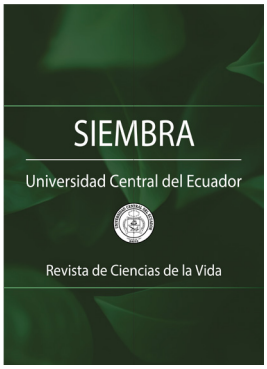
Biodegradation of pesticides by compost-isolated microorganisms

Biodegradación de pesticidas por microorganismos aislados de compost



Ana María Cunachi Pillajo[1], Jimmy Romario Clemente Rivera[2], Talía Luzmila Barragán Rodríguez[3]

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[1] Escuela Superior Politécnica de Chimborazo. Facultad Recursos Naturales. Laboratorio Ciencias Biológicas. Panamericana Sur Km 1 ½. CP 060106. Riobamba, Chimborazo, Ecuador.
✉ amcunachip@espoch.edu.ec.
https://orcid.org/0000-0001-6391-3489

[2] Escuela Superior Politécnica de Chimborazo. Facultad de Ciencias. Panamericana Sur Km 1 ½. 060106. Riobamba. Chimborazo. Ecuador.
✉ jimmy.clemente@espoch.edu.ec.
https://orcid.org/0009-0000-8671-3914

[3] Escuela Superior Politécnica de Chimborazo. Facultad de Ciencias. Panamericana Sur Km 1 ½. 060106. Riobamba. Chimborazo. Ecuador.
✉ talia.barragan@espoch.edu.ec.
https://orcid.org/0009-0004-1062-4586

* *Corresponding author:* animari4025@hotmail.com

Abstract

Soil degradation and low agricultural productivity have often been linked to the indiscriminate use of pesticides. In recent years, to restore soil fertility, farmers have increasingly turned to organic fertilizers, which supply both macronutrients and micronutrients to enhance crop production. However, the microbial load of these fertilizers can significantly impact soil biological populations, their diversity, and their activity. In this context, the objectives of this study were to assess the microbiological quality of compost samples and to conduct pesticide degradation tests. Microbiological analyses revealed that the compost's microbial load was primarily composed of phytopathogenic fungi, such as *Fusarium spp*. and *Cladosporium spp*., as well as phytopathogenic bacteria, including *Pseudomonas spp*. and Enterobacteriaceae, which are pathogenic to humans and animals. The population and diversity of actinomycetes were notably low. Previous analyses identified the persistence of pesticides such as glyphosate, chlorfenapyr, and difenoconazole in the samples. The most abundant bacteria and actinomycetes, identified as *Pseudomonas spp*. and *Streptomyces spp*. (ACP1 and ACP2), were effective in degrading these pesticides under in vitro conditions. Specifically, difenoconazole was degraded by up to 70%, chlorfenapyr by 44%, and glyphosate by 30%, both individually and in mixtures. These results demonstrate the potential of these microorganisms for use in decontamination and bioremediation processes by reducing pesticide concentrations in soil.

Keywords: agricultural production, pesticides, fertilizers, phytopathogenic fungi, microbiodiversity.

Resumen

La degradación del suelo y la baja productividad agrícola a menudo se han relacionado con el uso indiscriminado de pesticidas. En los últimos años, con el objetivo de restaurar la fertilidad del suelo, los agricultores han recurrido al uso de fertilizantes orgánicos, los cuales aportan tanto macronutrientes como micronutrientes para mejorar la producción agrícola. Sin embargo, la carga microbiana de estos fertilizantes también puede afectar a las poblaciones biológicas del suelo, su diversidad y su actividad. En este contexto, los objetivos de este estudio fueron evaluar

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la calidad microbiológica de muestras de compost y realizar pruebas de degradación de pesticidas. Los análisis microbiológicos revelaron que la carga microbiana del compost estaba compuesta principalmente por hongos fitopatógenos, como *Fusarium spp*. y *Cladosporium spp*., así como por bacterias fitopatógenas, incluidas *Pseudomonas spp*. y enterobacterias, las cuales pueden ser patógenas para humanos y animales. La población y diversidad de actinomicetos fue notablemente baja. Análisis previos identificaron la persistencia de pesticidas como glifosato, clorfenapir y difenoconazol en las muestras. Las bacterias y actinomicetos más abundantes, caracterizados como *Pseudomonas spp*. y *Streptomyces spp*. (ACP1 y ACP2), fueron eficaces en la degradación de estos plaguicidas en condiciones in vitro. Específicamente, el difenoconazol se degradó hasta en un 70 %, el clorfenapir en un 44 % y el glifosato en un 30 %, tanto de forma individual como en mezclas, reduciendo así la concentración de estos contaminantes y demostrando el potencial de estos microorganismos en procesos de descontaminación y biorremediación.

Palabras clave: producción agrícola, pesticidas, fertilizantes, hongos fitopatógenos, microbiodiversidad.

