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The role of *Bacillus cereus* and *Luteimonas* sp. as plastic degraders on phosphorus uptake by pak choi plants

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ABSTRACT

The accumulation of microplastics in agricultural land interferes with phosphorus nutrient uptake and inhibits plant growth, thereby reducing crop production. Microplastic-degrading bacteria can be used as biological agents to reduce microplastic accumulation and increase nutrient uptake. This study aimed to analyze the types of plastic-degrading bacterial isolates, the types of plastic, and their interactions with plastic degradation and phosphorus nutrient uptake in Pak choi plants grown in soil to identify the optimal treatment combination. The microplastic materials used consisted of silver plastic mulch made of high-density polyethylene (HDPE), polybags made of low-density polyethylene (LDPE), fertilizer bags made of polyethylene terephthalate (PET), and straws made of polypropylene (PP). The study was conducted using a factorial experimental design with a Complete Randomized Design (CRD) consisting of two factors, namely the type of bacterial isolate (*Bacillus cereus* ATCC 14579, *Luteimonas* sp. ZGLJ41, and control without bacteria), and plastic type (silver plastic mulch-HDPE, polybag-LDPE, fertilizer bag-PET, straw-PP) with 3 replicates, resulting in 36 experimental units. The results showed that the combination of *Bacillus cereus* ATCC 14579 and polybag-LDPE resulted in the highest plastic degradation (3.08%) and an increase in plant phosphorus uptake (66.6%) compared to the control. The *Bacillus* cereus ATCC 14579 isolate showed the highest microplastic reduction, with an average degradation of 2.43% over 45 days, supported by a high bacterial population density. The type of plastic that degraded most easily was LDPE polybags, with a degradation percentage of 1.78%.

Keywords: Andisol, biodegradable plastic, *Brassica rapa* L., degrading bacteria, microplastics.

INTRODUCTION

The accumulation of microplastics in agricultural soil interferes with nutrient uptake and reduces crop productivity. Microplastics measuring <5 mm are often found accumulated in agrarian soil, adhering to root surfaces, entering plant vascular tissue, and inhibiting water and nutrient uptake (Iqbal et al., 2023). Microplastics that attach to root hairs can disrupt root growth patterns and reduce nutrient uptake efficiency (Ge et al., 2021).

The use of various plastic materials, such as mulch, polybags, fertilizer sacks, and straws, on agricultural land is a significant source of microplastic pollution. Plastics such as HDPE, LDPE, PP, PVC, and PMMA are commonly found in the environment due to their widespread use (Fadhilah et al., 2023). Building on this, Ingraffia et al. (2022) found that soil response to microplastics depends on the physical and chemical characteristics of the soil.

The interaction between microplastics and soil microorganisms plays a vital role in maintaining ecosystem balance. The soil microbiome functions as a reservoir of microorganisms capable of degrading various types of microplastics (Wang et al., 2025), thereby supporting the development of biological approaches to reducing microplastic pollution in agricultural land.

In cultivation, Pak choi is a vegetable crop with rapid growth and high nutrient requirements (Han et al., 2024), making it highly sensitive to changes

in nutrient availability, including phosphorus. Efforts to increase P uptake in microplastic-contaminated soil can be achieved by using plastic-degrading microorganisms. Bacteria such as *Bacillus cereus* and *Luteimonas* sp. have been identified as capable of degrading plastic polymers (Rosariastuti et al., 2025), potentially reducing barriers to nutrient uptake caused by microplastics.

However, no specific research has evaluated the interaction between *Bacillus cereus* and *Luteimonas* sp. with various types of plastic in soil, nor its implications for phosphorus uptake in Pak choi plants. Therefore, this study was conducted to analyze the roles of these two bacterial isolates in degrading various types of plastic and their effects on phosphorus uptake in pak choi plants.

MATERIALS AND METHODS

Research location

The research was conducted from April to December 2024 in Sukosari Village, Jumantono Subdistrict, Karanganyar Regency, Central Java Province, Indonesia (7°37'48.3 "S; 110°56'51.2"E). Soil and plant analysis were performed at the Soil Chemistry and Soil Fertility Laboratory, while microorganism analysis was conducted at the Soil Biology and Biotechnology Laboratory, Faculty of Agriculture, Sebelas Maret University.

Experimental design and data analysis

The study used a factorial experimental design with a completely randomized design (CRD) as the base design consisting of two factors: bacterial isolate (I0 = without bacterial isolate, I1 = *Bacillus cereus* ATCC 14579, I2 = *Luteimonas* sp. ZGLJ41) and plastic type (P1 = silver plastic mulch-HDPE, P2 = polybag-LDPE, P3 = fertilizer packaging bag-PET, P4 = Straw-PP).

Soil and growth media preparation

The soil used as the plant growth medium was Andisol obtained from Kemuning Village, Ngargoyoso Subdistrict, Karanganyar Regency, Central Java Province, Indonesia, then sieved to 5 mm. The growing medium consisted of soil, burnt husks, sand, and manure (2:1:1:1) at a rate of 3 kg/pot. The medium was sterilized by steam for 2 hours/day for 3 consecutive days (Sutami et al., 2021). The sterilized medium was then placed in pots with a 30 cm diameter. The purpose of this study was to determine the effect of microplastic-degrading bacteria *Bacillus cereus* ATCC 14579 and *Luteimonas* sp. ZGLJ41 on soil containing microplastics on phosphorus (P) uptake by pak choi plants. In this case, no specific observations were made on P dynamics. Therefore, the growing medium was sterilized beforehand to kill or reduce the native bacteria present.

