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A Multi-agent Approach to Bioremediation of Organophosphate Pesticide Contaminated Soil: Bacteria, Fungi, Plant Extracts and Animal By- products.

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Abstract: Pesticides are potentially hazardous chemicals that have a long half-life in the environment. They can be toxic to both aquatic and terrestrial organisms, endangering human and environmental health . The current study was conducted to evaluate the efficiency of the bioremediation process. Bacterial strains(*Bacillus.coagulans,B.licheniformis,B.subtilis*)from(B1-B7;B1-*Bacilluscoagulans*),(B2-*B.licheniformis*),(B3-*B.subtilis*),(B4-B.C+B.L), to (B5-B.L+B.S), (B6-B.S+B.C),(B7-B.C+B.L+B.S), fungal strains (*Botrytis cinerea, Alternaria alternata, Saccharomyces cerevisiae, Rhizopus stolonifer, Penicillium chrysogenum*) Obtained from Tomato,yeast and bread (F1- Tomato cultures),(F2-Bread cultures),(F3-yeast),(F4- comb of Tomato+bread),(F5- bread+ yeast),(F6- Yeast+Tomato),(F7- Bread, Tomato,yeast), plant extracts (soap nuts, moringa seeds, fenugreek) from(P1-P7 P1- Soap Nut Extract; P2- Fenugreek Extract; P3- Moringa Extract; P4- Soap Nuts + Moringa; P5-Fenugreek Extract + Moringa Extract; P6- Soap Nuts Extract + Fenugreek Extract; and P7- Fenugreek + Moringa Extract + Soap Nut Extract), and animal-derived substrates (cow dung, fish scales, eggshells)from(A1-A7,A1-cowdung,A2-eggshells,A3-fishscales,A4-CD+ES,A5-ES+FS,A6-FS+CD,A7-CD+FS+ES).Along with these we also used combinations of two , in which first one is (plant + animal derivatives) and the second is(Bacterial+fungi specifies)were used in the experiment to treat the soil contaminated by 20% Emulsifiable Concentrate (EC) chlorpyrifos pesticide. The effectiveness of treatment was investigated using chemicals such as copper sulphate (CuSo4) and sodium hydroxide (NaOH), microbe growth measurements, organoleptic evaluation (including changes in the colour and smell of treated soil), and pH measurement methods. Certain combinations such as (B5,F3, F4, F5, F7,P6,A1,A2)of factors have been shown to degrade pesticides in soils by up to 90%.

Keywords: Bioremediation; Organophosphate Pesticide; Cow dung; Soil microbial degradation; Pesticides Contaminated Soil.

1.INTRODUCTION

Agriculture is a key pillar of the Indian economy. In terms of vegetable production, India is currently ranked second in the world (Diksha Gour et al.,2025).Pesticides are essential to modern agriculture because they increase crop yields while controlling weeds, illnesses, and pests (Gyanendra Dhakal et al., 2025).However, its extensive use results in serious health and environmental issues, such as contaminated soil and water, harm to food chains, non-target creatures, and human health (Gyanendra Dhakal et al., 2025).About 80–90% of pesticides that are sprayed remain in the soil for extended periods of time(Wang et al.,2006).Pesticides are a major contributor to soil degradation because of their propensity to change soil microbial populations and lower soil fertility (Maha Aljabri et al., 2025).

These substances can negatively affect the flow of nutrients and stop the growth of helpful microorganisms, according to Noorani and Singh.Despite its significance, inappropriate usage and management, which results in soil degradation, are among the factors endangering soil health (Raquel Carvalho et al., 2025). According to estimates, 60–70% of European soils are unhealthy, and 33% of the world's soil is already deteriorated (Raquel Carvalho et al., 2025).Additionally, according to UNESCO's (United Nations Educational, Scientific, and Cultural Organization) projections, this percentage will rise to 90% by 2050 (Raquel Carvalho et al. 2025). Chlorpyrifos (CP) 20%EC, a broad spectrum organophosphate insecticide that is categorised as moderately hazardous, was utilised in this study(Sharma et

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al .,2019). CP has been widely used in agriculture since the 1960s to manage pest infestations of crops like cotton, cereals, vegetables, and fruits (Shamsa Akbar et al. 2016).CP is known to have neurotoxic and immunotoxic qualities and has been demonstrated to be detrimental to both humans and animals, although being only moderately toxic (Shamsa Akbar et al., 2016).

