHODS

2.1. Sample Collection

A 20 g soil sample was taken from two sites of the Lakhodair landfill, with parameters 31˚3736.62" N and 74˚2507.64"E , at a depth of 5-7 cm, using a sterile spatula. The samples were collected in sterile, air-tight plastic bags and transferred to the lab. They were stored at 4°C till further processing [12].

2.2. Strain Isolation and Purification

The refrigerated soil samples were kept at room temperature. A total of 1g of soil sample was enriched in 50 ml BHI (Brain Heart Infusion) broth. Incubation was performed at 37°C for 24 hrs with a control [13]. Then, the standard serial dilution method was used to calculate the number of bacterial colonies in each dilution. The dilutions were spread on the Nutrient agar N-agar poured/nutrient agar plates. The incubation of the plates with the control was done for 24 hrs at 37°C [14]. After proper incubation, CFU was calculated. Distinct colonies on the spread plates were quadrant streaked on separate pour plates. A pure, isolated, and single colony was used for further biochemical screening via the methods stated in the Sherman Manual. The bacterial strains were Gram stained [15].

2.3. Low-Density Polyethylene Degradation

The isolated bacterial strains (bacterial consortia) were inoculated in L-broth along with the thin films of polyethylene sheets cut in the ratio of 1:1 cm (width: length). Plastic pieces were transferred to a solution containing sodium hypochlorite, Tween 80, and distilled water, which was kept stirring for 1 hour. Afterwards, they were transferred to a 70% ethanolic solution for almost half an hour. The ethanolic solution was used for sterilization. Then, polyethylene pieces were incubated for 16 hrs at 50°C. The bacterial strains and plastic pieces were incubated at 37°C for 2 months at 130 rpm in the shaking incubator. Another set was also used but as a control without any bacterial strain containing plastic pieces. This experiment was carried out in triplicates [16].

2.4. Light Microscope Analysis

After every 30 days, plastic pieces were removed from the bacterial consortia in sterile conditions and air dried in the fumehood. The plastic sheet was observed under the light microscope at 100X and the level of degradation by the bacterial consortia was calculated using the percentage degradation method [17].

$$\% \text{ Weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial Weight}} \times 100$$

2.5. Scanning Electron Microscope Analysis

Scanning electron microscopic analysis of plastic pieces was performed. The pieces from both the control and experimental groups were packed in sterile boxes and transported for SEM analysis. The effect of bacterial consortia on these pieces was observed at a magnification of 4000X, 130000X, 25000X, 50000X, and 100000X. They were examined with the scanning electron microscope while using high vacuum mode at 10kV [18].

2.6. Growth Kinetics of Bacterial Consortia

Selected bacterial strains were grown in L-broth, collectively. The experiment was carried out in 100 ml Erlenmeyer flasks and LDPE pieces were used as a sole carbon source by bacteria. The initial optical density was adjusted to 0.1 and optimized during incubation. The incubation time was 60 days at 37°C in a shaking incubator at 130 rpm. The optical density was measured using a spectrophotometer adjusted at 600 nm at the intervals of 0 hour, 7 days, 30 days, and 60 days. An increase in the optical density was observed after regular intervals [19].

2.7. pH dynamics of Bacterial Consortia

The pH of the bacterial consortia was adjusted to 7 at 0 hours. After the incubation of bacterial consortia, the pH was measured at regular intervals [11] using a pH meter.

2.8. FTIR Analysis of LDPE

The FTIR analysis of LDPE was performed before and after its incubation in the bacterial consortia. For this purpose, plastic pieces were removed from the media and air-dried. After drying, they were subjected to FTIR [20].

3. RESULTS AND DISCUSSION

3.1. Strain Isolation and Purification

Four bacterial strains (SZ-1, SZ-2, SZ-3, and SZ-4) were isolated from the soil sample after incubation. Two of them were Gram-positive, while the other two were Gram-negative.

