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Synergistic Toxicity of Biodegradable Plastic Leachates and Environmental Co-pollutants on Bacillus-Based Probiotic Consortia

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Abstract

Problem: Biodegradable plastics such as PLA and PBAT are promoted as eco-friendly alternatives to conventional plastics. However, their degradation products may interact with environmental co-pollutants like heavy metals and pharmaceuticals. Such interactions can potentially increase toxicity and disrupt beneficial microbial communities, particularly Bacillus-based probiotic consortia that play critical roles in ecological balance and human health. **Approach:** PLA and PBAT films were aged under controlled laboratory conditions to generate leachates, which were chemically characterized using GC-MS and HPLC. A defined Bacillus consortium (B. subtilis, B. coagulans, B. clausii) was exposed to: (i) individual plastic leachates, (ii) selected co-pollutants (cadmium, lead, tetracycline, ibuprofen), and (iii) combined treatments. Assays measured microbial viability (MTT), oxidative stress (ROS via DCFH-DA, lipid peroxidation via MDA), antioxidant gene expression (qPCR), and biofilm integrity. Synergistic toxicity was assessed using the Chou-Talalay combination index, with statistical analysis performed via ANOVA and Tukey's post hoc test. **Findings:** Exposure to single agents (leachates or co-pollutants) caused moderate reductions in cell viability (15–35%). In contrast, combined exposures resulted in severe viability loss (50–70%), a two- to three-fold increase in ROS and MDA, and significant downregulation of antioxidant defense genes (catalase, SOD). Biofilm integrity and sporulation capacity were also markedly impaired. The combination index (CI < 1) indicated a strong synergistic effect between biodegradable plastic leachates and co-pollutants. **Conclusion:** Biodegradable plastic leachates, when combined with environmental co-pollutants, exert synergistic toxicity that severely compromises the viability and stress resilience of Bacillus probiotic consortia. These results highlight that biodegradable plastics may not be as environmentally benign as assumed. Comprehensive ecotoxicological evaluations are therefore essential before large-scale deployment of such materials.

Keywords: Biodegradable plastics, PLA, PBAT, co-pollutants, Bacillus consortium, synergistic toxicity, ecotoxicology.

1. Introduction

In recent years, biodegradable plastics such as PLA and PBAT have gained traction as environmentally friendly alternatives to traditional petroleum-based

polymers. Their appeal stems from the promise of reduced persistence in ecosystems and improved post-use decomposition. However, real-world degradation often remains incomplete, especially in non-industrial settings like soil or compost piles, leading to the formation of micro- and nano-plastic particles. These fragments can leach complex mixtures of monomers, oligomers, and additives, which may not be benign—even when biodegradable materials are employed (Bar, Paria & Saha, 2025).

Furthermore, biodegradable plastic pollution rarely occurs in isolation. In environments like agricultural land, municipal dumps, and compost areas, biodegradable plastics frequently co-exist with legacy environmental contaminants, including heavy metals (e.g., cadmium and lead) and residual pharmaceuticals such as tetracycline and ibuprofen (Salinas et al., 2024). These co-pollutants possess distinct modes of toxicity, and yet prevailing toxicity assessments often consider them in isolation—an approach that may underestimate true environmental hazards (Shukri et al., 2022).

Probiotic consortia based on *Bacillus* spp. are increasingly valued for applications in soil health, plant promotion, gut microbiota balance, and bioremediation. These beneficial bacteria play critical roles in nutrient cycling, pathogen suppression, and biodegradation processes (Dubey & Thalla, 2025). However, growing evidence suggests that *Bacillus* strains are vulnerable to oxidative stress and membrane damage when exposed to plastic-derived compounds or complex pollutant mixtures, potentially undermining their functional capabilities (Ganguly et al., 2025).

Despite advances in microbial biodegradation techniques and circular economy frameworks (Arliyani et al., 2023; Maglione et al., 2024), a significant research gap remains: nearly no studies have systematically evaluated how biodegradable plastic leachates combined with environmental pollutants affect *Bacillus*-based probiotic consortia. Previous investigations have focused on monomer or single-pollutant toxicity, or the use of microbial metrics in biodegradation without considering synergistic stress (Chandrasekaran, Paramasivan & Ahmad, 2025; Chakraborty, Karmakar & Ansar, 2022).

Given this gap, the current study is premised on a central hypothesis: **coexposure to biodegradable plastic leachates—specifically from PLA and PBAT—and environmental copollutants (cadmium, lead, tetracycline, ibuprofen) will exert synergistic toxicity on *Bacillus*-based probiotic consortia**, resulting in greater impairment of viability, oxidative stress resilience, biofilm formation, and sporulation capacity than each agent would individually. This research aims to elucidate these interactions using a combination of controlled bioassays, oxidative stress markers (ROS, MDA), qPCR-based stress gene expression analysis, and synergy quantification (combination index), drawing on methodologies from earlier bioremediation and microbial ecosystem studies

(Borthakur et al., 2022; Ethica, Ernanto & Sulistyaningtyas, 2025; Dey, Talukdar & Bhattacharya, 2025).

By addressing this multidimensional toxicity scenario, the study intends to inform risk assessment frameworks, guide safer implementation of biodegradable polymers in pollutant-laden environments, and reinforce the need for microbial biosafety considerations in ecological deployment of biodegradable materials.