1. Introduction

Agrochemicals are compounds widely used in agriculture to control weeds, diseases, and pests in crops. Their mode of action includes repelling, preventing, mitigating, or destroying weeds, pests, and diseases. Based on their purpose, agrochemicals can be classified into insecticides, herbicides, bactericides, fungicides, miticides, molluscicides, nematicides, wood preservatives, and rodenticides (Ahamad & Kumar, 2023; Durán-Lara et al., 2020; Parra-Arroyo et al., 2022).

Herbicides are commonly used to improve crop yield and quality by reducing or inhibiting weed growth. They also serve as desiccants for various grain crops, including cereals (e.g., wheat, barley, oats, corn, sorghum), oilseeds (e.g., soybean, canola), pulses (e.g., beans, peas, chickpeas, lentils), and pseudocereals (e.g., buckwheat, quinoa). Among herbicides, glyphosate-based herbicides (GBHs) are globally recognized and widely used to control perennial weeds such as quackgrass and thistle, as well as to accelerate crop drying for harvest (Fuchs et al., 2022; Xu et al., 2019).

However, glyphosate is considered toxicologically harmful and has been potentially linked to human carcinogenesis and other chronic diseases, including reproductive and mental health disorders. The challenges in analyzing and demonstrating its toxicity are likely due to its metal-chelating properties, interference from organic compounds in the environment, and the structural similarity between glyphosate and its by-products (Peillex & Pelletier, 2020; Valle et al., 2019).

Other pesticides, such as chlorfenapyr, have also raised concerns. After its initial registration for use on green onions in 1996, chlorfenapyr usage in South Korea increased significantly, from 5,539 metric tons in 2006 to 15,821 metric tons in 2014. This increase has raised concerns about the potential health risks of pesticide residues, despite the establishment of maximum residue limits and preharvest intervals for crops (An et al., 2024; Jeong et al., 2019).

Similarly, overuse of difenoconazole may cause chronic and irreversible harm to human health and adverse ecological effects. Previous studies have shown that difenoconazole binds to human serum albumin and is associated with an increased incidence of hepatocellular adenomas and carcinomas in mice following long-term dietary exposure. As a result, determining difenoconazole residues in postharvest fruits and vegetables has become essential to ensure food safety for producers, regulatory authorities, and consumers (Qin et al., 2021; Shalaby et al., 2022; Wang et al., 2019).

The behavior of pesticides in soil and the environment is governed primarily by two processes: adsorption/desorption and degradation. Pesticides applied to the soil are absorbed by organic matter, which is often added in the form of organic fertilizers (Rassol et al., 2022).

Organic fertilizers, or manures, are considered biodegradable and are mostly derived from plant or animal sources, municipal solid waste, food industry by-products, crop residues, and various types of composts, such as vermicompost, kitchen waste compost, and distillery effluents (Jakubus & Michalak-Oparowska, 2022). These organic materials not only supply essential nutrients to plants but also help reduce the need for chemical fertilizers - particularly micronutrients - and eliminate the need for waste management or disposal (Singh et al., 2020b; Van Gijn et al., 2021).

However, organic materials may also contain traces of pesticides and their metabolic by-products, which can accumulate in soil in excessive amounts due to diffusion through water and air, or due to their persistence in plantations (Lau et al., 2023). Pesticide residues can degrade in both soil and organic waste materials

through microbial activity, chemical interactions, or ultraviolet radiation. The degradation rate and removal of pesticides are influenced by environmental factors such as water availability, soil pH, temperature, and the chemical properties of the pesticides. Key factors include solubility, volatility, ionization, hydrophilicity, and lipophilicity. Pesticide breakdown occurs via three primary mechanisms: microbiological, chemical, and thermal (Carpio et al., 2021; Lau et al., 2023; Rassol et al., 2022).

In this context, the objectives of this study were to evaluate the microbial quality of compost samples and to assess pesticide degradation mediated by microbial activity.

2. Materials and Methods

2.1. Microbiological quality of compost

Compost samples were collected using the quartering method from piles totaling 12 tons of product, with five sub-samples taken (Buol et al., 2011; Salazar Calvo et al., 2020). In the Biological Sciences Laboratory, Faculty of Natural Resources, ESPOCH, the pH of the samples was determined. Serial dilutions were prepared from 10^{-1} to 10^{-6}, and 100 µL from each dilution was used to inoculate nutrient agar [NA], potato dextrose agar [PDA], Sabouraud dextrose agar [SDA], and selective media: eosin methylene blue [EMB] agar for enterobacteria, *Pseudomonas* agar [PA] for the *Pseudomonas* genus, and glucose yeast extract agar [GYM] for actinomycetes. All isolates were incubated at room temperature, ranging from 17°C to 22°C. Bacterial growth was evaluated at 72 hours, while fungal and actinomycete growth was evaluated at 120 hours. The number of colony-forming units per gram of soil was calculated (Bhimani et al., 2024; Harrigan & McCance, 2014; Ibrahim & Hameed, 2015; O'Hara, 2005; Wellington & Toth, 1994; Williams & Cross, 1971).

