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## Assessing Biodegradation of Commercially Available Bioplastics in Garden and Agriculture Soil Using Soil Burial test.

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**ABSTRACT**

Bioplastics are potentially promising and sustainable alternative to commercially available conventional plastics. They offer biodegradability and are far less harmful to the environment. However, the biodegradation of bioplastics is observed to significantly vary in different soil environments. This study presents a comparative assessment that focusses on microbial biodegradation of commonly used bioplastics, in garden soil and agriculture soil using the soil burial method. The study observed slightly enhanced biodegradation in garden soil than in agriculture soil. This was confirmed by weight loss measurements and structural analysis by FTIR and changes in few of the physicochemical properties like pH, organic carbon content and water holding capacity were also observed. However, other properties were not altered much which proves that soil microflora does have effect on biodegradation process.
**Keywords:** Bioplastics, biodegradation, soil burial method, FTIR, physico-chemical properties of soil.

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## INTRODUCTION

Plastics are an indespensible part of human life and can be found in almost every sector of day-to-day life, owing to their high performance in variety of applications, , excellent mechanical potential, durability, and low cost [1]. Plastic pollution is a major global problem that needs an urgent collaborative effort. According to the United Nations, the annual production of plastics is expected to double by 2035 (approx. 800 Mt), and reach 1600 Mt by 2050 [19]. The non-biodegradable and highly resistant nature of the material leads to its prolonged presence in the ecosystems. This has shown many negative impacts on the biotic as well as abiotic components of the environment [20]. Bioplastics, a biodegradable and sustainable alternative to conventional plastics has recently garnered a lot of interest. These are bio-based polymers produced from combination of different renewable sources such as starch, cellulose, lignin, pectin, materials originating from microbes and polyhydroxyalkanoates (PHA). These bioplastics made out of such material have exhibited biodegradable property [2]. Unlike regular plastics, the main aim of bioplastics is to reduce environmental impact of non-biodegradable plastics, as it offers a sustainable alternative that lowers the carbon footprints. Higher production costs, multiple mechanical limitations, and inadequate recycling facilities pose a challenge for widespread bioplastic use [21].

Formulations of bioplastics needs improvement and optimization to enhance durability while maintaining its biodegradable nature. Research shows that bioplastics have huge potential in sectors like packaging, agriculture, and biomedical applications, thus reinforcing their role in circular economy models. Though bioplastics are known to be biodegradable in nature, its biodegradation is primarily carried by microbial activity in the soil. This microflora facilitates the breakdown of polymeric structure of bioplastic into simpler compounds. In reality, biodegradation of bioplastics varies prominently soil composition, microbial activity, and other abiotic factors. Researchers are working on gaining detailed understanding of these dynamics as it plays a crucial role in the process of optimizing bioplastic formulations for industrial applications. [13,22]

The most commonly used method to assess the potential of bioplastic biodegradation is the soil burial method. This method helps in mimicking the natural conditions and provide insights into plant as well as microbial interactions, rate of biodegradation, and effects of other environmental factors. Many prior studies have mentioned the importance of soil type and biodegradation efficiency. Various factors such as moisture, pH, organic matter, and microbial diversity play a crucial role in degradation kinetics [6, 9].

This study focussed on comparative assessment of microbial biodegradation of bioplastics in garden and agriculture soil using the soil burial method. Physical and Chemical properties of the garden and agriculture soil was evaluated along with the characterization of the control and biodegraded samples of bioplastic over a span of 40 days. This research seeks to gain insights into the efficiency and variability of biodegradation under garden and agriculture soil conditions as both the soil samples exhibit distinct microbial composition, along with distinct physicochemical properties.