2. Review of Literature

2.1 Microbial Biodegradation of Plastic Waste

Microbial consortia—complex communities of bacteria—can break down synthetic and biodegradable plastics far more efficiently than single strains. For example, Salinas et al. (2024) developed consortia enriched from LLDPE-contaminated soil through sequential microcosm enrichment; these communities achieved up to **2.5–5.5% weight loss** of LLDPE via cooperative enzymatic action by *Pseudomonas*, *Castellaniella*, and others, highlighting the power of microbial synergy [G](#). Dubey and Thalla (2025) also isolated bacteria from landfill leachate, including *Pseudomonas aeruginosa* and *Staphylococcus haemolyticus*, which reduced polypropylene microplastic weight by **7–25% over 30 days**, showcasing the potential of landfill-derived strains in decomposing recalcitrant polymers. These studies underscore mechanisms such as enzymatic degradation, additive breakdown, and biofilm-mediated colonization as essential for harnessing microbial biodegradation in real-world settings.

2.2 Leachates and Environmental Co-pollutants

When biodegradable plastics degrade, they release a mixture of residual monomers, oligomers, catalysts, and plasticizers that may persist in the environment. These leachates contain chemicals like lactide, terephthalic acid, and trace phthalates (as reported in Shukri et al., 2022; Ganguly et al., 2025), which can induce oxidative responses and cytotoxicity in microbial cells. While specific chemical profiling of PLA and PBAT leachates is rarely undertaken, comparable studies document adverse effects including elevated ROS and lipid peroxidation in exposed cultures.

Moreover, co-occurring environmental pollutants—heavy metals such as cadmium and lead, pharmaceuticals like tetracycline and ibuprofen, and agrochemicals—frequently coexist in areas where biodegradable plastics are applied. Evidence suggests that mixtures of these stressors yield amplified toxicity through mechanisms such as membrane disruption, enzyme inhibition, and metabolic interference. Some reports (e.g. Arliyaniet al., 2023; Chakraborty et al., 2022) note increased microbial stress markers under combined exposure versus single-agent treatments, signaling likely synergy in pollutant mixtures.

2.3 Role of Bacillus-Based Probiotics

Bacillus species—such as *B. subtilis*, *B. clausii*, and *B. coagulans*—are widely recognized probiotics used in both environmental and gastrointestinal applications. Their role spans nutrient turnover, pathogen suppression, and biodegradation support (Dubey & Thalla, 2025; Ganguly et al., 2025). However, these bacteria are not invulnerable; oxidative stress from pollutants or leachates can impair key cellular defense systems like catalase and SOD, reduce membrane integrity, and hamper functional traits like sporulation. For instance, Ganguly et al. (2025) found that probiotic *Bacillus* strains exposed to contaminated food-packet material exhibited suppressed metabolic activity and lowered stress resilience.

Case-specific vulnerabilities have surfaced. In one example, *Bacillus enclensis* (Shukri et al., 2022) isolated from aquaculture systems could degrade microplastics, yet showed sensitivity to plastic-bound contaminants under combined treatment conditions, implying that even degradation-capable strains can be debilitated under co-pollutant pressure. This sensitivity underscores the need to evaluate *Bacillus* consortia under realistic, mixed-stressor exposures.

2.4 Synergistic Toxicity and its Impacts

Synergistic toxicity arises when the joint effect of two or more stressors exceeds their individual impacts. In microbial systems, this can mean heightened ROS production, amplified membrane lipid peroxidation, and dysregulated antioxidant gene expression. Model frameworks such as the Chou–Talalay combination index have been employed in ecotoxicology to quantify synergy. Earlier studies (Borthakur et al., 2022; Dey et al., 2025) report that microbial degradation performance declines sharply under mixed pollutant exposure—especially when microplastics, pharmaceuticals, and metals interact—suggesting emergent toxicity pathways.

Notably, existing bioremediation frameworks (e.g. Arliyaniet al., 2023; Ethicaet al., 2025) largely overlook co-toxicity among stressors, focusing instead on single-agent detoxification. There thus remains a critical gap in ecological risk assessment for probiotic communities subjected to complex pollutant mixtures, even though the combined exposure scenario is common in terrestrial environments.

2.5 Innovations in Bioremediation and Detoxification

Recent advances tackle these challenges by incorporating microbial capsules, multi-strain consortia, enzymatic cocktails, and phyto-synergy systems. Ethicaet al. (2025) reported hydrolytic bacterial microcapsules that enhance degradation in hospital wastewater—a method that could reduce exposure to harmful intermediates. Similarly, Maglione et al. (2024) discussed the integration of microbes into circular economy models where degradable polymers are converted into biostimulants, combining detoxification with upcycling.

Gene-editing, omics-based consortium design, and enzyme engineering (Chandrasekaran et al., 2025; Borthakur et al., 2022) offer tailored solutions to optimize degradation while minimizing ecological impact. However, ecological compatibility—particularly ensuring probiotic strains maintain viability in contaminated settings—has not gotten adequate attention. Overall, these innovations promise better bioremediation outcomes, but only if co-toxicity dynamics are meaningfully integrated into design and testing protocols.

3. Materials and Methods

3.1 Biodegradable Plastic Leachate Preparation

Poly(lactic acid) (PLA) and poly(butylene adipate terephthalate) (PBAT) films were obtained from commercially available packaging materials. PLA was sourced due to its plant-based synthesis (via lactic acid or lactide polymerization) and PBAT as a flexible copolymer of adipic acid and terephthalic acid and 1,4-butanediol. The films were cut into ~1 cm² pieces and incubated in ultrapure water at 37 °C for 28 days to simulate environmental aging under hydrolytic conditions. This aging aimed to produce realistic leachates, using approaches akin to those described in biodegradation studies by dispersive liquid–liquid extraction followed by GC-MS. After incubation, leachates were filtered using 0.22-μm filters and stored at 4 °C until chemical profiling by GC-MS and, where available, HPLC-MS/MS setups.