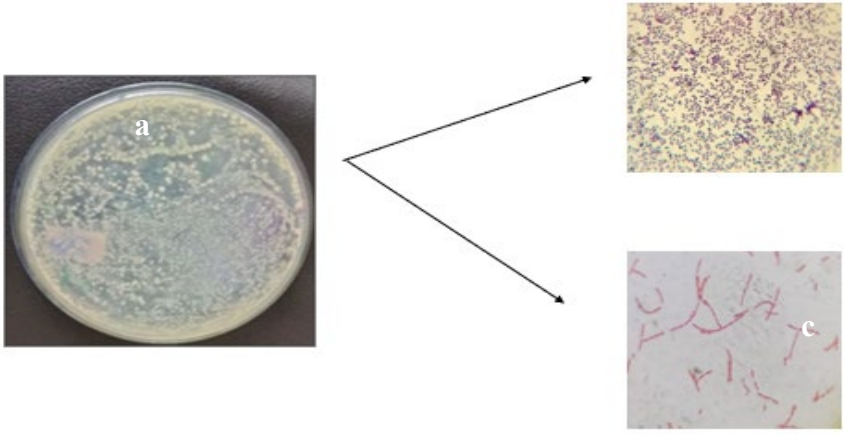


Figure 1. Bacterial Colonies after Spreading a) Gram-negative Bacteria and b) Gram-positive Bacteria

In this study, 50% Gram-positive and 50% Gram-negative bacteria were isolated. The results of [21] also showed the same percentage isolation of Gram-positive and Gram-negative bacterial species purified from the landfill samples. However, another study [22] indicated the isolation of only Gram-positive bacteria from the landfill soil. Different types of Bacillus species were isolated in that study.

3.2. Low-Density Polyethylene Degradation

The pretreatment of plastic pieces was carried out in the 250ml Erlenmyer flask.

3.3. Light Microscope Analysis

Under the 100X microscope lens, degradation was observed after regular intervals. The holes on the surface of the plastic sheet were observed.

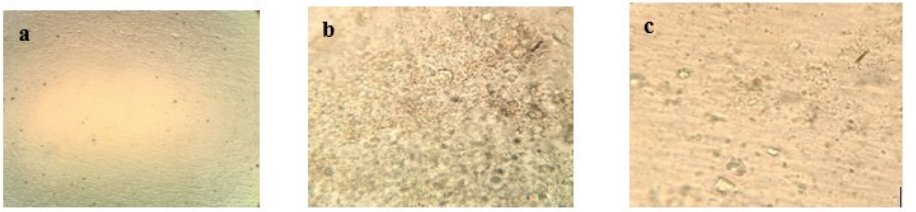


Figure 2. Light Microscopic Analysis (1000X) of the LDPE Sheet a) At 0 Hours of Incubation, b) After 40 Days of Incubation, and c) After 60 Days of Incubation

A study [23] also showed the holes on the surface of LDPE plastic sheet after its incubation in the bacterial consortia. These holes indicated that the bacteria used this LDPE sheet as a source of carbon to grow, resulting in its degradation.

3.4. Scanning Electron Microscopic Analysis

The control (plastic sheet without consortia) and the plastic sheet with bacterial consortia were used for SEM analysis. The excretion of extracellular substances by the bacteria was observed via SEM.

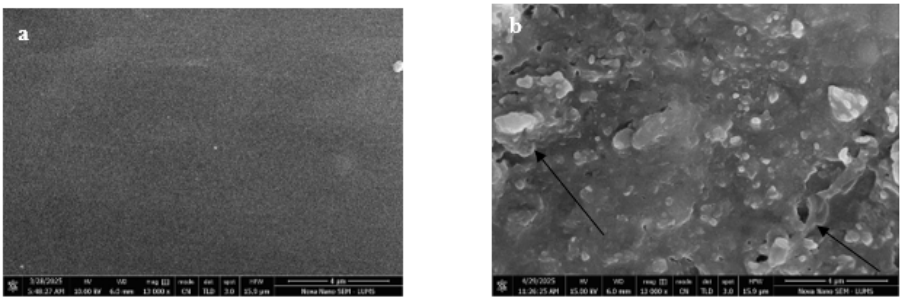


Figure 3. Scanning Electron Micrograph of LDPE Sheet, a) Control b) Colonization of Bacteria-Secreting Extracellular Polysaccharides. The Arrows Show the Holes and Polymeric Substances on the Surface of the Plastic Sheet