### Biodegradation Mechanisms and Influencing Factors

Biodegradation of bioplastics involves microbial colonization that leads to enzymatic hydrolysis resulting in biodeterioration i.e. the structure is weakened. This is followed by fragmentation of the polymer wherein the microorganisms breakdown the material and finally assimilating the molecules. Commonly occurring biopolymer-degrading microorganisms belong to the *Pseudomonas* and *Bacillus* species, and their presence significantly impact the rate of degradation. Also, various physical and chemical properties of the soil, such as texture, moisture content, water holding capacity, pH, temperature, alkalinity and organic matter, are also known to influence microbial activity thereby, polymer breakdown. The soil acts as a reservoir for millions of microorganisms, of which approximately more than 85% are beneficial for plant life. Thus, the soil is a resilient eco system and soil microorganisms provide precious life to soil systems catering to plant growth [9,7,12]. The process of biodegradation of bioplastics is depicted in Figure no. 1 below.

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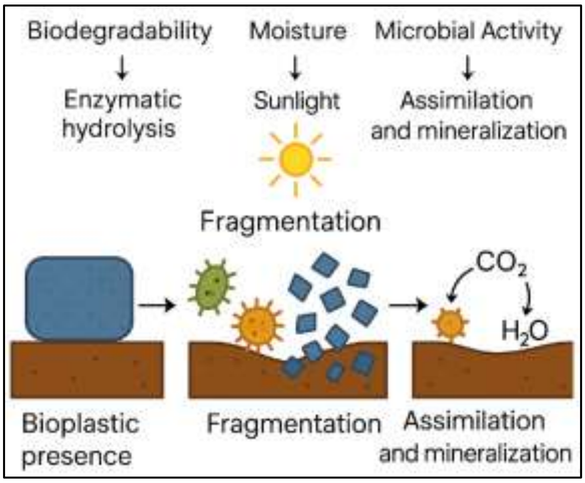


**Figure 1: Process of biodegradation of bioplastics in soil**

Garden soil is rich in organic matter and diverse microflora, which may facilitate enhanced biodegradation as compared to agriculture soil as it is subjected to fertilizers and pesticides more often. This can lead to alterations in the microbial diversity of the soil. Composition of the bioplastic also, affects the degradation rate. Polylactic acid (PLA), polyhydroxyalkanoates (PHA), or starch blends have shown distinct susceptibility to microbial attack [8].

**Significance of Comparative Assessment**

The real-world challenges and performance of the bioplastics can be better understood by a comparative analysis of biodegradation in garden and agriculture soil. This study employed tests as weight loss measurements of the bioplastics, structural analysis by FTIR and soil analysis to evaluate degradation patterns.

## MATERIALS AND METHODS

**Sample collection**

Soil samples from a garden (MIDC, Bhosari) and an agriculture land (Alandi, Pune) were collected in thoroughly cleaned plastic bags, transferred to the college. These soils were kept in separately labelled containers of dimension (40x30x20) cm and moisture was maintained by periodical spraying of water. Commercially available PLA bioplastics were collected from households, washed thoroughly with detergent, dried and stored in Ziplock bags.

**Table 1: Type and Area of the Soil samples collected from PCMC, Pune**

| Soil Type | Location |
|---|---|
| Garden | MIDC, Bhosari |
| Agriculture | Alandi |

**Soil Analysis**

Various physical and chemical properties of the garden soil and agriculture soil samples were performed using standard protocols. Both the soil samples were analysed for their physical properties like colour, texture, temperature and water holding capacity; chemical properties like pH, organic carbon content and alkalinity. The colour of the soil samples was confirmed by using Munsell colour chart, the texture was studied by Jar method and using soil texture triangle. Water holding capacity was calculated by collecting the excess water drained through the soil samples and weighing the soil samples before. pH was detected by using a digital pH meter and temperature of the soil was checked using a laboratory thermometer. Organic carbon content and was determined by Walkley-Black chromic acid wet oxidation method and alkalinity was determined by using two color indicators (Phenolphthalein and methyl orange).

**Biodegradation test by Soil Burial Method**

In this method, the thoroughly cleaned strips of the bioplastic samples were buried in the two different soils i.e. garden and agriculture soil. To assess the microbial biodegradation of the samples weight loss, surface morphology and chemical structure are analysed. Starch-grafted polyethylene exhibited increasing weight loss with prolonged burial, supported by microbial colonization and structural breakdown confirmed via SEM and TGA analyses [17]. One of the studies reported enhanced degradation of plastic bags using *Pseudomonas* and *Bacillus* in beach sand and mangrove sediments, and FTIR analysis indicating significant polymer breakdown over 16 weeks [18]. This method mimics the real conditions in the nature and thus is capable of predicting the fate of biodegradable materials in the nature.