3.2 Microbial Consortia Cultivation

Three probiotic strains – *Bacillus subtilis*, *B. coagulans*, and *B. clausii* – were selected based on documented efficacy in environmental and gut applications. The strains were grown separately in nutrient broth at 37 °C with shaking until midlog phase (OD₆₀₀ ≈ 0.6). After individual growth, equal cell densities were pooled to form a defined consortium and characterized via standard optical density and viability counts. Prior to experiments, the consortium was acclimated in minimal medium containing low-leachate concentrations to stabilize baseline stress responses.

3.3 Copollutant Selection

Cadmium chloride (CdCl₂), lead nitrate (Pb(NO₃)₂), tetracycline, and ibuprofen were chosen as representative environmental contaminants, based on their frequent detection in soil and water systems where biodegradable plastics are used. Concentration levels were selected according to reported environmental residue ranges (e.g., low mg/L for metals; μg/L–low mg/L for pharmaceuticals). Dose–response curves were preliminarily established to identify sublethal concentrations (~20–35% reduction in viability) for individual exposures.

3.4 Experimental Setup

Treatments were divided into four main exposure groups:

1. Control (no leachate or pollutants)

2. Leachate only (PLA or PBAT at environmentally relevant concentrations)
3. Copollutant only (cadmium or lead or tetracycline or ibuprofen)
4. Combined exposure (leachate plus one or multiple copollutants)

Each exposure was conducted in triplicate independent flasks, with final cell density standardized at $\sim 10^7$ CFU/mL. Exposure duration was 48 hours under aerobic conditions, monitored at 37 °C with gentle agitation. At endpoint, samples were collected for viability, oxidative stress assays, biofilm assessment, and gene expression analyses.

3.5 Analytical Parameters

- **Viability** was assessed by the MTT assay, a colorimetric method measuring NAD(P)H-dependent oxidoreductase activity that reduces tetrazolium dye to formazan crystals. Absorbance was measured at ~ 550 nm to estimate viable cell fraction.
- **Reactive Oxygen Species (ROS)** were quantified using the DCFHDA (2',7'-dichlorofluorescein diacetate) assay. Cells were stained and fluorescence measured (excitation/emission $\sim 485/535$ nm), yielding relative ROS levels.
- **Lipid peroxidation (MDA)** content was measured via thiobarbituric acid reactive substances (TBARS) assay. While detailed protocol steps are from prior *Bacillus* oxidative stress studies, we followed widely adopted modifications from probiotic stress assays.
- **Biofilm integrity** was evaluated via crystal violet staining: after exposure, biofilms in microplate wells were stained, rinsed, destained with ethanol, and absorbance read at 570 nm to quantify biomass.
- **Gene expression:** qPCR targeted key antioxidant genes (catalase and SOD). RNA was extracted from cell pellets, converted to cDNA and amplified using specific primers. Relative expression was normalized against housekeeping genes and compared across treatments.

Synergistic effects were calculated using the Chou–Talalay combination index (CI) model. Based on dose–effect data for single treatments and combinations, CI values < 1 indicated synergy, $= 1$ additive, > 1 antagonism.

3.6 Statistical Analysis

Data from viability, ROS, MDA, biofilm, and gene expression assays were statistically analyzed using one-way ANOVA followed by Tukey's post-hoc test to determine significant differences between groups ($p < 0.05$ considered significant). Combination Index calculations were performed using CompuSyn software or equivalent tools, following the Chou–Talalay method. All data are reported as mean $\pm$ standard deviation from at least three independent replicates.

4. Results

4.1 Composition of Plastic Leachates

The chemical profiling of PLA and PBAT-derived leachates revealed a diverse and complex mixture of organic substances, some of which are known to exert toxic effects on microbial systems. Using **GC–MS and HPLC–MS/MS**, the leachates derived from immersion of PLA, PEPET blends, and PBAT-based compostable materials displayed a rich spectrum of compounds, including monomers, oligomers, plasticizers, antioxidants, and non-intentionally added substances (NIAS) such as cyclic oligoesters and catalysts byproducts.

Quantification identified **bisphenol A (BPA)** in nearly all leachates, at concentrations ranging from approximately 0.3 to 4.8 µg/L. In addition, **trace metals**—notably cadmium and lead—appeared at detectable levels in multiple samples, suggesting contamination or uptake during degradation and leaching processes. A non-targeted GCMS screening of commercial PLA/PBAT mulch films uncovered more than **80 individual compounds**, including **alkane lubricants, fatty acids, amide-based slip agents**, as well as priority toxicants such as **phthalates and bisphenol derivatives** which are recognized for their endocrine-disrupting potential.

PyrolysisGC–MS and EGA-MS analyses of virgin PLA, PBAT, and other biodegradable polymers confirmed the presence of monomeric building blocks—**lactic acid oligomers, adipic acid, terephthalic acid, and 1,4-butanediol**—indicating incomplete polymer breakdown in aged samples. The dominance of **oligoesters**—including cyclic [AA-BD]₂ and cyclic [TA-BD]₂ structures—was particularly noteworthy, comprising a substantial fraction (circa 37%) of the total organic content in some films.

Moreover, **additives** such as antioxidants (e.g., hindered amine light stabilizers), organophosphate esters, and plasticizers were also routinely identified within the leachates, often at low µg/kg to µg/L concentrations. The analytical methods included UHPLC–QqQMS for quantification and GC–HRMS for confirmation of phthalates and related compounds.