The results of the study [24] showed the colonization of the surface of LDPE sheet by extracellular substances. These polymeric substances helped the bacteria to colonize the surface of the plastic sheet. While, the secreted polymeric substances helped in the degradation of the plastic sheet.

3.5. Growth Kinetics of Bacterial Consortia

The bacterial consortia were grown in the enriched media along with LDPE. Bacteria used this LDPE as the only carbon source. An increase in the optical density was observed after 60 days of incubation. The optical density increased in a gradual manner.

Table 1. Optical Density of SZ Consortia Measured at 600nm after Regular Intervals

Strains	OD						
	Ohr	7 days	14 days	21 days	28 days	45 days	60 days
A Consortia	0.289± 0.0167	0.572± 0.0330	0.796± 0.0640	0.887± 0.0543	1.00± 0.0621	1.332± 0.0735	1.772± 0.0879
Control	0.1± 0.001	0.1± 0.001	0.1± 0.001	0.15± 0.001	0.15± 0.001	0.2± 0.002	0.2± 0.002

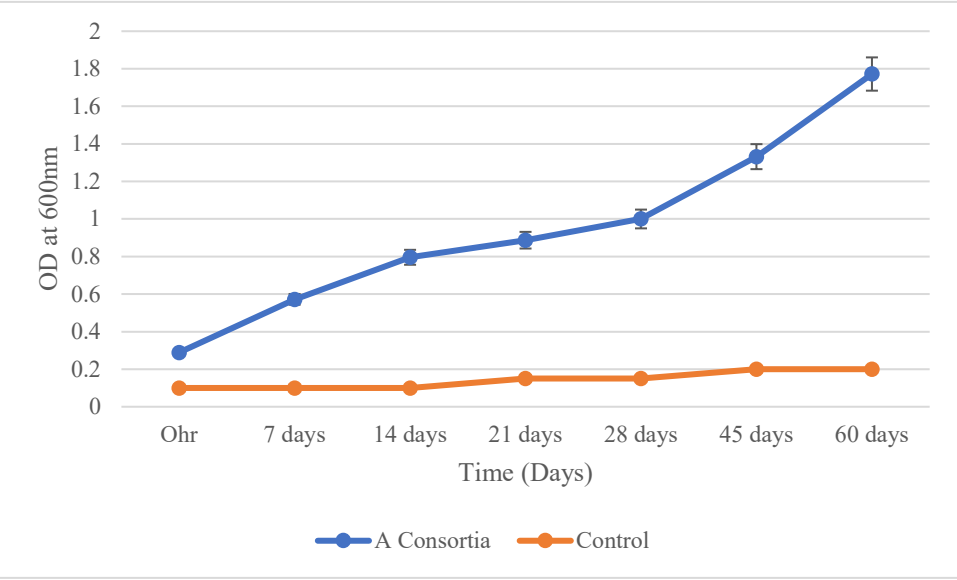


Figure 4. Gradual Increase in Optical Density after 60 Days of Incubation

The initial optical density was 0.289 which increased to 1.772, indicating thriving bacterial growth. The results of another study [23] also correspond to the results of this study. An increase in optical density indicates that bacterial consortia used the LDPE sheet as their only carbon source and the growth of bacteria thrived in this medium.

3.6. pH Dynamics of Bacterial Consortia

A gradual decrease in the pH was observed during the experiment. This pH was measured after regular intervals.