Preparation and application of microplastics

Four types of plastic were used: HDPE (silver mulch), LDPE (polybag), PET (fertilizer bag), and PP (straw). Each was cut into <5 mm pieces and weighed at 6 g per pot (Han et al., 2024). Microplastics are sterilized with 70% alcohol, dried, and then evenly mixed into the planting medium before transplanting Pak choi seedlings (7–14 days old).

Preparation and application of bacterial inoculum

Two isolates were used: *Bacillus cereus* ATCC 14579 and *Luteimonas* sp. ZGLJ41. The first step was to inoculate bacterial isolates from stock cultures on slant media into nutrient agar (NA) using the streak method. The next step was to prepare the bacterial inoculum through gradual liquid culture. Each bacterial isolate from a single slant medium was inoculated into 25 mL of nutrient broth (NB) and shaken at 70 rpm for 48 hours. The culture was then gradually transferred to 250 mL of NB medium and shaken again for 48 hours. Next, the culture was placed in 1 L of NB medium in two Erlenmeyer flasks (total volume 2 L) and shaken at 80 rpm for 7 days to increase the bacterial density to 10^{12}, in accordance with the principle of *stepwise inoculum* scale-up procedure (Barrena and Gea, 2025). Bacterial density was calculated by counting cells under a microscope using a hemocytometer.

Seedling preparation

Mix soil and fertilizer in a 1:1 ratio to prepare the planting medium. Grow seedlings in wooden boxes and make furrows for sowing. Maintain seedlings for 7–14 days, then transfer them to experimental pots.

Preparation of liquid organic fertilizer

Liquid organic fertilizer (LOF) was produced by composting a mixture of organic waste from households, campuses, and markets, which was ground and composted in a 30 kg closed composter bag. Each composter bag was placed on a plastic bucket that serves to collect leachate. This leachate was used as LOF. The application method involves diluting the LOF with well water at a 1:10 ratio. Diluted LOF is applied to plants at 100 mL per pot.

Planting

Pak choi seedlings aged 7–14 days with 3–4 true leaves are transferred from the nursery to pots containing planting media after the nursery media has been moistened, to facilitate removal of the seedlings from the nursery.

Plastic-degrading bacteria application

Plastic-degrading bacteria were applied in pots after planting pak choi seedlings. The bacterial application dose was 10^9 cells g^{-1} of soil. The application was carried out using a bacterial inoculum at a density of 10^{12} cells mL^{-1}, so that 3 mL of the bacterial inoculum solution was dissolved in 97 mL of distilled water and applied to each pot containing 3 kg of soil. The pouring process was carried out evenly, resulting in a final inoculum density of 10^9 cells g^{-1} of soil.

Plant maintenance

Plant maintenance involved watering regularly in the morning and evening to maintain soil moisture. At the beginning of planting, basal fertilization was applied using autoclaved compost at a rate of 22.2 g pot^{-1}, equivalent to approximately 20 t ha^{-1} based on a soil bulk density of 0.83 g cm^{-3} and a soil depth of 32.5 cm. Diluted LOF at a dose of 100 mL per pot^{-1} was applied every 7 days. Inorganic fertilization was carried out using compound NPK fertilizer (16:16:16) at a rate of 0.5 g per pot^{-1}, applied on the 7th and 21st days after transplanting (DAT). Pest control uses botanical pesticides (plus biopesticides) if pest attacks are found. Pak choi plants were harvested at 45 DAT.

Sampling and laboratory analysis

Microplastic separation

The plants were harvested, then their roots were washed slowly with running water over a container to remove any soil particles and microplastics that were attached. The wash water was filtered through a 60-mesh sieve, and the retained solids were returned to the treatment pots for drying again. The plants are then weighed to obtain their fresh weight, and then oven-dried to obtain their dry weight. After drying, plant samples are analyzed to determine the P uptake contained in the plants. Meanwhile, 50 g of soil samples are taken from each pot around the roots for soil biological and chemical analysis. The soil samples were sieved to remove microplastics, and the remaining soil in the pots was separated from the microplastics using a multi-stage sieve (5 mm, 3 mm, 2 mm, and 1 mm) according to the method described by Möller et al. (2020). Particles passing through the 1 mm sieve were not analyzed because they may have contained nanoparticles and had a low recovery rate. To remove contaminants attached to microplastics, gradual washing is carried out. Fragments that are still mixed with soil and residues attached to their surfaces are soaked in pure water for 12 hours; the microplastics then float to the surface, where they are separated and cleaned of any remaining soil particles. Next, they were soaked in an alcohol-water mixture (75% alcohol, 25% water). The microplastics that floated were then filtered and collected with tweezers, then dried for analysis based on their final weight.