Initially, clean containers were half filled with the soil samples and commercially available clean PLA bioplastics and a sample of conventional plastic were cut into (3×3) cm fragments. These fragments were carefully placed in the designated rows (marked with help of wooden sticks) made in the soil and covered with soil till the container was completely filled. Soil was watered regularly to maintain the moisture. The samples were checked around 20th day and again covered and kept for further biodegradation and they were collected for the final analysis on 40th day.

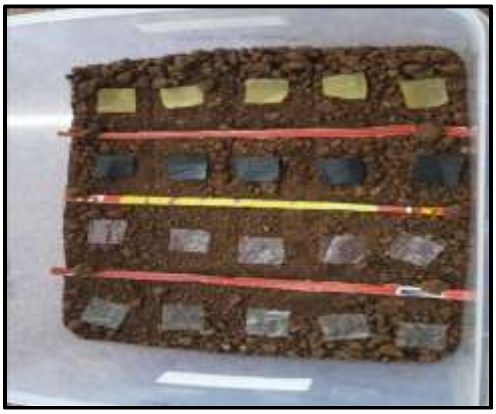

**Figure 1a: Biodegradation test of bioplastics by Soil burial method in Garden S**

**oil.**

**Figure 1b: Biodegradation test of bioplastics by Soil burial method in Agriculture Soil.**

**Analysis of the Weight Loss after biodegradation**

The plastic and bioplastic samples were washed with distilled, dried and weighed multiple times on a digital weighing machine till a constant weight was observed. The weights were taken before and after the process of biodegradation. The loss in the overall weight was calculated by the below formula:

$$\text{Percent weight loss (\%)} = \frac{\text{(Initial weight of the bioplastic sample - Final weight of the bioplastic sample)}}{\text{Initial weight of the bioplastic sample}} \times 100$$

**Characterization of the bioplastic samples by FTIR**

Fourier Transform Infrared (FTIR) analysis is an important and useful tool to identify any formation of new functional groups or disappearance of the existing functional groups in the bioplastic samples. Using this technique, we can identify any degradation products or any new chemical molecules that has been incorporated in polymer structure as a result of biodegradation process [10].

## RESULTS AND DISCUSSION

The present study deals with the comparative analysis of microbial biodegradation in garden and agriculture soil. Microflora in the soil has reported many useful abilities like degradation of wastes, petroleum, plastics and other xenobiotic materials. Microbes such as *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes,* and *Aspergillus niger* are reported to show plastic degrading activity. [10]

The physico-chemical analysis of soil shows that the both the soil samples were loamy in nature but shown a small textural difference after the biodegradation process. Water holding capacity of both the soils was significantly reduced while, temperature showed a drop of ~ 3̊ C. Organic carbon content was increased after biodegradation, positively in both the soils, on the other hand alkalinity was not much altered. The results of the above tests are mentioned in the table number 2 and 3.

**Table 2: Analysis of Physico-Chemical Properties: Garden Soil**

| Properties | Before test | After test |
|---|---|---|
| Sand (%) | 50% | 40% |
| Silt (%) | 33% | 33% |
| Clay (%) | 16% | 26% |
| Textural Class | Loam | Silt Loam |
| Water Holding Capacity | 65% | 35% |
| Soil Colour | Reddish Brown | Reddish brown |
| Temperature | 29°c | 26°c |
| PH Value | 7 | 6 |
| Organic Carbon Content | 1.4 % | 2.5 % |
| Alkalinity | PA= 0 mg (CaCO3)/litre. MOA = 210mg (CaCo3)/litre | PA= 0 mg (CaCO3)/litre. MOA = 205mg (CaCo3)/litre |

*Phenolphthalein Alkalinity (PA); Methyl Orange Alkalinity (MOA)