Table 2. pH of Bacterial Consortia SZ Measured at Regular Intervals

Strains	pH						
	Ohr	7 days	14 days	21 days	28 days	45 days	60 days
A Consortia	7.0± 0.05	7.0± 0.04	6.4± 0.05	6.0± 0.04	5.9± 0.03	5.6± 0.02	5.3± 0.0
Control	7.0± 0.00	7.0± 0.00	7.0± 0.00	6.9± 0.00	6.9± 0.00	6.9± 0.00	6.8± 0.00

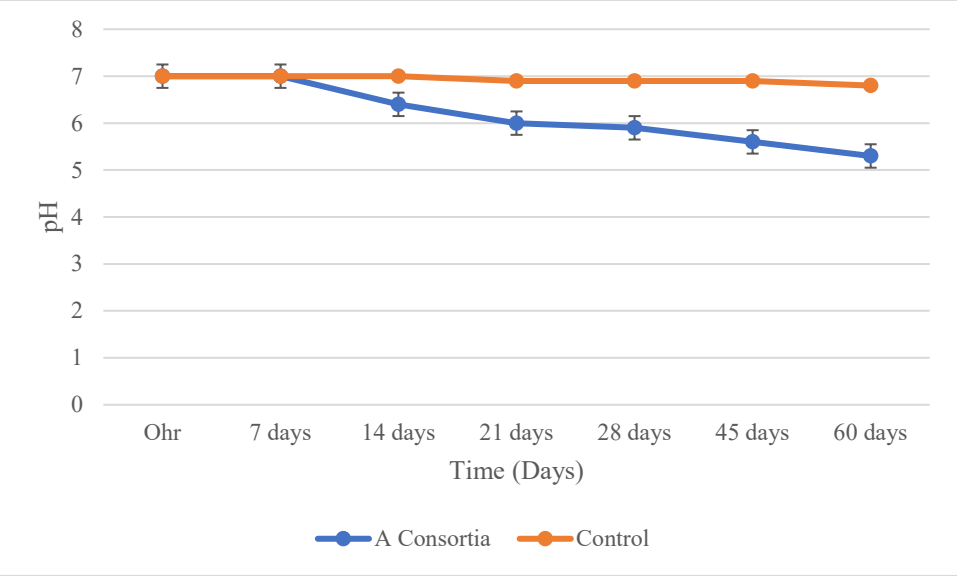


Figure 5. Gradual Decrease in pH after 60 Days of Incubation

The study [25] showed an increase in pH, while decreasing the microbial activity. However, this study showed that a decrease in pH causes an increase in the metabolic rate of bacterial consortia. The given formula was used for the percentage of weight loss estimation:

$$\% \text{ Weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial Weight}} \times 100$$

$$\% \text{ Weight loss} = \frac{1\text{g} - 0.65\text{g}}{1\text{g}} \times 100$$

$$= 35\%$$

A 35% weight loss was calculated as a result of 60 days of incubation of the plastic sheet in the bacterial consortia. Another study [26] showed the same promising results of plastic degradation by the bacterial consortia.

3.7. FTIR Analysis of LDPE

The FTIR analysis of LDPE was also performed. The control and the plastic sheet grown in the bacterial consortia were analyzed by using FTIR. Sharp peaks were observed in the LDPE sheet after incubation in the bacterial consortia.