2.2. Isolation and characterization of microorganisms with degradative capacity from compost samples

The most abundant bacterial colonies were selected for pure culture isolation, and the isolates were characterized based on macroscopic and microscopic morphology, as well as biochemical tests. For identification of the Pseudomonas genus, the API E20 test was used (Mashi, 2018; Mohammad et al., 2020), along with MacConkey agar (Midhat & Abed, 2023; Pant et al., 2022). The predominant fungal population was isolated and characterized through both macroscopic and microscopic morphology (Agu & Chidozie, 2021; Fischer & Dott, 2002). Additionally, the most abundant actinomycete colonies were isolated to obtain pure cultures. Using GYM medium, their aerial mycelium, substrate mycelium, diffusible pigments, microscopic morphology, and growth on different amino acids were characterized (Kumar & Jadeja, 2016; Rodríguez et al., 2018; Sapkota et al., 2020).

2.3. Pesticide degradation assays under in vitro conditions

Three of the most abundant microorganisms from the compost were selected for pesticide degradation tests: a bacterium identified as *Pseudomonas spp*. and two actinomycete cultures, ACP1 and ACP2. A fungus from the DCB collection was used as a reference for degradation.

The culture medium used in the degradation tests consisted of a commercial pesticide dissolved in distilled water and solidified with bacto agar. The concentrations were 4.6 ppm of glyphosate, 4.4 ppm of chlorfenapyr, and 4.1 ppm of difenoconazole, while the pesticide mixture reached a concentration of 13.21 ppm. In each culture medium, 50 µL of microbial suspension was added. The concentrations of the microorganisms were as follows: (1) *Pseudomonas spp*. (5.25×10^7 CFU μL^{-1}), (2) ACP1 (3.80×10^7 CFU μL^{-1}), (3) ACP2 (1.20×10^7 CFU μL^{-1}), (4) *T. harzianum* (2.75×10^7 CFU μL^{-1}), and (5) the control treatment, which received 50 µL of sterile distilled water (Esimbekova et al., 2022; Pizzul et al., 2009; Rossi et al., 2021).

The tests were incubated at room temperature (17°C ± 5°C) for 15 days. The degradative capacity of the microorganisms was evaluated by measuring the diameter of the colonies and assessing their morphology. Pesticide residues in the culture medium were analyzed using gas chromatography and mass spectrometry [GC-MS/MS], following the Lab 1-01-105 procedure by Agrolab Company (Lab Innovation Analytical, a Tentamus Company) (Hernández-Mesa & Moreno-González, 2022; Riedo et al., 2023).

3. Results and Discussion

3.1 Microbiological quality of compost suge

The microbial load of the compost samples was dominated by fungi and bacteria, with lower levels of actinomycetes (Table 1). These findings are consistent with previous studies describing microbial communities during composting, which typically include bacteria, fungi, and protozoa. The composition of these communities varies depending on factors such as temperature, moisture content, C/N ratio, and the nature of the organic materials used. Bacteria and fungi are generally the most abundant and rapidly emerging microorganisms during composting, as the substrates and indigenous microbiota significantly influence the quality of the final compost (Palaniveloo et al., 2020; Rastogi et al., 2020; Singh et al., 2022).

Table 1. Quantification of Colony Forming Units per gram (CFU g^{-1}) in the compost samples.

Sample	Bacteria			Fungí		Actinomycetes
	NA	PA	EBM	PDA	SDA	GYM
Compost 1	5.73E+05	7.80E+05	3.59E+05	5.30E+05	2.50E+04	3.63E+05
Compost 2	4.52E+06	6.14E+05	3.81E+05	4.87E+05	2.32E+04	3.30E+05
Compost 3	6.02E+05	7.10E+05	3.09E+05	4.95E+05	2.41E+04	3.17E+05
Compost 4	5.11E+05	6.57E+05	3.47E+05	5.53E+05	2.55E+04	3.84E+05
Compost 5	5.81E+05	7.80E+05	3.24E+05	5.76E+06	2.76E+04	3.69E+05
$\dot{x}$	**1.36E+06**	7.08E+05	3.44E+05	**1.57E+06**	2.51E+04	3.53E+05

It has been demonstrated that during the mesophilic and thermophilic phases of composting, the relative abundance of plant-pathogenic fungi decreases significantly due to high temperatures, which are critical for their elimination. However, during the cooling and maturation stages, the abundance of pathogenic fungi can return to initial levels, suggesting that some species survive the composting process. While certain fungi lose viability, others can recolonize the compost as temperatures drop during maturation. Our results align with previous findings reporting increased fungal abundance during the cooling phase, likely due to nutrient conditions in mature compost that favor fungal growth (Danish et al., 2021; Lv et al., 2023; Xu et al., 2024).