Soil chemical analysis

Methods for analyzing soil chemical properties include pH H_2O (electrometric), KTK (ammonium saturation), organic C (Walkley–Black), and available P (Olsen) (Soil and Fertilizer Instrument Standard Testing Center, 2023).

Microbiological and plant parameter analysis

Analysis methods include: Percentage of microplastic degradation (gravimetry) and bacterial population density (total plate count). Plant growth analysis methods include fresh and dry weight (direct measurement) and phosphorus uptake (wet digestion).

Data analysis

The data were analyzed using a two-way ANOVA, which had been tested for normality and homogeneity of variance to ensure ANOVA assumptions were met. Significant differences were tested using Duncan's test, and Pearson's correlation was used to assess the relationship between parameters.

RESULT AND DISCUSSION

Initial soil characteristics

The soil characteristics analysis in Table 1 showed that the Andisol used has a sandy clay loam texture, consisting of 53.22% sand, 18.90% silt, and 27.88% clay. Additionally, the H_2O pH value of 6.88 (neutral) and NaF pH of 11.10 support the interpretation that these soils have a high content of amorphous minerals, as indicated by the Andisol identification method (Widyawati et al., 2024). The CEC value of 24.51 cmol(+) kg^{-1} was in the high category. The organic C content (3.10%) and available P (2.54 ppm) indicate high phosphate retention, consistent with Andisol, which is rich in allophane (Napitupulu et al., 2025). After sterilization and the addition of organic matter, several parameters changed. The pH decreased to 6.67. Sterilization released H^+ ions from the decomposition of organic matter (Hyun et al., 2023). Organic C increased to 3.42%, as a result of the formation of stable organo-metal complexes (Lyu et al., 2025). Available P content increased to 7.45 ppm, influenced by the complexation of Al and Fe by organic acids and changes in the structure of organic matter during sterilization (Manea et al., 2024; Jin and Dai, 2024). The total bacterial population decreased from 6.40 to 2.73 log10 CFU/g after sterilization, due to a reduction in microbial biomass caused by heating (Ding et al., 2023).

Effect of treatments on final soil characteristics

Based on the ANOVA conducted, the results can be seen as follows.

pH H_2O

The soil pH values in Table 3 showed that the various treatments ranged from 6.33 to 6.64 (slightly acidic–neutral) and were all lower than the initial pH after sterilization (6.67). The control treatment had a higher pH (6.54–6.66) than all treatments with bacterial isolates. The decrease in pH was related to the activity of microorganisms that produced acidic metabolites (Wang et al., 2025; Feng and Deng, 2025).

The ANOVA results (Table 2) show that the type of bacteria, the type of plastic, and their interaction have a highly significant effect on soil pH ($p<0.01$). The highest pH was observed in the treatment without isolate + silver plastic mulch-HDPE (I0P1), while the lowest pH was observed in *Bacillus cereus* ATCC 14579 + fertilizer

Table 1. Initial soil characteristics

No	Parameter	Value		Unit	Classification
		Initial soil	Sterilized growth medium		
1.	Texture			%	Sandy clay loam
	Sand	53.22			
	Silt	18.90			
	Clay	27.88			
2.	pH H_2O	6.88	6.67	-	Neutral
3.	pH NaF	11.10	-	-	-
4.	Cation exchange capacity (CEC)	24.51	24.43	cmol(+) kg^{-1}	High
5.	Soil organic C	3.10	3.42	%	High
7.	Soil available P	2.54	7.45	ppm	Very low; Low
9.	Total soil bacterial population	6.40	2.73	Log10 CFU g^{-1}	-

Note: Classification based on the Soil and Fertilizer Instrument Standard Testing Center (Ministry of Agriculture, Republic of Indonesia, 2023).

Table 2. Two-way ANOVA results for soil chemical properties

Variable	Source	df	*F*-value	*p*-value
pH	I	2	102.889	0.000**
	P	3	18.054	0.000**
	I*P	6	5.901	0.001**
CEC	I	2	353.316	0.000**
	P	3	28.108	0.000**
	I*P	6	36.444	0.000**
Soil organic C	I	2	796.415	0.000**
	P	3	23.859	0.000**
	I*P	6	87.501	0.000**
Soil available P	I	2	54.184	0.000**
	P	3	3.890	0.021*
	I*P	6	12.643	0.000**

Note: ** highly significant ($p<0.01$), * significant ($0.01 \leq p<0.05$), ns – not significant ($p>0.05$), I – type of bacteria, P – type of plastic, I*P – interaction.

bag-PET (I1P3). The type of HDPE plastic without isolation showed a relatively small change in pH compared to the initial soil pH.

Based on DMRT, the treatment without bacterial isolate + silver plastic mulch-HDPE (I0P1) differed significantly from all other treatments. This difference was associated with changes in the polymer's chemical and physical properties, which influenced soil H^+ concentration by altering soil acidity or related mechanisms (Zhao et al., 2021). The lowest pH value in *Bacillus cereus* ATCC 14579 + fertilizer bag-PET (I1P3) showed plastic-degrading activity, producing acidic compounds during metabolism – the accumulation of organic acids correlated with increased microbial metabolic activity (Liu et al., 2025).