**Table 3: Analysis of Physico-Chemical Properties: Agriculture Soil**

| Properties | Before test | After test |
|---|---|---|
| Sand (%) | 33% | 36% |
| Silt (%) | 50% | 50% |
| Clay (%) | 16% | 13.3% |
| Textural Class | Clay Loam | Loam |
| Water Holding Capacity | 75% | 40% |
| Soil Colour | Black | Black |
| Temperature | 31°c | 27°c |
| PH Value | 12 | 8 |
| Organic Carbon Content | 0.69% | 0.98% |
| Alkalinity | PA= 0 mg (CaCO3)/litre. MOA= 232.5mg (CaCo3)/litre | PA = 0 mg (CaCO3)/litre. MOA = 227.5mg (CaCo3)/litre |

*Phenolphthalein Alkalinity (PA); Methyl Orange Alkalinity (MOA)

**Biodegradation test by Soil Burial Method**

This method is popular when it comes to assessing the microbial biodegradation of polymers in the soil under natural conditions. The bioplastic samples were placed under the soil for 40 days and the moisture in it was maintained throughout the period. They were cleaned, thoroughly washed with distilled water, dried and weighed. When the weight of all samples appeared to be constant, they were noted down and percent weight loss was calculated [17, 18].

**Weight Loss Assessment**

Multiple environmental factors chemical composition of biopolymers like bioplastics plays a crucial role in their biodegradation. These biopolymer structures undergo varying degrees of weight loss during their biodegradation. Here, weight loss observed on garden soil was more than in agriculture soil which is presented in the table no. 3. Bioplastics with polylactic acid (PLA) or polyhydroxyalkanoates (PHAs), are known to show more pronounced degradation as they are more susceptible to microbial attack owing to the presence of ester linkages. Hence, a measurable weight loss over few weeks to months can be observed and this majorly depends on the factors like soil moisture, temperature, pH, and microbial diversity. Weight loss corelates with the functionality of the bioplastics and their environmental compatibility. Polyethylene or polypropylene resists microbial attack due to its recalcitrant nature and hydrophobicity and thus are known to exhibit minimal weight reduction. Percent weight loss is a quantitative indicator of the amount of material that is degraded or broken down and also can help in assessing formulations for alternative packaging materials [9, 3, 14, 15, 16].

**Table 4: Weight loss observed in the bioplastics after 40 days of Biodegradation test.**

| SAMPLE NO. | DAYS OF TREATMENT | INITIAL WEIGHT (g) | FINAL WEIGHT (g) | Difference in the weight (g) | PRECENTAGE WEIGHT LOSS (%) |
|---|---|---|---|---|---|
| **GARDEN SOIL** | | | | | |
| **Control** | 0 | 0.0078g | 0.0075g | 0.0003g | 3.84 |
| **Sample 1** | 39 | 0.0078g | 0.0063g | 0.0015g | 19.23 |
| **Sample 2** | 39 | 0.0080g | 0.0071g | 0.0009g | 11.25 |
| **Sample 3** | 39 | 0.0079g | 0.0064g | 0.0015g | 18.98 |
| **AGRICULTURE SOIL** | | | | | |
| **Control** | 0 | 0.0091g | 0.0089g | 0.0002g | 2.19 |
| **Sample 1** | 39 | 0.0095g | 0.0082g | 0.0013g | 13.68 |
| **Sample 2** | 39 | 0.0093g | 0.0082g | 0.0011g | 11.82 |
| **Sample 3** | 39 | 0.0089g | 0.0082g | 0.0007g | 7.86 |