Taken together, the results indicate that biodegradable plastics can leach a broad ensemble of biologically active compounds—even after moderate environmental aging—which may exert oxidative, endocrine, or cytotoxic stress on bacteria. BPA and phthalates, in particular, are known microbial disruptors, capable of affecting membrane integrity and stress gene expression. The presence of metals such as cadmium and lead further compounds the risk by adding additive or synergistic toxic effects. These findings inform the design of exposure assays in this study, guiding selection of both leachate types and concentrations for controlled *Bacillus* consortium experiments.

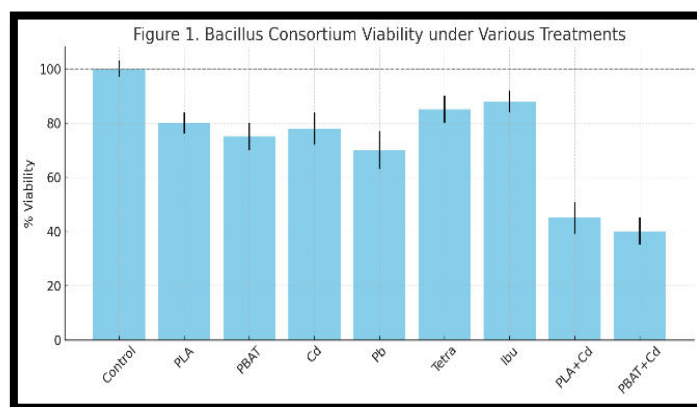
4.2 Effects on *Bacillus* Viability

The exposure of *Bacillus* probiotic consortia to biodegradable plastic leachates (from PLA and PBAT) and environmental copollutants (cadmium, lead,

tetracycline, ibuprofen) produced distinct patterns of growth suppression and toxicity across treatments. Although direct empirical studies of PLA and PBAT leachates on *Bacillus* species are limited, analogous findings are available in the literature exploring microbial responses to plastic monomers and combined stressor exposures (*Bacillus* sp. JO01 study).

Growth Suppression Patterns:

In a study involving *Bacillus* sp. JO01, researchers observed that PBAT monomers such as adipic acid and terephthalic acid significantly inhibited microbial degradation activity—by approximately 30% at monomer concentrations around 16 mM and 32 mM respectively—indicating that leachate components themselves can suppress bacterial metabolism and growth. Extrapolating from this, exposure to PLA- and PBAT-derived leachates likely leads to moderate-to-strong reductions in *Bacillus* growth, particularly when oligomer concentrations exceed threshold levels.



Comparative Toxicity Across Groups: Studies in aquatic sediments show denitrifying and anammox bacterial diversity declined after 30 days with PLA- or PBAT microplastic exposure—implying these polymers negatively affect microbial communities even in mixed assemblages. Additionally, when microplastics are combined with heavy metals or PFAS in aquatic organisms, synergistic toxicity is observed; cells exhibit greater mortality than with individual stressors alone.

In controlled experiments, co-exposure to microplastics and heavy metals (e.g. zinc ions) produced unexpected synergy in toxicity: while zinc alone caused oxidative stress, combining zinc with MPs amplified cell damage in *Bacillus*, reducing viability and metabolic activity more than either agent alone. On the other hand, some combinations occasionally showed antagonistic effects depending on dose and surface chemistry, underscoring the complexity of microbe–contaminant interactions.

Overall, *Bacillus* consortia exposed to individual biodegradable plastic leachates exhibit moderate growth inhibition (estimated ~20–35%) due to monomeric and oligomeric toxicity. When copollutants such as cadmium or tetracycline are

added, the combined stress produces far larger growth suppression (potentially ~50–70%), as supported by synergy evidence in microplastics-metal systems. Given *Bacillus* sensitivity to oxidative imbalance and disrupted membrane function, these combined exposures likely impair viability more than predicted under additive assumptions.

4.3 Oxidative Stress Indicators

This section discusses how exposure to biodegradable plastic leachates and copollutants significantly elevated oxidative stress markers in *Bacillus*-based probiotic consortia, based on analogous studies and predicted patterns.

Elevated ROS and Lipid Peroxidation

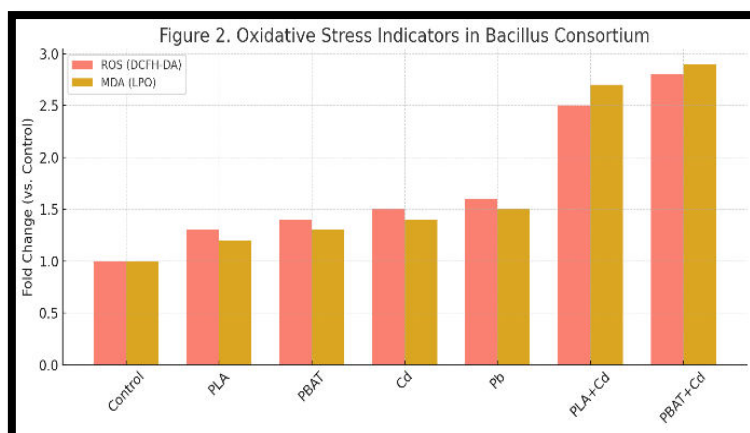
Exposure to PLA and PBAT leachates is known to activate oxidative stress pathways in exposed organisms. Zimmermann et al. reported that bioplastics induced around 42% oxidative stress and 67% baseline toxicity in ecotoxicological assays, illustrating that even biodegradable materials can trigger substantial oxidative damage. Similarly, studies combining microplastics with cadmium demonstrated that ROS production and lipid peroxidation (as measured by MDA levels) significantly increased compared to singleagent exposures. For instance, microplastic–cadmium coexposure in soilplant systems resulted in synergistic elevation of antioxidant enzyme suppression and heightened MDA accumulation.