Notably, the fungal population was predominantly composed of phytopathogenic species, which contrasts with other reports indicating fungal absence due to inhibition during decomposition (Boiu-Sicuia et al., 2021). Morphological and microscopic characterization of fungal colonies revealed the presence of *Fusarium sp*., *Aspergillus sp*., *Penicillium sp*., and *Cladosporium sp*. (Ameen & Al-Homaidan, 2020; Chorolque et al., 2021). The abundance of *Aspergillus* and *Penicillium* species in our samples is consistent with other studies reporting these genera as the most common and widespread during various composting processes (Akyol et al., 2019; Kurakov & Bilanenko, 2023; Zhang et al., 2022).

The bacterial population in the samples analyzed showed diversity levels comparable to those reported in the literature. Bacterial richness tends to increase as composting progresses, typically peaking in mature compost. However, microbial diversity is also strongly influenced by the types of waste used, which directly and indirectly affect compost quality, particularly in terms of stability and phytotoxicity, due to their broad biochemical capabilities (Chen et al., 2024; Liu et al., 2023; Singh et al., 2023; Zhan et al., 2023; Zhao et al., 2018). Based on morphological, physiological, and biochemical traits, most bacterial colonies in our samples were identified as belonging to the Enterobacteriaceae family, *Pseudomonas spp*., and other Gram-negative bacteria (Liu et al., 2020; Meng et al., 2019; Naeem et al., 2022).

Actinomycetes, another key microbial group, were also identified in the compost samples. These microorganisms are well-documented in mature compost and natural environments. Many genera are known for promoting seed germination, root development, and biological control (Ayed et al., 2021; Ćwiertniewicz-Wojciechowska et al., 2023; Kannan & Kallapiran, 2022). Our findings are consistent with those of Buzón-Durán et al. (2020), who reported the proliferation of actinobacteria during low-temperature periods and their presence across a wide variety of composts, agricultural residues, and soils (Chopkova et al., 2023). Several studies suggest that actinomycete abundance increases with rising compost temperatures. The low diversity of

actinomycetes observed in our samples may be attributed to physicochemical factors, particularly pH and temperature, which influence microbial communities throughout different composting phases (Grigorova-Pesheva et al., 2024; Narsing Rao & Li, 2022).

3.2 Isolation and characterization of microorganisms with degradative capacity from compost samples

Fungal isolates were excluded from the pesticide degradation study due to their pathogenic characteristics. The microbiota present in fertilizers such as compost can create adverse environmental conditions for native soil microbiota, adding a level of suppression beyond naturally occurring ones (Araujo, 2022; de Corato, 2020; Kraut-Cohen et al., 2023). While some phytopathogenic fungi have been reported to perform bioremediation, preserving microbial biodiversity remains essential for maintaining soil quality and promoting crop health (Ahmad, 2020; Esparza-Naranjo et al., 2021; Fauriah et al., 2021; Leskovac & Petrović, 2023; Matúš et al., 2023; Rigolin et al., 2024; Sharma, 2021; Tawfik Ali ElHaty et al., 2022).

The most abundant bacterial and actinomycete strains were re-isolated and characterized. Bacterial colony morphology (small, round colonies), microscopic features (curved bacilli, Gram-negative), and biochemical tests (catalase and oxidase positive), along with the production of a greenish-yellow fluorescent pigment under UV light, indicated that the isolate belonged to the genus *Pseudomonas spp*. (Figure 1), consistent with descriptions by several authors (de Sousa et al., 2021; Girard et al., 2021; Lakshmanan et al., 2020; Maleki et al., 2010; Ruiz-Hernandez et al., 2024; Nazem Shirazi et al., 2023).