Bioremediation of pesticides contaminated soil is shown in this graph from 2016 to 2026. Counts remained relatively stable in the 270-290 range through 2020, then dropped to a lower plateau of around 248-250 between 2021 and 2023, before surging to a peak of 325 in 2024 and 312 in 2025, the highest points in the entire series. The 2026 figure (189) is noticeably lower, but because the year has not yet ended, this is most likely due to incomplete data rather than a genuine decline in interest.(Fig7)

Four different classifications: fungal species, bacterial species, animal, and plant extracts were used in this research paper's bioremediation of pesticide-contaminated soil. First, we can talk about the significant role that fungal species play in this process.Utilizing microorganisms (fungi) to degrade contaminants in contaminated soil is known as bioremediation. Fungi are good bioremediators because they can: secrete potent extracellular enzymes, degrade complex organic substances (hydrocarbons, insecticides, colours) and tolerate harsh environmental conditions.Perform in situ (in place) work on polluted soil (Randika et al.,2022)(Fig. 3).

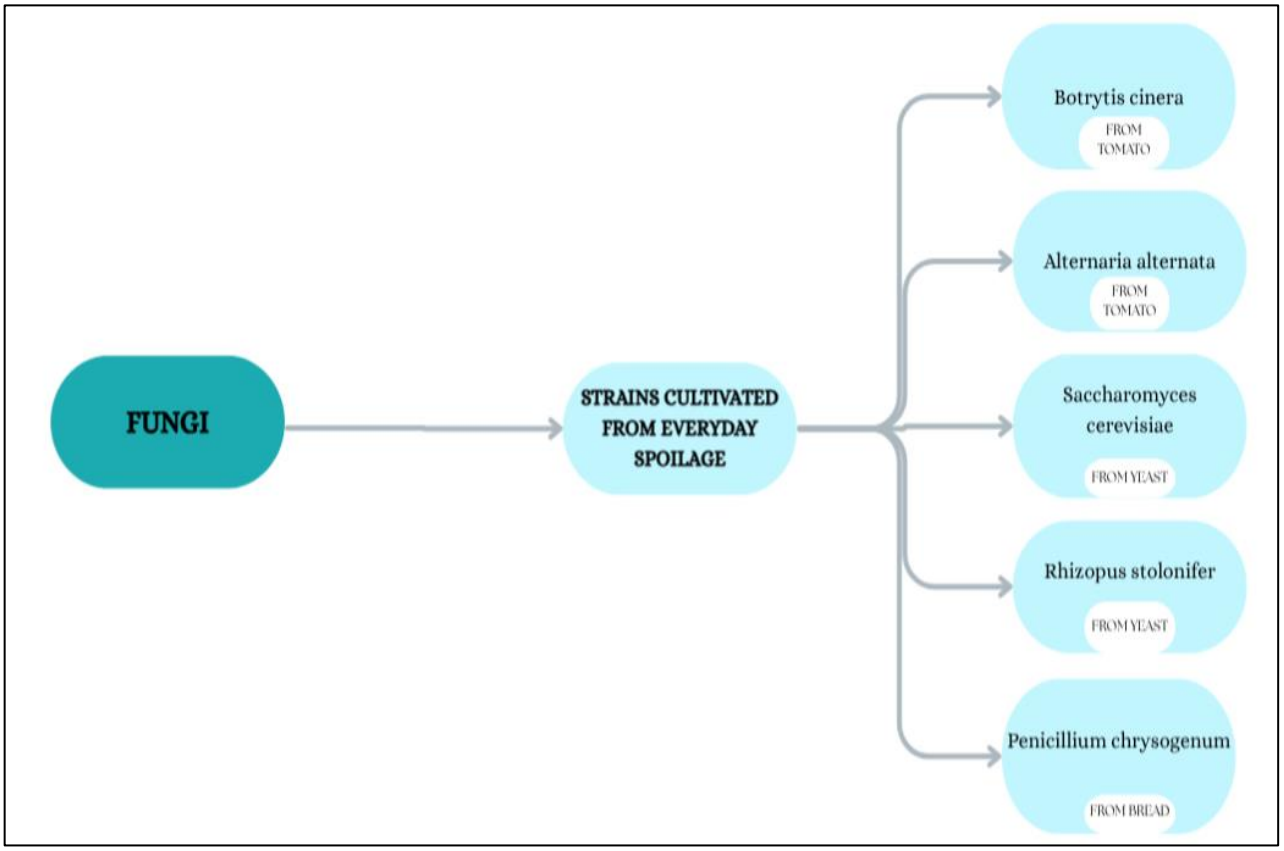

Fig1: Fungal species Cultivated from every day Spoilage

In our lab,used cultivated strains of fungus such as *Botrytis cinerea, Alternaria alternate, Saccharomyces cerevisiae, Rhizopus stolonifer,* and *Penicillium chrysogenum* from natural foods that contain fungi as they ripen or perish, such as bread, tomatoes, and yeast(Aljabri et al., 2025)(Fig1).The main aims for selecting fungi include: i) Isolating and identifying fungus from soil.ii) To assess fungal ability to breakdown contaminants (e.g., dyes, hydrocarbons)iii) To establish bioremediation in samples of polluted soil(Matúš et al.,2023).Sample preparation, serial dilution, media preparation, plating, isolation and purification, setting up bioremediation reactors for the liquid culture method (dye degradation), safety precautions, data recording and calculations, and troubleshooting are the procedures .This research followed when bioremediating soil using fungi(Perumbakkam et al.,2006)(Fig2).