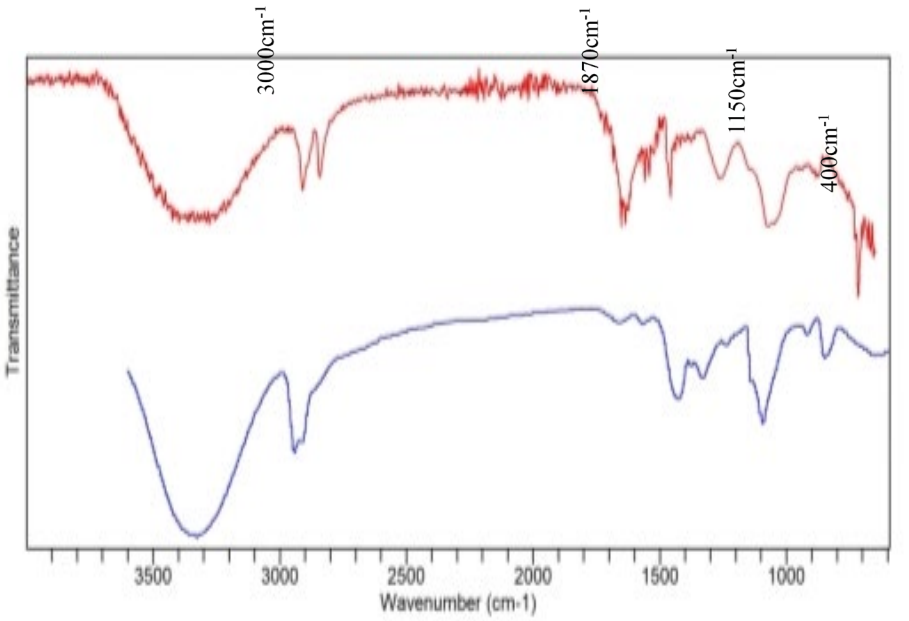


Figure 6. Fluctuation in the Peaks of the Functional Group of LDPE Inoculated in the Bacterial Consortia

In the control, the peak was at 2900 cm^{-1} but in the consortium, the peak went up to 3000cm^{-1}. No peak was visualized in the control at 1870 cm^{-1}. However, in the test LDPE sheet a sharp peak was visualized at 1870 cm^{-1}. The sharpness in peaks indicates the secretion of extracellular substances by bacterial consortia.

For the current study, the soil samples were collected from a landfill containing bacterial isolates. In previous studies, a limited bacterial consortium was landfill-based. The current experiment was limited to 60 days and showed very clear results for degradation apart from the other studies.

The bacterial consortium developed in this study demonstrated a significant potential in degrading LDPE. Such microbial consortia can be effectively applied in the bioremediation of plastic-contaminated environments, especially in landfill sites. By converting plastic waste into biodegradable compounds, mainly polyhydroxyalkanoates (PHA), this approach provides a sustainable solution to plastic pollution.

3.8. Future Research Directions

In the future, large-scale field studies should be conducted to evaluate the efficiency of this bacterial consortium under real environmental conditions. Metagenomic and proteomic analyses can be employed to identify the key genes and enzymes involved in degradation. Furthermore, optimization of growth conditions and consortia composition may enhance degradation efficiency and PHA yield. The integration of such microbial consortia into waste management systems could become a sustainable alternative to conventional plastic disposal methods.

3.9. Conclusion

The current study carried out the initial screening of the bacteria that could degrade low-density polyethylene (LDPE). Microscopic analysis showed the degradation of bacteria through the visualization of holes on the surface of LDPE. SEM analysis showed the colonization of bacteria of the surface of the plastic sheet via the secretion of extracellular substances. Hence, it was concluded that there is a key role of bacterial consortia in LDPE degradation when LDPE is used as a carbon source.

3.10. Limitations

This study has some limitations. The degradation experiments were carried out under controlled laboratory conditions, which don't represent the complexity of the natural environment. Moreover, the metabolic pathways involved in plastic degradation and PHA synthesis require molecular characterization to optimize this process for industrial applications.

Author Contribution

Nazia Jamil: conceptualization, data curation, investigation, methodology, project administration, resources, supervision, validation, visualization, writing – review & editing. **Rida Batool:** data curation, resources, formal analysis, writing – review & editing. **Aleena Zahid:** methodology, software, writing – review & editing.

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request.

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Generative AI Disclosure Statement

The authors did not used any type of generative artificial intelligence software for this research.

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