Soil cation exchange capacity (CEC)

Table 3 showed that all treatment CEC values increased after sterilization (24.43 cmol(+) kg^{-1}). The control CEC values (24.44–32.30 cmol(+) kg^{-1}) were generally lower than those from treatments combining bacterial isolates and microplastics. The increase in CEC is related to the production of

Table 3. Effects of treatments on observed soil parameters

Treatment code	Soil pH	CEC (cmol (+) kg^{-1})	Soil organic C (%)	Soil available P (ppm)
I0P1	6.64f	26.08ab	3.35a	2.75a
I0P2	6.58d	24.44a	3.48b	4.12bc
I0P3	6.56d	27.70b	3.69c	4.63bcd
I0P4	6.54d	32.30c	3.48b	2.62a
I1P1	6.40b	41.79f	3.95d	5.60d
I1P2	6.42bc	39.27e	4.63g	7.06e
I1P3	6.33a	31.57c	4.79h	5.02cd
I1P4	6.47c	39.03e	4.52f	7.43e
I2P1	6.52d	41.11f	4.17e	7.13e
I2P2	6.43bc	35.39d	4.05d	5.76d
I2P3	6.35a	38.00e	3.50b	5.73d
I2P4	6.44bc	35.24d	3.77c	3.65ab

Note: I0: without bacterial isolate, I1: *Bacillus cereus* ATCC 14579, I2: *Luteimonas* sp. ZGLJ41, P1: Silver plastic mulch (HDPE), P2: Polybag (LDPE), P3: Fertilizer packaging bag (PET), P4: Drinking straw (PP).
Values followed by the same letter are not significantly different, while different letters indicate significant differences based on DMRT at a significance level of 5% ($p \leq 0.05$).

Extracellular Polymeric Substances (EPS). These substances contain negatively charged groups, such as carboxyl and hydroxyl. These groups act as cation exchange sites by attracting and binding positively charged ions (Costa et al., 2018). Microplastics also enhance biofilm formation by providing a substrate for colonization and EPS accumulation (Ma et al., 2023).

The ANOVA results (Table 2) showed that the type of bacteria, the type of plastic, and their interaction had a highly significant effect on the CEC value (p<0.01). The best treatment was *Bacillus cereus* ATCC 14579 + silver plastic mulch-HDPE (I1P1) with a value of 41.79 cmol(+) kg^{-1}, which is very high. HDPE is known to affect soil physical properties and to provide a surface for biofilm formation, thereby stimulating EPS production (De Souza Machado et al., 2019; MacLean et al., 2024). EPS carries negatively charged groups, increasing the number of cation-exchange sites on soil colloids (Nkoh et al., 2022).

The DMRT results showed that *Bacillus cereus* ATCC 14579 + silver plastic mulch-(HDPE; I1P1) was not significantly different from *Luteimonas* sp. ZGLJ41 + silver plastic mulch-HDPE (I2P1). Oxidized plastic surfaces can form C=O and –OH groups, increasing reactivity and polarity and strengthening interactions with EPS (Poursat et al., 2024). In addition, *Luteimonas* possesses EPS-forming genes that contribute to biofilm formation (Zhang et al., 2015). Negatively charged groups in EPS, such as –COOH, $–OPO_3H$, and $–OSO_3H$, participate in cation exchange (Kistriyani et al., 2020).

Soil organic carbon

Based on Table 3, the treatment of *Bacillus cereus* ATCC 14579 + fertilizer packaging bags–PET (I1P3) produced the highest organic C value of 4.79%, an increase of 40.06% compared to the soil after sterilization (3.42%) and 29.81% compared to the I0P3 control (3.69%). This increase is due to enzymatic depolymerization, which breaks down PET polymers into oligomers and monomers that are more readily utilized as a carbon source by microorganisms (Patel et al., 2024; Zhou et al., 2024). The relatively limited extent of PET mineralization indicates that the increase in organic C is not solely attributable to polymer breakdown. Rather, the presence of PET stimulates bacterial activity, enabling the utilization of dissolved carbon, thereby supporting increased bacterial metabolism (Ni et al., 2021), which in turn promotes bacterial biomass turnover and soil organic C accumulation.

The ANOVA results (Table 2) showed that bacterial type, plastic type, and their interaction have a significant effect (p<0.01) on organic carbon. Microplastics increase microbial metabolism and trigger accelerated soil carbon decomposition (Yu et al., 2023). However, microplastics also trigger a priming effect by increasing dissolved carbon, thereby accelerating decomposition (Aralappanavar et al., 2024).

The DMRT results (Table 3) showed that the treatment with *Bacillus cereus* ATCC 14579 + *fertilizer* bags-PET (I1P3) was significantly different from all other treatments, including the control. This is because PET degradation can produce low-molecular-weight organic compounds (alcohols, esters, carboxylic acids) (Kopecká et al., 2022). PET degradation releases ethylene glycol (EG) and terephthalic acid (TPA) monomers, which are readily metabolized by microorganisms (Mohanan et al., 2020; Liu et al., 2023). The assimilation of these compounds stimulates microbial growth and soil residue formation. Microbial-derived residues, particularly necromass, represent a dominant and stable component of soil organic carbon fractions (Barrena and Gea, 2025).