**FTIR Analysis**

Analysis of the biodegraded and non-biodegraded samples was carried out by Fourier Transform Infrared Spectroscopy, to assess any alterations in the functional groups (Milstein et al., 1994). The collected samples were subjected to FTIR characterization for (in the range of 3500-500cm-1). Figure no. 3 shows the FTIR spectra of bioplastic before degradation, while figure 4, and 5 represents the FTIR spectra of the samples after biodegradation. Although no significant changes in the existing functional groups were observed in all the three FTIR spectra, the peaks in the biodegraded bioplastic samples in both the soils were seen to be sharper. Lowered transmittance suggests breakdown of the structure and increased porosity along which makes it more suitable for microbial enzyme attack. Peaks observed at 2913 $cm^{-1}$ and 2846 $cm^{-1}$ are associated with C-H stretching in aliphatic compounds in alkane groups and asymmetric stretch of -$CH_2$ groups respectively, both commonly present in bioplastics [10,11]. 1464 $cm^{-1}$relates to C-H bending vibrations, especially of methylene group present in aliphatic carbon structures. 1017 $cm^{-1}$ peak is attributed to C-O stretching vibrations, indicating the presence of alcohol or ether groups in a biopolymer. 873 $cm^{-1}$ and 716 $cm^{-1}$ corresponds to out-of-plane C-H bending vibrations, suggesting the presence of aromatic or cyclic structures. The peak observed at 524 $cm^{-1}$ in typically corresponds to skeletal vibrations of molecular structures, such as C–C bonds found in polymers. It may also be indicating the presence of specific functional groups, depending mostly on the composition of the bioplastic [3,4,5].