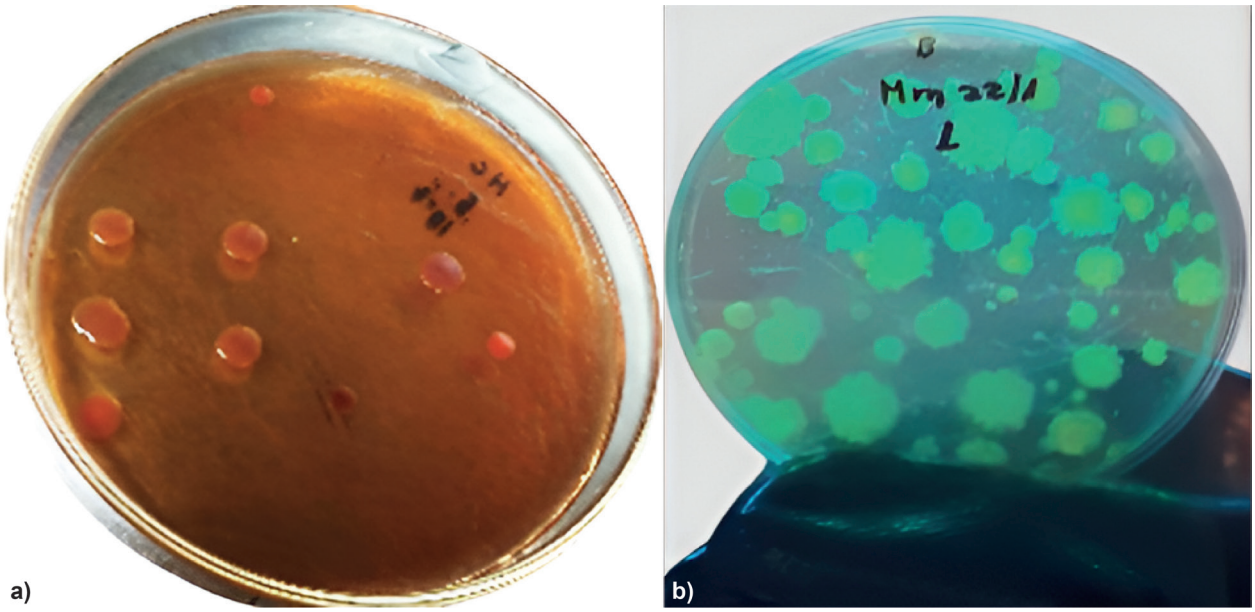

Figure 1. a) Colonies of *Pseudomonas* spp., on MacConkey agar, 72 hours of incubation at 26°C; b) Fluorescence on *Pseudomonas* agar.

Numerous *Pseudomonas* species have been reported as pesticide degraders, due to their genetic flexibility and metabolic versatility. These bacteria use a variety of mechanisms for degradation, including aerobic and anaerobic respiration, chemolithotrophy, extracellular enzyme activity, and fermentation. Although some limitations exist—such as incomplete mineralization or low efficiency in degrading complex pesticide mixtures—their molecular processes and catabolic potential are key to bioremediation (Ahmad et al., 2022; Randika et al., 2022; Rodríguez et al., 2020; Xu et al., 2023).

The actinomycete isolates were also characterized. Their aerial mycelium and diffusible pigments (Figure 2) described using the British Standard BS 381C color chart, along with colony morphology and spore microscopy (Table 2), confirmed that isolates ACP1 and ACP2 displayed typical characteristics of the genus *Streptomyces*. Growth assays on various carbon sources showed that both isolates could metabolize five of the eight tested substrates (Table 3). This ability to utilize different amino acids and organic sources is characteristic of actinomycetes, which play a key role in the carbon cycle by breaking down complex polymers and supporting microbial community dynamics (Grgas et al., 2023; Mehta & Jadeja, 2022; Oyedoh et al., 2023; Pérez-Corral et al., 2022; Rodríguez-Fonseca et al., 2021; Salem et al., 2023).