Available phosphorus

Based on Table 3, all treatments experienced a decrease in available P compared to the initial soil (7.45 ppm). The highest treatment was *Bacillus cereus* ATCC 14579 + straw-PP (I1P4) with a value of 7.43 ppm, an increase of 183.5% compared to the control without isolate (2.62 ppm). Microplastics affect phosphorus availability through physicochemical interactions and changes in soil microorganism activity (Wang et al., 2024).

The ANOVA results (Table 2) showed that the type of bacteria and their interaction have a highly significant effect on available P (p<0.01), while the type of plastic has a considerable impact on available P (p<0.05). Microplastics such as PE and PP can alter pH and surface properties during degradation, which has implications for phosphorus dynamics in soil. Microplastics act as vectors in the adsorption and desorption of various compounds (Li et al., 2022; Zhang et al., 2020).

Based on DMRT (Table 3), the best treatment was *Bacillus cereus* ATCC 14579 + straws-PP (I1P4), which was not significantly different from *Luteimonas* sp. ZGLJ41 + silver plastic mulch-HDPE (I2P1) and *Bacillus cereus ATCC 14579* +

polybag- LDPE (I1P2). *Bacillus* increases phosphorus availability by producing organic acids, phosphatase, and EPS, thereby enhancing phosphate release and mobilization (Liu et al., 2023; Nasrin et al., 2022). Availability was positively correlated with organic C (r=0.653**) and total bacterial population (r=0.786**), indicating that increased organic matter and microbial activity accelerate phosphorus mineralization and enhance its availability (Niederberger et al., 2019; Lemanowicz, 2018).

Effect of treatments on bacterial activity and microplastic degradation percentage

Based on the ANOVA conducted, the results can be seen as follows.

Total bacterial population

The total bacterial population in Table 5 increased after sterilization, to 2.76 Log10 CFU/g. In the treatment without isolates (I0P1–I0P4), the bacterial population ranged from 5.67 to 5.95 Log10 CFU/g. The highest total bacterial population (I1P4) increased by 166.43% compared to the control. Autoclaving proved effective in reducing the number of microorganisms, but it did not eliminate them. This is due to the presence of microorganisms capable of surviving in extreme conditions (King et al., 2024). Some organisms can survive by forming spores and become active again when environmental conditions are favorable (Perek et al., 2019). In the soil, microplastics provide a colonization surface (plastisphere) that supports biofilm formation, allowing plastic-degrading bacteria to utilize plastic fragments as a carbon source (Dhiman et al., 2024; Hooda and Mondal, 2023).

The ANOVA results (Table 4) showed that bacterial type, plastic type, and their interaction have a highly significant effect on the total bacterial population (p<0.01). Differences in the chemical characteristics and hydrophobicity of plastics regulate the level of colonization and the availability of carbon for bacteria (Hansen et al., 2021; Salini et al., 2024). Based on the DMRT results, the highest value was obtained in *Bacillus* cereus ATCC 14579+straw–PP (I1P4), which was not significantly different from *Bacillus cereus* ATCC 14579+polybag–LDPE (I1P2) and *Luteimonas* sp. ZGLJ41+silver plastic mulch–HDPE (I2P1). This increase reflects the ability of bacteria to form biofilms intensively on PP, LDPE, and HDPE surfaces, thereby strengthening microbial colonization (Afify et al., 2025).

The correlation test revealed a strong positive relationship between the total bacterial population and C-organic (r=0.751**) and microplastic degradation (r=0.900**). The availability of organic carbon increases bacterial metabolic activity (Liddle et al., 2020). There was also a strong negative correlation with pH (r = –0.737**), indicating that microbial activity produces organic acids that lower soil pH (Macias-Benitez et al., 2020). Genera such as *Bacillus*, *Streptomyces*, *Bradyrhizobium*, and *Lybsobacter* play roles in decomposing organic matter, nitrogen fixation, and in producing enzymes and bioactive compounds involved in the degradation of complex soil compounds (Rosariastuti et al., 2023).

Microplastic degradation percentage

Table 5 showed that microplastic degradation increased in treatments with bacterial isolates compared to those without isolates (I0). The I0P1–I0P4 treatments showed very low degradation values of 0.26–0.44%, with the highest value in the treatment without isolates + polybag (I0P2) (0.44%) and the lowest in the treatment without isolates + fertilizer bag (I0P3) (0.26%). Degradation occurred even in the absence of isolates

Table 4. Two-way ANOVA results for bacterial activity and degradation percentage

Variable	Source	df	*F*-value	*p*-value
Total bacterial population	I	2	150037.853	0.000**
	P	3	623.323	0.000**
	I*P	6	1841.533	0.000**
Degradation percentage	I	2	2287.825	0.000**
	P	3	92.238	0.000**
	I*P	6	25.311	0.000**

Note: ** highly significant (p<0.01), * significant (0.01≤p<0.05), ns – not significant (p>0.05), I – type of bacteria, P – type of plastic, I*P – interaction.

because the soil contained non-specific microorganisms and was influenced by abiotic factors such as temperature, humidity, and soil chemistry (Blesa et al*.,* 2024). The assessment of microplastic degradation in this study was based solely on gravimetric weight loss, thereby limiting the ability to confirm actual polymer degradation. The observed mass loss could be influenced by other factors such as the release of small fragments, the loss of additives, and potential bias due to the accumulation of biofilm, EPS, or soil particles adhering to the plastic surface. Furthermore, no confirmatory chemical or structural analyses, such as FTIR, SEM, or carbonyl index measurements, were performed to verify changes in polymer composition and structure. Therefore, the results of this study are best interpreted as observed mass loss, indicating potential degradation, but not definitive proof of chemical polymer breakdown.