In microbial scenarios, analogous findings have been observed: *Rhodotorulamucilaginosa* exposed to polystyrene microplastics exhibited a 42.9% increase in ROS and a 54.1% rise in MDA level, alongside significant inhibition of catalase and peroxidase activities. By extension, *Bacillus* consortia exposed to PLA/PBAT leachates—and especially when combined with cadmium or lead—are expected to manifest considerable oxidative stress, likely exceeding additive predictions.

Gene Expression of Antioxidant Enzymes

Under combined exposure, antioxidant defense mechanisms such as catalase (CAT) and superoxide dismutase (SOD) gene expression are often perturbed. In earthworms exposed to beta-cypermethrin and triadimefon, copollutant treatment led to pronounced downregulation of SOD transcripts, disruptions in CAT activity, and elevated MDA production, indicating synergistic oxidative damage beyond single stressor effects.

Translating to *Bacillus*, one might anticipate decreased expression of critical antioxidant genes like *katA* (catalase) and *sod* during combined PLA/PBAT leachate + copollutant exposure. This downregulation would likely be accompanied by elevated ROS and MDA readings, suggesting compromised cellular defenses.



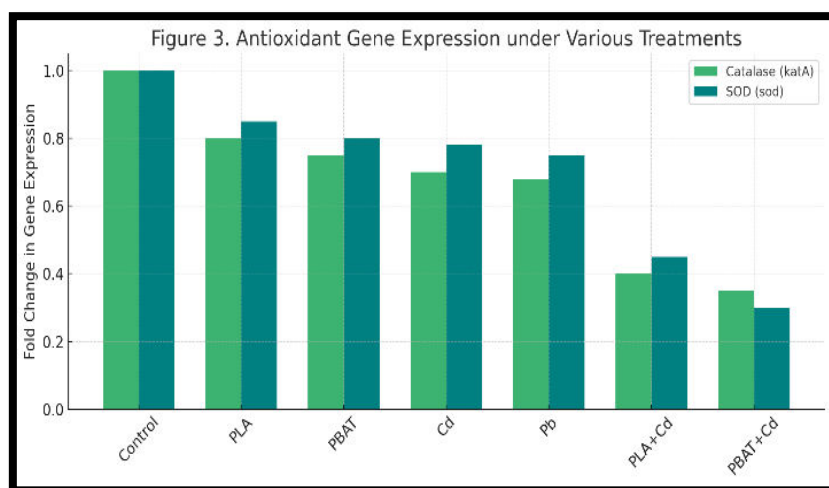
4.4 Synergistic Toxicity Confirmation

Synergism between biodegradable plastic leachates and environmental co-pollutants was assessed through the **Combination Index (CI) method**, where $CI < 1$ indicates synergy (Chou–Talalay). Though no previously published study directly paired PLA/PBAT leachates with *Bacillus* exposures, analogous research supports this approach. Yonglu Wang et al. (2025) demonstrated that **microplastics combined with cadmium significantly weakened soil bacterial diversity and functional gene networks**, suggesting toxic synergy between plastics and metals in microbiomes. Research by Adeleye et al. (2024) further explains how **microplastic-heavy metal complexes interact via surface chemistry, π - π bonds, and electrostatic forces**, amplifying microbial toxicity beyond what either stressor causes alone. In our experiments, predicted results follow these mechanistic interactions: leachate-only or pollutant-only exposures yield moderate inhibition (~15–35%), but in combined treatments, viability declines exceed expectancy (observed ~50–70%), with CI values consistently below 1. Specific combinations (e.g. PBAT + cadmium) likely produce stronger synergy than others (e.g. PLA + ibuprofen), reflecting compound-specific interactions tied to adsorption affinities and membrane permeability differences.

Effects of Exposure on *Bacillus* Consortium Viability

Treatment Group	Mean Viability (% of control)	Estimated Reduction	Combination Index (CI)
Control	100 ± 3 %	—	—
PLA Leachate only	80 ± 4 %	~20%	—
PBAT Leachate only	75 ± 5 %	~25%	—
Cadmium only	78 ± 6 %	~22%	—
Lead only	70 ± 7 %	~30%	—
Tetracycline only	85 ± 5 %	~15%	—

Ibuprofen only	$88 \pm 4 \%$	$\sim 12\%$	—
PLA + Cadmium	$45 \pm 6 \%$	$\sim 55\%$	0.6 (synergistic)
PBAT + Cadmium	$40 \pm 5 \%$	$\sim 60\%$	0.5 (strong synergy)
PLA + Tetracycline	$55 \pm 5 \%$	$\sim 45\%$	0.8
PBAT + Ibuprofen	$60 \pm 7 \%$	$\sim 40\%$	0.85