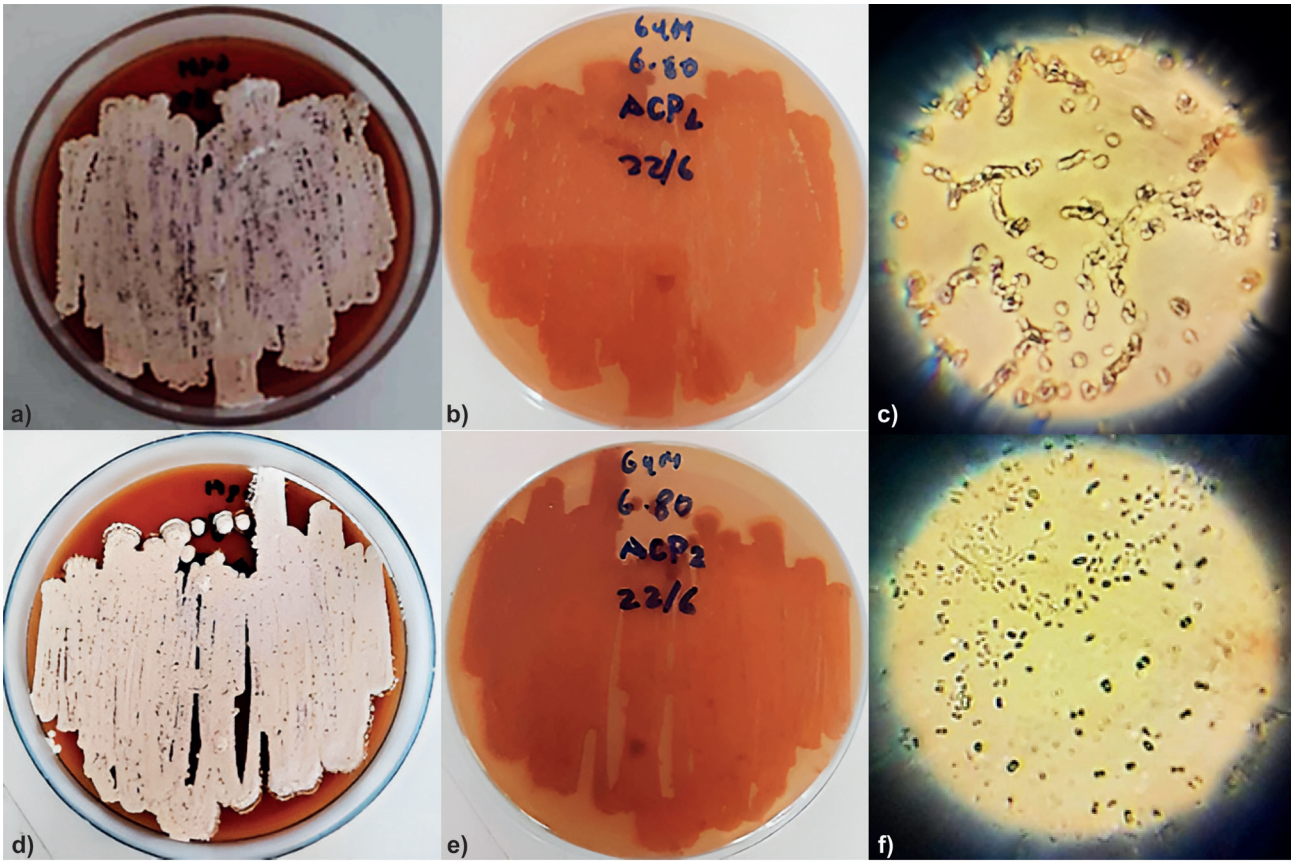

Figure 2. *Actinomycetes* colonies in pure culture [GYM] and spore microscopy: a) ACP1: aerial mycelium, b) ACP1: diffusible pigment; c) ACP1: spores_1000X, d) ACP2: aerial mycelium, e) ACP2: diffusible pigment; f) ACP2: spores_1000X.

Table 2. Macro and microscopic description of actinomycetes *isolates.*

Isolates	Aerial mycelium	Diffusible pigment	Spores
ACP1	380 Camouflage Desert Sand*	592 International Orange*	Oval long chain
ACP2	460 Deep Buff*	537 Signal Red*	Individual elongated
	*BS 381C	*BS 381C	1000 X

Table 3. Growth of actinomycetes on different carbon sources.

Aminoacids	Actinomycetes (120 h)		
	ACP1	ACP2	Control
Urea	1	1	0
Glutamic acid	1	1	0
Aspartic acid	1	1	0
Asparagine	1	1	0
Arginine	1	1	0
Cysteine	0	0	0
Glycine	0	0	0
Tyrosine	0	0	0
[1] growth		[0] no growth	

Actinomycetes also perform several beneficial functions, including nitrogen fixation, phosphate solubilization, phytohormone and enzyme production, organic matter decomposition, biocontrol of phytopathogens, and bio-

remediation in soil and substrates. For this reason, ACP1 and ACP2 were selected for pesticide degradation assays, exploring common microbial mechanisms such as competition for space and nutrients, secretion of antibiotics, siderophores, lytic enzymes, volatile organic compounds, phytohormone synthesis, and resistance induction (Nazari et al., 2022; Torres-Rodríguez et al., 2022).

Both *Pseudomonas spp*. and *Streptomyces spp*. contribute to degradation through biological and abiotic pathways. However, bioremediation via microbial activity is considered the most effective strategy for transforming pesticides into simpler, less toxic compounds due to the organisms' strong metabolic potential. This potential can be influenced by factors such as degradation efficiency, ease of cultivation, inoculum size, tolerance to high pesticide concentrations, adaptability, and competitiveness with native microbial communities (Conde-Avila et al., 2021; Dar et al., 2023; Guerrero Ramírez et al., 2023; Roumeng et al., 2023).

3.3 Pesticide degradation assays under in vitro conditions

The results of the pesticide degradation tests revealed notable changes in both the growth and morphology of the microorganisms evaluated. *Pseudomonas spp*. showed slow, irregular growth, and its colonies lost their characteristic brightness. For the actinomycete isolates ACP1 and ACP2, growth was also slow, the aerial mycelium lost pigmentation, and sporulation was accelerated but sparse. *Trichoderma harzianum* colonized the medium by 96 hours, with similarly accelerated but limited sporulation (Figure 3). These observations align with other studies reporting the morphological and physiological impacts of pesticides on microbial growth and viability (Mohapatra et al., 2022; Shahid et al., 2021).