The ANOVA results (Table 4) showed that bacterial type, plastic type, and their interaction have a significant effect on microplastic degradation ($p<0.01$), consistent with differences in enzymatic ability among bacterial species to break polymer bonds (Tania and Anand, 2025). The chemical characteristics of plastic also affect degradation rates; PET degrades more slowly than PE and PP due to the higher stability of its ester bonds (N. Zhang et al., 2022).

Based on DMRT (Table 5), the highest value was observed in *Bacillus cereus* ATCC 14579+polybag-LDPE (I1P2) at 3.08%, which was significantly higher than all other treatments. *Bacillus cereus* ATCC 14579 demonstrated high degradation activity. The *Bacillus cereus* ATCC 14579 isolate was obtained from the Putri Cempo Landfill and demonstrated a microplastic degradation capacity of 8.83% after 20 days of incubation (Rosariastuti et al., 2025). The degradation mechanism involves oxidative and hydrolytic enzymes, such as laccase, lipase, and esterase, as well as biosurfactants, which increase adhesion and biofilm formation (Zeenat et al., 2021). Polyethylene (PE) in the early stages of degradation can begin with abiotic oxidation that forms carbonyl and hydroxyl groups, followed by bacterial colonization and the activity of manganese peroxidase (MnP) and laccase enzymes that break C–C bonds, then small fragments are assimilated through β-oxidation (Ghatge et al., 2020).

Figure 1 showed that the treatment without isolates (I0) resulted in the lowest degradation of 0.35%, while *Bacillus cereus* ATCC 14579 (I1) exhibited the highest degradation of 2.43%, followed by *Luteimonas* sp. ZGLJ41 (I2) at 1.54%. These results confirm that the presence of bacterial isolates enhances microplastic degradation compared to the absence of bacterial treatment. The mechanism of accelerated LDPE degradation through biofilm formation and polymer surface damage has also been demonstrated in other *Bacillus cereus* strains (Jebashalomi et al*.,* 2024). However, bacterial biofilms that adhere to microplastic surfaces form an exopolysaccharide (EPS)

Table 5. Effect of treatments on observed parameters

Treatment code	Total bacterial population (Log10 CFU g^{-1})	Degradation percentage (%)
I0P1	5.67a	0.40bc
I0P2	5.95c	0.44c
I0P3	5.71a	0.26a
I0P4	5.84b	0.31ab
I1P1	13.90e	2.28h
I1P2	15.55g	3.08i
I1P3	14.35f	2.27h
I1P4	15.56g	2.10g
I2P1	15.54g	1.71f
I2P2	14.41f	1.80f
I2P3	13.91e	1.22d
I2P4	12.04d	1.42e

Note: I0: without bacterial isolate, I1: *Bacillus cereus* ATCC 14579, I2: *Luteimonas* sp. ZGLJ41, P1: Silver plastic mulch (HDPE), P2: Polybag (LDPE), P3: Fertilizer packaging bag (PET), P4: Drinking straw (PP).
Values followed by the same letter are not significantly different, while different letters indicate significant differences based on DMRT at a significance level of 5% ($p \leq 0.05$).

matrix (Gaylarde et al., 2023), which causes irreversible adhesion, making biofilms difficult to remove from polymer surfaces (Chen et al., 2025). Thus, the accumulation of biomass on the particle surface can increase the effective mass of microplastics and affect the weighing results (Semcesen and Wells, 2021).

Based on Figure 2, the ANOVA results indicate that microplastic type has no significant effect on microplastic degradation, and the DMRT reveals no significant differences among microplastic types or across all treatments. However, based on the trend, the polythene bag (LDPE)-P2 type showed the highest degradation rate of 1.78%, followed by silver mulch (HDPE) at 1.46%, fertilizer bags (PET) at 1.25%, and straws (PP) at 1.28%. This tendency aligns with the chemical structure of LDPE, which consists of a saturated aliphatic backbone that facilitates alkyl radical formation and promotes autoxidation reactions (Ahn and Colin, 2021).

Correlation tests showed that microplastic degradation has a strong positive relationship with total bacterial population (r=0.900**) and organic C (r=0.853**). An increase in the bacterial population accelerates degradation by producing more hydrolytic and oxidative enzymes that break down polymer chains into oligomers and monomers, which are then assimilated and mineralized (Nakei et al., 2022; Heris, 2024). Higher degradation rates also contribute to increased organic carbon, as carbon fragments resulting from polymer breakdown serve as a source of energy for microorganisms (Wang et al., 2024).