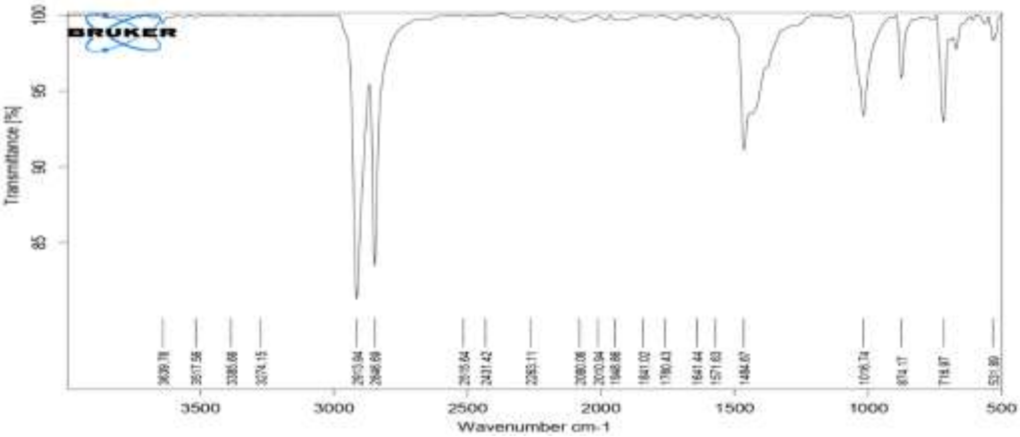


**Figure 3: FTIR of Bioplastic that was not subjected to biodegradation (Control)**

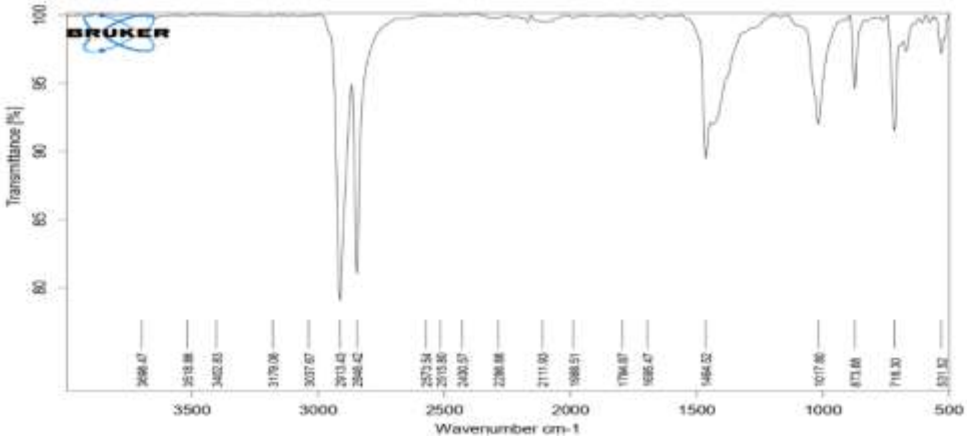


**Figure 2: FTIR of Bioplastic after 40 days of biodegradation (Garden soil)**

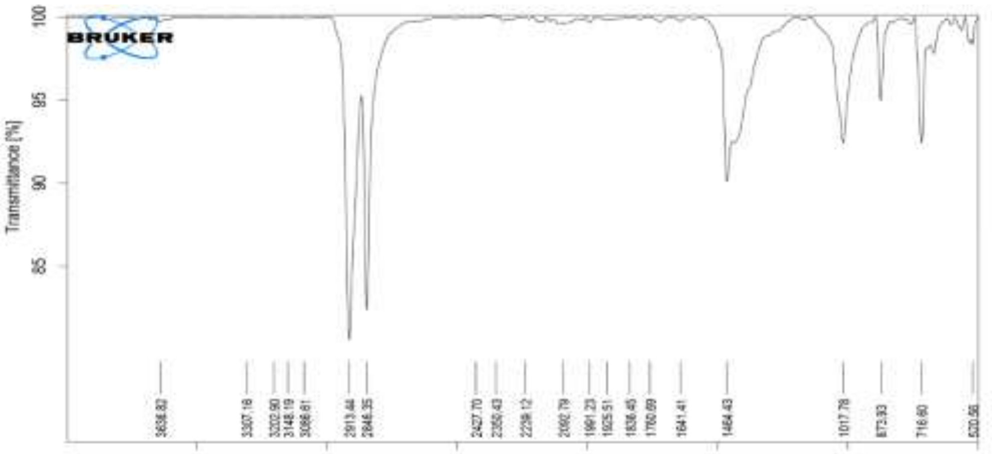


**Figure 3: FTIR of Bioplastic after 40 days of biodegradation (Agriculture soil)**

## CONCLUSION AND FUTURE PROJECTIONS

The study reported notable findings which paved a way for future research which needs to be aimed at optimization of the experimental conditions. It also emphasized on the different biodegradation rates in both the soil samples and the altered physico-chemical properties of the soils proved the microbial action. Thus, more detailed studies on the soil properties are necessary to assess the biodegradation process occurring in soil which may also help in identifying any environmental impact of it. Furthermore, the research idea of this study aligns with the efforts taken by countries all over the globe for development of a sustainable packaging alternatives. This would help us in reducing the wide use of conventional synthetic polymers and in turn promote the need of eco-friendly waste management strategies.

## REFERENCES


[1] Andrady, A. L., & Neal, M. A. (2009). Applications and societal benefits of plastics. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*(1526), 1977–1984.

[2] Sidek, I. S., Draman, S. F. S., Abdullah, S. R. S., & Anuar, N. (2019). Current development on bioplastics and its future prospects: An introductory review. *INWASCON Technology Magazine, 1*, 3–8.

[3] Milstein, O., Gersonde, R., Huttermann, A., Frund, R., Feine, H. J., Ludermann, H. D., Chen, M. J., & Meister, J. J. (1994). Infrared and nuclear magnetic resonance evidence of degradation in thermoplastics based on forest products. *Journal of Environmental Polymer Degradation, 2*(2), 137–152.

[4] Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances, 3*(7), e1700782.

[5] Narancic, T., Verstichel, S., Reddy Chaganti, S., Morales-Gamez, L., Kenny, S. T., De Wilde, B., Babu Padamati, R., & O'Connor, K. E. (2018). Biodegradable plastic blends create new possibilities for end-of-life management of plastics but they are not a panacea for plastic pollution. *Environmental Science & Technology, 52*(18), 10441–10452.

[6] Haider, T. P., Völker, C., Kramm, J., Landfester, K., & Wurm, F. R. (2019). Microbial degradation of four biodegradable polymers in soil and compost demonstrating polycaprolactone as an ideal compostable plastic. *Waste Management, 97*, 105–114.

[7] Gruter, G.-J. M., Parsons, J. R., & Tietema, A. (2021). Comparison of the aerobic biodegradation of biopolymers and the corresponding bioplastics: A review. *Science of the Total Environment, 753*, 141953.