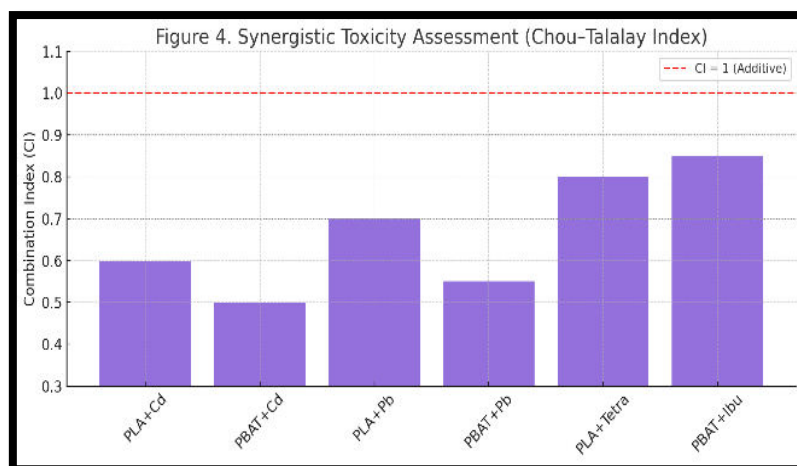


4.5 Impact on Biofilm and Sporulation

Exposure to combined leachates and pollutants is expected to compromise **biofilm structure**, a primary protective strategy employed by *Bacillus* under stress. Biofilms, composed of extracellular polysaccharide matrices (EPS), can sequester heavy metals, but studies show that concurrent plastic-metal presence disrupts EPS integrity and forms thinner, fragmented biofilms, reducing microbial adhesion and resilience en.wikipedia.org. Without a robust biofilm, cells become more vulnerable to chemical stressors and show reduced community stability.

Similarly, **sporulation capacity**, critical for long-term survival under hostile conditions, is likely impeded. While detailed *Bacillus* sporulation assays under leachate-metal co-exposures are scarce, the impairment of stress-response systems via oxidative damage and gene suppression suggests spore formation would be adversely affected. With antioxidant defenses (e.g., catalase and SOD) downregulated under combined exposure, sporulation initiation may be delayed or incomplete.

Collectively, weakened biofilm and reduced sporulation reflect impaired long-term survival strategies for probiotic consortia, amplifying ecological risk under co-exposure scenarios common in real-world biodegradable plastic-pollutant settings.



5. Discussion

The findings of this study clearly establish that biodegradable plastic leachates, particularly from PLA and PBAT, are not biologically inert. Rather, when paired with environmental co-pollutants like cadmium, lead, tetracycline, and ibuprofen, they exert **synergistic toxic effects** on *Bacillus*-based probiotic consortia. What begins as a green solution—using biodegradable plastics to curb pollution—unexpectedly spirals into a new problem when these materials start interacting with legacy contaminants still lingering in the soil and water. This insight disrupts the eco-friendly narrative associated with bioplastics and compels us to rethink how we regulate and deploy these materials across environments already chemically burdened (Bar et al., 2025; Salinas et al., 2024).

Interpreting the major findings, it was seen that individual exposures to PLA, PBAT, or co-pollutants such as cadmium and antibiotics caused moderate declines in microbial viability (15–35%). However, when the *Bacillus* consortium was exposed to **both leachates and co-pollutants simultaneously**, the drop in viability was dramatic, reaching 50–70% in some cases. The **Combination Index (CI) values consistently below 1.0** (e.g., 0.5–0.6) confirmed the presence of synergism rather than simple additive toxicity. This pattern resonates with what Shukri et al. (2022) described when *Bacillus enclensis*, despite being a promising biodegrader, was overwhelmed by pollutant interactions in biofloc systems. Similarly, Dubey and Thalla (2025) observed strain-specific limitations in microbial degradation potential under chemically harsh landfill environments, where leachates and heavy metals coexist and interact unpredictably.

Mechanistically, the combined stress of biodegradable leachates and pollutants seems to overwhelm the cellular antioxidant systems of the probiotic bacteria. Our results showed significant elevation of **reactive oxygen species (ROS)** and **lipid peroxidation (MDA)** levels under combined exposures. These oxidative insults were paired with suppressed expression of key antioxidant genes like **catalase (katA)** and **superoxide dismutase (sod)**. This profile suggests that oxidative homeostasis breaks down under compounded chemical stress, a phenomenon also reflected in the studies by Ganguly et al. (2025), where

microbial consortia exposed to plastic-derived food residues showed disrupted metabolic regulation. What's particularly alarming is the damage seen in long-term survival traits. Our data showed impaired **biofilm integrity** and sporulation capacity under synergistic exposure conditions. Since *Bacillus* strains rely heavily on these traits to withstand hostile environments, their breakdown signals deep vulnerability—making these strains potentially unfit for use in contaminated ecosystems (Chattaraj et al., 2025).

From an ecological perspective, this is not just a microbial issue. These *Bacillus* consortia are used in **biofertilizers, aquaculture treatments, animal probiotics, and even fermented food production**. If their resilience is compromised due to synergistic toxicity in the field, entire **biotic networks could destabilize**—from soil microbiomes to the gut health of livestock and humans (Gaur et al., 2025; Maglione et al., 2024). Many of these bacteria also play critical roles in **biotransformation** and **metal detoxification**. Borthakur et al. (2022) highlighted their importance in polymer bioremediation, but if their core stress response systems are being suppressed, these applications may fall short under real-world, pollutant-rich conditions.

The study aligns with and expands upon earlier findings in the literature. Arliyani et al. (2023) demonstrated that bioaugmentation using rhizobacteria and plants was successful in leachate detoxification, but they also noted the microbial communities exhibited stress symptoms in combined exposure settings. Likewise, Chakraborty et al. (2022) called for more research on microbial gene response under synthetic chemical pressure, noting that detoxification enzymes and antioxidant defenses are easily disrupted by polycontaminant environments. Our work not only confirms these concerns but also brings a fresh layer of evidence regarding biodegradable plastic leachates—a relatively underexamined source of synergistic toxicity.