Effect of treatments on plant growth and P uptake

Based on the ANOVA conducted, the results can be seen as follows

Fresh and dry weight

The ANOVA results (Table 6) indicated that the type of bacteria, the type of plastic, and their interaction had a highly significant effect on plant fresh and dry weight ($p < 0.01$). Based on Table 7, the best treatment for fresh weight was *Luteimonas* sp. ZGLJ41 + polybag-LDPE (I2P2) at 43 g, an increase compared to the control I0P2 (27.33 g). The highest dry weight value was obtained in *Bacillus cereus* ATCC 14579 + polybag-LDPE (I1P2), at 3.61 g, while the control (I0P2) was only 2.62 g. Polyethylene microplastics reduce fresh and dry weight by blocking root growth, disrupting water and nutrient uptake, causing mechanical damage to root tissue, and inhibiting photosynthesis (Zhang et al., 2025). The role of bacteria in mitigating stress caused by microplastics has also been demonstrated in several strains of *Bacillus* and *Enterobacter*, which are capable of enhancing plant height and dry weight by counteracting the toxic effects of microplastics (Zhao et al., 2025).

The DMRT results showed that the highest fresh weight was found in I2P2 (43 g) and was not significantly different from I2P4 (42.67 g) and I2P3 (40 g). The highest dry weight was observed in I1P2 (3.61 g) and was not significantly different from that in I2P3. The increase

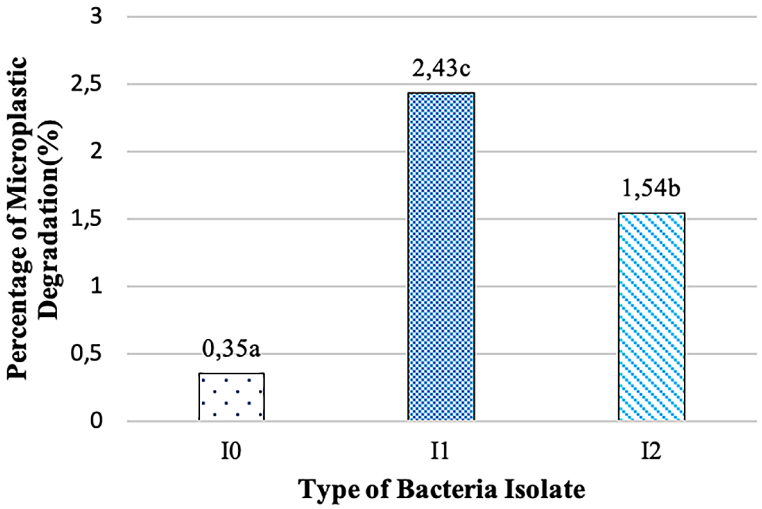

Figure 1. Effect of bacterial isolate type on the percentage of microplastic degradation
Note: I0: without bacterial isolate, I1: *Bacillus cereus* ATCC 14579, I2: *Luteimonas* sp. ZGLJ41.

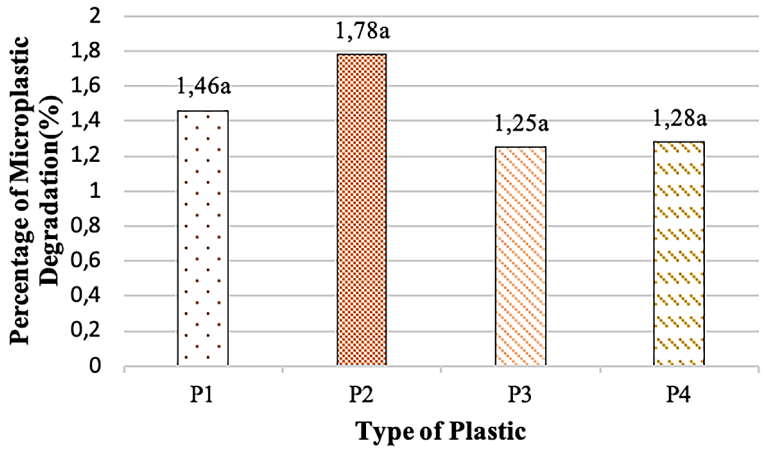

Figure 2. Effect of plastic type on microplastic degradation percentage
Note: P1: Silver plastic mulch (HDPE), P2: Polybag (LDPE), P3: Fertilizer packaging bag (PET), P4: Drinking straw (PP).

Table 6. Two-way ANOVA results for plant growth and P uptake`

Variabel	Source	df	*F*-value	*p*-value
Fresh Weight	I	2	50.716	0.000**
	P	3	9.606	0.000**
	I*P	6	13.916	0.000**
Dry Weight	I	2	93.254	0.000**
	P	3	29.053	0.000**
	I*P	6	61.479	0.000**
P Uptake	I	2	200.961	0.000**
	P	3	53.886	0.000**
	I*P	6	35.259	0.000**

Note: ** highly significant ($p<0.01$), * significant ($0.01 \leq p<0.05$), ns – not significant ($p>0.05$), I – type of bacteria, P – type of plastic, I*P – interaction.