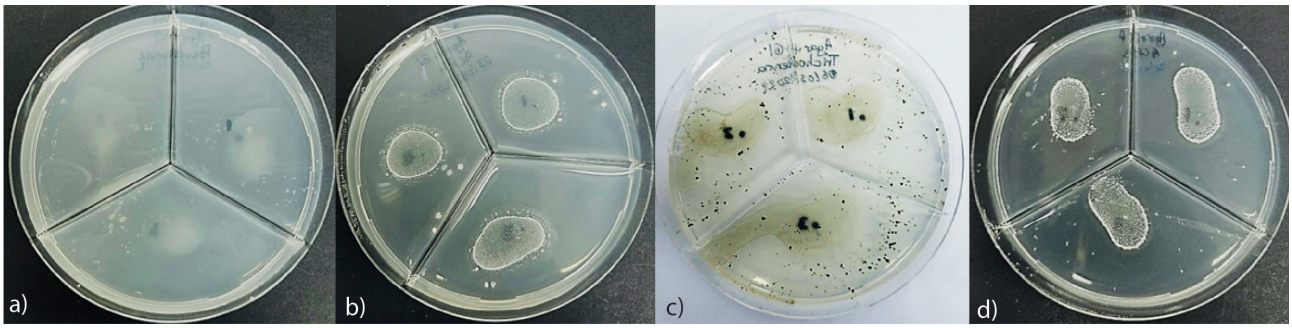

Figure 3. Morphological alterations of pesticide-degrading microorganism: a) *Pseudomonas* / Chlorfenapyr, b) ACP1 / Glyphosate c) *Trichoderma harzianum* / Glyphosate, d) ACP2 / Difenoconazole.

Residue analysis (Table 4) confirmed that all tested microorganisms effectively reduced pesticide concentrations (Figure 4). Among the compounds, difenoconazole was the most readily degraded, with a reduction of up to 87%. This is likely due to both microbial efficiency and aerobic conditions, which are known to favor rapid degradation. Aerobic composting processes have also been associated with enhanced difenoconazole breakdown, whereas anaerobic conditions hinder it. This is especially relevant considering the pesticide's high toxicity, which includes neurotoxic effects and oxidative stress in eukaryotic organisms (Liu et al., 2022; Man et al., 2021).

Chlorfenapyr was degraded by 52%, suggesting that the tested microorganisms lack some of the enzymatic pathways necessary for complete breakdown. Given its link to neurotoxic, gastrointestinal, and leukoencephalopathic effects, its persistence poses a potential risk. Although few microorganisms are reported to degrade chlorfenapyr, the observed reduction may involve enzymatic pathways—such as oxygenases—commonly found in *Pseudomonas spp*. (Cheng et al., 2022; Schaeffer & Wijntjes, 2022; Sun et al., 2023; Wu et al., 2020; Yang et al., 2020).

Glyphosate degradation reached only 50%, which is relatively low given the molecule's toxicological risks to human health and its documented lethality to plants and mammals at low concentrations (Singh et al., 2024). These findings are consistent with previous studies showing partial degradation of glyphosate by *Pseudomonas spp*. and *Trichoderma harzianum* (Rafieenia et al., 2022; Singh et al., 2020).

In the pesticide mixture, degradation was higher, reaching 62%, suggesting synergistic degradation effects. This aligns with other studies highlighting the ability of bacterial, fungal, and actinomycete genera to degrade pesticide mixtures via enzymatic and metabolic pathways (Bamdad et al., 2022; Raffa & Chiampo, 2021). In this study, *Pseudomonas spp*. demonstrated its capacity to break down pollutants across different

environments. Other genera—such as *Flavobacterium*, *Achromobacterium*, *Sphingomonas*, *Arthrobacter*, and *Bacillus*—have also shown promising results in pesticide degradation (Esikova et al., 2023; Huang et al., 2023; Mustapha et al., 2019).

Table 4. Percentage of pesticide degradation by microbial action.

Pesticide	Microorganism	IC initial concentration ppm	FC final concentration ppm	Standard Deviation σ	Degradation (%)
Chlorfenapyr	*Pseudomona* spp.	4.4	2.1	0.33040379	52.2
	ACP1	4.4	2.8		36.3
	ACP2	4.4	2.7		38.6
	Trichoderma harzianum	4.4	2.3		47.7
	Control	4.4	4.4		0
Difenoconazole	*Pseudomona* spp.	4.13	0.91	0.67406108	77.9
	ACP1	4.13	2		51.5
	ACP2	4.13	1.6		61.2
	Trichoderma harzianum	4.13	0.5		87.8
	Control	4.13	4.13		0
Glyphosate	*Pseudomona* spp.	4.68	3.2	0.71355915	31.6
	ACP1	4.68	2.3		50.8
	ACP2	4.68	3.7		20.9
	Trichoderma harzianum	4.68	3.9		16.6
	Control	4.68	4.68		0
Mix_3	*Pseudomona* spp.	13.21	5.79	0.42762912	56.1
	ACP1	13.21	5		62.1
	ACP2	13.21	5.17		60.8
	Trichoderma harzianum	13.21	5.84		55.7