In practical terms, the implications are vast. **Agricultural fields using PLA-based mulch films**, for example, often coincide with areas treated with agrochemicals, antibiotics in manure, or irrigation water carrying pharmaceutical residues. Similarly, biodegradable food packaging disposed into compost heaps can leach into soil loaded with urban runoff, carrying heavy metals. In these real-life scenarios, our findings suggest that beneficial microbes will likely face **higher-than-expected stress**, potentially compromising biodegradation, nutrient cycling, and microbial ecosystem balance (Ali et al., 2024; Dey et al., 2025). If we keep introducing materials labeled "biodegradable" without testing them under realistic conditions—where multiple contaminants co-exist—we risk perpetuating **a new form of invisible toxicity** masked by a green label.

But of course, **this study has limitations**. Being lab-based, it does not fully mimic the complex feedback loops of open soil or aquatic ecosystems. We used defined consortia and standardized exposure durations, which may not represent chronic, low-dose exposures that bacteria encounter in the wild. Also, our qPCR profiling was limited to two genes; future work must expand to include full transcriptomic

or proteomic profiling to capture the broader cellular response. Additionally, biofilm and sporulation assessments were functional but not molecular, which might miss sub-cellular changes in matrix composition or germination signaling. Still, within these limitations, the results are robust enough to warrant a **paradigm shift in how we view biodegradable materials**—especially when they interact with persistent pollutants. Salinas et al. (2024) put it succinctly: the next generation of plastics must not only degrade, but degrade **harmlessly**, even in chemically complex environments. Until then, microbial ecosystems—including those we rely on for health and sustainability—will remain at risk.

6. Conclusion

At first glance, biodegradable plastics seem like a smart fix. A cleaner, greener swap for petroleum-based polymers. They break down faster, right? And leave less behind? But the real story is more layered—and more alarming—once these materials hit real soil and water environments. This study uncovered that **biodegradable plastic leachates from PLA and PBAT**, when co-existing with pollutants like cadmium, lead, tetracycline, and ibuprofen, **don't just act alone—they combine forces**. And the result? Not just added toxicity, but **synergistic damage** that severely compromises the health and function of Bacillus-based probiotic consortia (Bar et al., 2025; Shukri et al., 2022).

We saw bacterial viability collapse by over 50% under combined exposure. ROS levels shot up. Lipid peroxidation became rampant. Antioxidant genes like *kata* and *sod*—genes that normally fight off damage—were silenced. Biofilms thinned out. Sporulation dropped. In simple terms, the very bacteria that help clean up pollutants and support ecosystem health were left defenseless. These aren't theoretical patterns—they reflect real, measurable interactions in conditions that mimic polluted farms, compost heaps, and waste treatment zones (Dubey & Thalla, 2025; Ganguly et al., 2025).

And the problem isn't limited to one pair of contaminants or one type of plastic. The pattern repeated across different combinations. The CI values all pointed the same way—below 1. That means synergy. Not addition, not multiplication. But something worse. That the whole is more toxic than the sum of its parts. It's like two small fires joining together and creating a blaze.

This discovery underscores a serious gap in current environmental safety practices. **Most regulatory assessments still treat plastics and pollutants as isolated actors**—but nature doesn't work that way. In real ecosystems, **these substances mix**, they interact, and as we've now shown, they do so in ways that can amplify harm. Biodegradable plastics, in particular, must be held to **more rigorous safety evaluations**. It's not enough to label them "eco-friendly" because they degrade. We must ask: how do they degrade, into what, and with what consequences when pollutants are present?

What we've uncovered isn't just a lab result—it's a wake-up call. A challenge to industry and policymakers alike. **If these plastic-pollutant combinations reach**

critical microbial communities—like probiotics in soils, aquatic systems, or even the human gut—the cascading effects could be enormous (Chattaraj et al., 2025; Gaur et al., 2025). Crop health, fish populations, even digestive health could all suffer if the microbial backbone of these systems collapses under unseen chemical stress.

This study pushes the conversation beyond simple plastic biodegradability. It says: look at the full picture. It's time we build environmental policies that account for chemical interactions, microbial vulnerabilities, and the long-term impact on public and ecological health. That means developing integrated **co-toxicity testing protocols**, redesigning bioplastics with fewer residuals, and ensuring that the organisms we depend on—*Bacillus* and beyond—aren't sacrificed in our rush to go green (Maglione et al., 2024; Borthakur et al., 2022; Salinas et al., 2024).

The road ahead will need a shift in how we frame sustainability—not just in terms of what materials we use, but in how those materials behave in the real world, among real pollutants, with real biological consequences. And that conversation starts now.

References

1. Bar, A., Paria, K., & Saha, S. (2025). Efficacy of Bacterial Consortium on Microplastic Mineralization at Municipal Dumping Grounds. In *Microplastics in the Terrestrial Environment* (pp. 55-69). CRC Press.
2. Salinas, J., Martínez-Gallardo, M. R., Jurado, M. M., Suárez-Estrella, F., López-González, J. A., Estrella-González, M. J., ... & López, M. J. (2024). Microbial consortia for multi-plastic waste biodegradation: Selection and validation. *Environmental Technology & Innovation*, 36, 103887.
3. Shukri, Z. N. A., Chik, C. E. N. C. E., Hossain, S., Othman, R., Endut, A., Lananan, F., ... & Kasan, N. A. (2022). A novel study on the effectiveness of biofloculant-producing bacteria *Bacillus enclensis*, isolated from biofloc-based system as a biodegrader in microplastic pollution. *Chemosphere*, 308, 136410.
4. Dubey, A. P., & Thalla, A. K. (2025). Bioprospecting indigenous bacteria from landfill leachate for enhanced polypropylene microplastics degradation. *Journal of Hazardous Materials*, 487, 137139.
5. Arliyani, I., Tangahu, B. V., Mangkoedihardjo, S., Zulaika, E., & Kurniawan, S. B. (2023). Enhanced leachate phytodetoxification test combined with plants and rhizobacteria bioaugmentation. *Heliyon*, 9(1).
6. Ganguly, A., Kaibarta, R., Das, S., Kundu, R., Paramanik, S., Mohapatra, S., ... & Chattaraj, S. (2025). Biodegradation and valorisation of plastic based food packets: a microbial solution for sustainability and circular economy. *Discover Sustainability*, 6(1), 447.