Table 7. Effect of treatments on observed parameters

Treatment code	Fresh weight (g)	Dry weight (g)	P uptake (g plant^{-1})
I0P1	32b	2.92bc	0.0097a
I0P2	27.33a	2.62a	0.0102a
I0P3	31ab	2.94bc	0.0124b
I0P4	31.67b	3.13d	0.0122b
I1P1	32b	2.92bc	0.0137d
I1P2	38.67c	3.60f	0.0170f
I1P3	32b	2.99c	0.0137d
I1P4	31.33b	2.86b	0.0133cd
I2P1	30ab	2.99c	0.0125bc
I2P2	43d	3.41e	0.0157e
I2P3	40cd	3.53f	0.0161e
I2P4	42.67d	3.15d	0.0126bc

Note: I0: without bacterial isolate, I1: *Bacillus cereus ATCC 14579*, I2: *Luteimonas* sp. *ZGLJ41*, P1: Silver plastic mulch (HDPE), P2: Polybag (LDPE), P3: Fertilizer packaging bag (PET), P4: Drinking straw (PP).
Values followed by the same letter are not significantly different, while different letters indicate significant differences based on DMRT at a significance level of 5% ($p \leq 0.05$).

in biomass in the treatment with added bacteria was consistently high because the presence of *Bacillus* strengthened plant resistance to microplastic stress by increasing antioxidant levels and enzyme activity, thereby supporting biomass accumulation (Liu et al., 2023). Plants with optimal height have higher photosynthesis and biomass accumulation (Wu et al., 2025).

P Uptake

The ANOVA results presented in Table 6 indicate that the type of bacteria, type of plastic, and their interaction have a highly significant effect on P uptake ($p < 0.01$). The best treatment (Table 7) was *Bacillus cereus* ATCC 14579+ polybag-LDPE (I1P2), with a value of 0.017 g/plant, representing a 70% increase compared to the control with the same type of microplastic (I0P2) (0.010 g/plant). Microplastics reduce P uptake by mechanically inhibiting root growth and by binding phosphate ions to their surfaces, thereby reducing P availability in the soil solution (Zhou et al., 2024). Microplastics also affect alkaline phosphatase (APA) activity, which plays a role in P mineralization; this activity decreases at high concentrations and exposure, especially in PE, PP, and PET polymers (Dindar, 2025).

The DMRT results indicate that *Bacillus cereus* ATCC 14579+polybag-LDPE (I1P2) was the most effective treatment and was significantly different from all other treatments. Microplastics trigger oxidative stress and membrane damage, which reduces the absorption and translocation efficiency of phosphorus in plants (Wang et al., 2024). Conversely, *Bacillus cereus* (UFT42*)* was capable of dissolving phosphate and increasing phosphatase enzyme activity, thereby supporting increased P absorption (Soares et al., 2023).

The correlation test results showed that P uptake was strongly correlated with available P (r=0.585**), total bacterial population (r=0.722**), and plastic degradation (r=0.700**). Adding fertilizer to the soil increases the number of microorganisms, thereby enhancing the solubilization of bound phosphorus (P) into available P, which can increase P uptake by plants (Guo et al., 2025). In addition, microplastics affect the P cycle by altering microbial communities, phosphatase activity, and interactions between polymer surfaces and soil colloids, thereby enhancing phosphate ion adsorption and the formation of insoluble phosphate compounds, thereby reducing P availability to plants (Lan et al., 2025).

CONCLUSIONS

Based on the data and analysis above, it can be concluded that treatment with bacterial isolates increased microplastic degradation and phosphorus uptake in Pak choi plants in Andisol compared to the treatment without isolates. The *Bacillus cereus* ATCC 14579 isolate showed the highest degradation result of 2.43%, followed by *Luteimonas* sp. ZGLJ41 at 1.54%, while without the isolate it was only 0.35%. Each type of plastic showed different degradation rates. Polybag plastic (LDPE) experienced the highest degradation rate of 1.78%, followed by silver mulch (HDPE) at 1.46%, straws (PP) at 1.28%, and fertilizer bags (PET) at 1.25%. The best combination was found in the treatment of *Bacillus cereus* ATCC 14579 + polybag (LDPE), with a degradation value of 3.08% after a 45-day planting period in Andisol and P uptake of 0.017 g plant^{-1}, the highest value among all treatments. The growth and yield of Pak choi also showed consistently high values, with a fresh weight of 38.67 g and a dry weight of 3.60 g.

These findings confirm that the use of plastic-degrading bacteria accelerates degradation by reducing the accumulation of plastic particles in the root zone, thereby mitigating the negative impact of microplastics on plant growth. Therefore, bacterial isolates have the potential to serve as a biological strategy for managing agricultural soil contaminated with microplastics. The limitations of this study include experiments conducted under controlled conditions on a pot scale over a relatively short 45-day period, as well as the lack of quantitative analysis of microplastic accumulation in plant tissues, which means the safety aspects of agricultural products still require further study. Further field-scale research is needed to evaluate the effectiveness of bacterial isolates under real conditions and in studies with long-term incubation periods. In addition, quantitative analysis of microplastic accumulation in plant tissues is needed to ensure the safety of agricultural products. Furthermore, future studies are recommended to include confirmatory chemical and structural analyses, such as Fourier transform infrared spectroscopy, scanning electron microscopy, and carbonyl index measurements, to verify changes in polymer composition and structure during degradation.

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