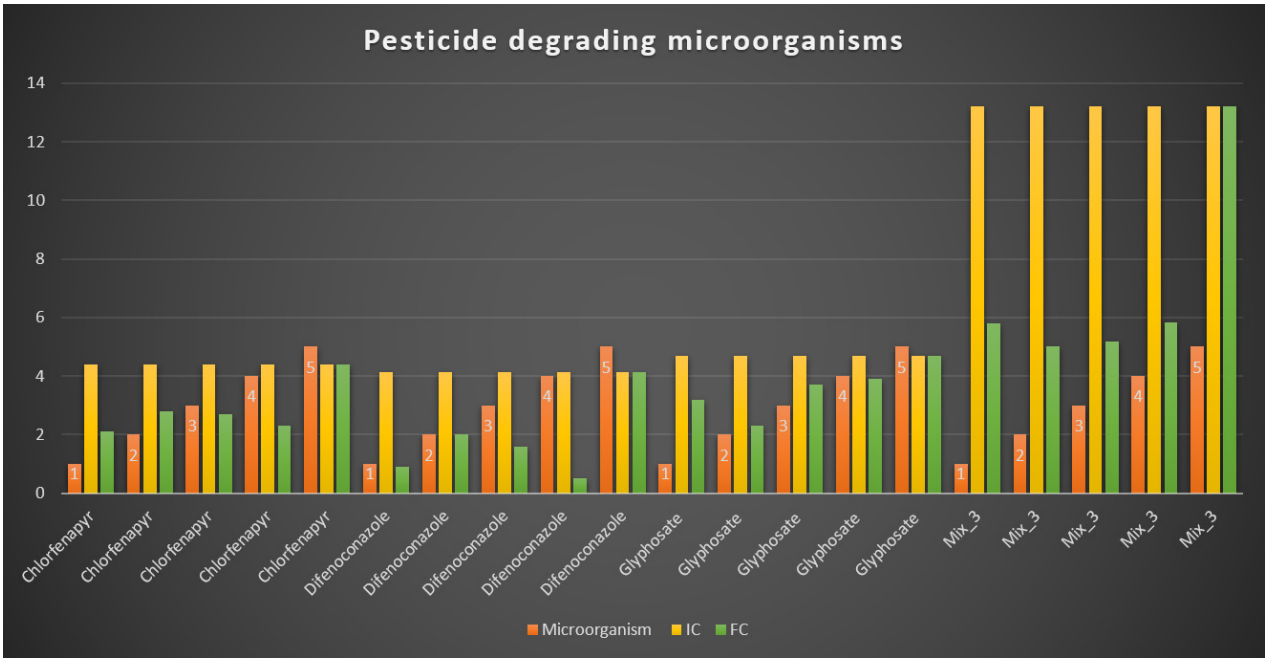

Figure 4. Degradation of pesticides by microbial action.

Actinomycetes, particularly *Streptomyces spp*. isolates ACP1 and ACP2, also contributed significantly to degradation. ACP1 was most efficient at degrading difenoconazole, both alone and in combination, while ACP2 showed notable activity against difenoconazole alone and chlorfenapyr in the mixture. These results support previous findings that actinomycetes play key roles in degrading pesticides across various chemical classes—such as organophosphates, carbamates, triazinones, acetanilides, and sulfonylureas—through mechanisms including nutrient cycling and polymer decomposition (Castrejón-Godínez et al., 2021; Das et al., 2021; Srinivasulu et al., 2024).

Fungi such as *Trichoderma spp*. have also been reported for their bioremediation potential. In this study, *T. harzianum* efficiently degraded chlorfenapyr and difenoconazole. These findings align with recent reports of *Trichoderma spp*. acting as hyperaccumulators capable of absorbing heavy metals and degrading pesticide residues in soil (Correa et al., 2021; Khazal & Suhail, 2022; Kunanbayev et al., 2019; Sun et al., 2020; Wang et al., 2024; Zin & Badaluddin, 2020). Interestingly, while degradation of difenoconazole and chlorfenapyr was high, glyphosate concentrations in the mixture increased, possibly due to *T. harzianum's* hyperaccumulation behavior.

Statistical analysis of standard deviations (Table 4) revealed low variability across treatments, indicating consistent pesticide degradation by the microbial isolates used in this study.

4. Conclusions

The presence of abundant phytopathogenic fungi and enterobacteria indicates low microbiological quality in the compost samples analyzed, and their use poses a risk to the health of the soil, crops, and farmers. It is recommended to carry out pretreatments before use to reduce the presence of pathogens.

Pesticide degradation tests using microbial isolates obtained from compost—identified as *Pseudomonas spp*. and *Streptomyces* (ACP1 and ACP2)—demonstrated their potential as bioremediation agents for residues of glyphosate, chlorfenapyr, and difenoconazole.

Additionally, the reference strain *Trichoderma harzianum* was effective in pesticide degradation, confirming its potential role in bioremediation processes. Therefore, it is recommended to enrich compost with a defined concentration of fungal spores before application to promote biological activity, reduce phytopathogen populations, and enhance the additional beneficial mechanisms this fungus provides to soil and crops.

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Contributor roles

- Ana María Cunachi Pillajo: conceptualization, formal analysis, investigation, methodology, resources, supervision, writing – review & editing.
- Jimmy Romario Clemente Rivera: investigation, funding acquisition, methodology.
- Talía Luzmila Barragán Rodríguez: investigation, funding acquisition, methodology.

Ethical implications

Ethics approval not applicable.

Conflict of interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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