[8] My Experiment. (2014, June 25). *How to isolate plastic degrading micro-organisms from soil*. http://www.instructables.com/id/How-to-isolate-plastic-degrading-bacteria-from-soi/step4/

[9] Thakur, P. (2012). *Screening of plastic degrading bacteria from dumped soil area* (M.Sc. thesis). Department of Life Science, National Institute of Technology - Rourkela, Odisha, India.

[10] Jumaah, O. S. (2017). Screening of plastic degrading bacteria from dumped soil area. *IOSR Journal of Environmental Science, Toxicology and Food Technology, 11*(5), 93–98.

[11] Kamble, A., Tanwar, S., & Shanbhag, T. (2015). Isolation of plastic degrading micro-organisms from soil samples collected at various locations in Mumbai, India. *International Research Journal of Environment Sciences, 4*(3), 77–85.

[12] Mörtl, M., Damak, M., Gulyás, M., Varga, Z. I., Fekete, G., Kurusta, T., Rácz, Á., Székács, A., & Aleksza, L. (2024). Biodegradation assessment of bioplastic carrier bags under industrial-scale composting conditions. *Polymers, 16*(24), 3450. https://doi.org/10.3390/polym16243450

[13] Adhikari, D., Mukai, M., Kubota, K., Kai, T., Kaneko, N., et al. (2016) Degradation of Bioplastic in Soil and their Degradation Effects on Environmental Microorganisms. Journal of Agricultural Chemistry and Environment, 5, 23-24. https://doi.org/10.4236/jacen.2016.51003

[14] Folino, A., Karageorgiou, A., Calabrò, P. S., & Komilis, D. (2020, August 1). Biodegradation of wasted bioplastics in natural and industrial environments: A review. *Sustainability (Switzerland)*. MDPI. https://doi.org/10.3390/su12156030

[15] Kumar, B. M., Noobia, S., & Mythri, S. (2016). Studies on biodegradation of plastic packaging materials in soil bioreactor. *Indian Journal of Advances in Chemical Science, S1*, 297–299.

[16] Saeed, M., Ahmed, A., Aftab, M. N., Afzal, M., & Naveed, M. (2022). Biodegradation of polyethylene by bacterial and fungal consortia isolated from plastic-contaminated soil. *Archives of Microbiology, 204*, 358. https://doi.org/10.1007/s00203-022-03050-8

[17] Gautam, R., & Kaur, J. (2013). Biodegradation of starch grafted polyethylene using microorganisms. *Journal of Environmental Research and Development, 8*(1), 12–20.

[18] Shovitri, M., Yuliani, S., & Mahmudah, R. A. (2017). Biodegradation of plastic bags using *Pseudomonas PL-01* and *Bacillus PL-01* isolated from mangrove and beach sand. *Journal of Environmental Chemical Engineering, 5*(5), 5025–5031. https://doi.org/10.1016/j.jece.2017.09.025

[19] Barra, R., Leonard, S., Whaley, C., & Bierbaum, R. (2018, June). *Plastics and the circular economy: A STAP document*. https://doi.org/10.13140/RG.2.2.11515.57128

[20] Yang, Y., Zhang, X., Li, Q., Chen, J., & Liu, M. (2023). Environmental persistence and impacts of non-biodegradable plastics on terrestrial and aquatic ecosystems. *Environmental Pollution, 319*, 121013. https://doi.org/10.1016/j.envpol.2022.121013

[21] Yadav, K., & Nikalje, G. C. (2024). Comprehensive analysis of bioplastics: Life cycle assessment, waste management, biodiversity impact, and sustainable mitigation strategies. *PeerJ, 12*, e18013. https://doi.org/10.7717/peerj.18013

[22] Kaur, R., Pathak, L., & Vyas, P. (2024). Biobased polymers of plant and microbial origin and their applications: A review. *Biotechnology for Sustainable Materials, 1*, Article 13. https://doi.org/10.1007/s44154-023-00013-9