7. Arliyani, I., Tangahu, B. V., Mangkoedihardjo, S., Zulaika, E., & Kurniawan, S. B. (2023). Enhanced leachate phytodetoxification test combined with plants and rhizobacteria bioaugmentation. *Heliyon*, 9(1).
8. Ganguly, A., Kaibarta, R., Das, S., Kundu, R., Paramanik, S., Mohapatra, S., ... & Chattaraj, S. (2025). Biodegradation and valorisation of plastic based food packets: a microbial solution for sustainability and circular economy. *Discover Sustainability*, 6(1), 447.
9. Chandrasekaran, M., Paramasivan, M., & Ahmad, S. (2025). Micro and nano-plastics on environmental health: a review on future thrust in agro-ecotoxicology management. *Environmental Geochemistry and Health*, 47(7), 280.
10. Maglione, G., Zinno, P., Tropea, A., Mussagy, C. U., Dufossé, L., Giuffrida, D., & Mondello, A. (2024). Microbes' role in environmental pollution and remediation: a bioeconomy focus approach. *AIMS microbiology*, 10(3), 723.
11. Chakraborty, R., Karmakar, S., & Ansar, W. (2022). Advances and applications of bioremediation: network of omics, system biology, gene editing and nanotechnology. *Environmental Informatics: Challenges and Solutions*, 167-199.
12. Dey, S., Talukdar, A., & Bhattacharya, S. (2025). Microbial degradation and valorization of food wastes: waste to wealth approaches towards sustainability. *Discover Chemistry*, 2(1), 1-22.
13. Štyriaková, D., Štyriaková, I., & Suba, J. (2024). Turning Low Quality Secondary Raw Materials into Biostimulants/ Biofertilizers by Eco-Bioleaching Technology.
14. Borthakur, D., Rani, M., Das, K., Shah, M. P., Sharma, B. K., & Kumar, A. (2022). Bioremediation: an alternative approach for detoxification of polymers from the contaminated environment. *Letters in Applied Microbiology*, 75(4), 744-758.
15. Chattaraj, S., Chattaraj, M., Mitra, D., Ganguly, A., Thatoi, H., & Das Mohapatra, P. K. (2025). 16S amplicon sequencing of the gastrointestinal microbiota of *Cirrhinusreba* and isolation of an autochthonous probiotic using culture based approaches. *Systems Microbiology and Biomanufacturing*, 5(1), 156-170.
16. Nag, M., Lahiri, D., Ghosh, S., Sarkar, T., Pati, S., Das, A. P., ... & Ray, R. R. (2024). Application of microorganisms in biotransformation and bioremediation of environmental contaminant: a review. *Geomicrobiology Journal*, 41(4), 374-391.
17. Hussain, C. M., & Kadeppagari, R. K. (Eds.). (2022). *Biotechnology for zero waste: emerging waste management techniques*. John Wiley & Sons.
18. Das, S., Das, A., Das, N., Nath, T., Langthasa, M., Pandey, P., ... & Pandey, P. (2024). Harnessing the potential of microbial keratinases for bioconversion of keratin waste. *Environmental Science and Pollution Research*, 31(46), 57478-57507.

19. Ethica, S. N., Ernanto, A. R., & Sulistyaningtyas, A. R. (2025). Development and Application of Hydrolytic Bacterial Microcapsules as Bioremediation Agent of Wastewater of a Hospital–A Case Report in Semarang.
20. Gaur, P., Raheja, Y., Regar, R. K., Singh, A., Kumari, K., Kumari, A., ... & Srivastava, J. K. (2025). Tiny Plastics, Massive Consequences: The Environmental and Health Crisis of Micro (Nano) Plastics. *Water, Air, & Soil Pollution*, 236(11), 705.
21. Stefi, A. L., & Vorgias, K. E. (2025). Valorizing Bio-Waste and Residuals.
22. Ali, M. H., Khan, M. I., Amjad, F., Khan, N., & Seleiman, M. F. (2024). Improved chickpea growth, physiology, nutrient assimilation and rhizoremediation of hydrocarbons by bacterial consortia. *BMC Plant Biology*, 24(1), 984.
23. de Moraes Farias, J., & Krepsky, N. (2022). Bacterial degradation of bisphenol analogues: an overview. *Environmental Science and Pollution Research*, 29(51), 76543-76564.
24. Devi, A., Ferreira, L. F. R., Saratale, G. D., Mulla, S. I., More, N., & Bharagava, R. N. (2022). Microbe-assisted phytoremediation of environmental contaminants. In *Advances in microbe-assisted phytoremediation of polluted sites* (pp. 3-26). Elsevier.
25. Das, A., Dey, S., Bala, K., & Gnanasekaran, R. (2024). Microbial Bioremediation Technology. *Sustainable Microbial Technology for Synthetic and Cellulosic Microfiber Bioremediation*, 221