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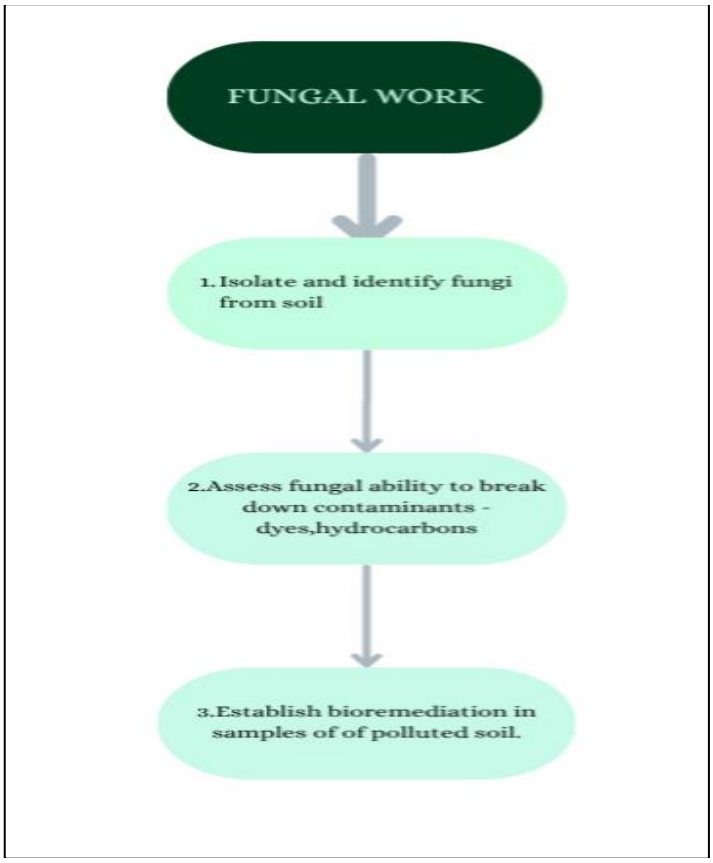

Fig2: What the fungal work set out to do.

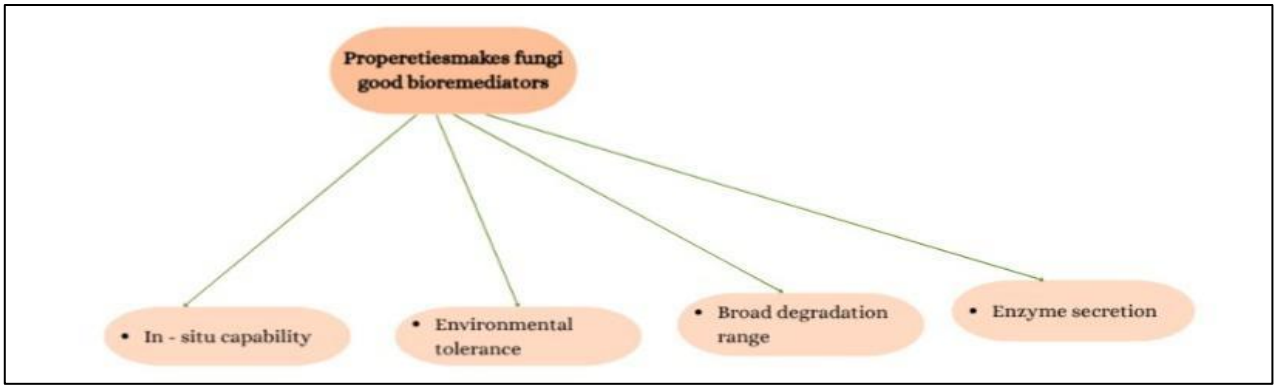

Fig3: Four properties that make Fungi good Bioremediators

This article now discusses how bacterial species play an important role in the bioremediation of pesticide-contaminated soil. The bacterial species strains that used to reduce soil bioremediation are (*Bacillus coagulans, B. licheniformis, B.subtilis*). Certain bacteria have enzymatic machinery that breaks down pesticide molecules via metabolic pathways. Pseudomonas species are very good at breaking down synthetic pyrethroid pesticides, whereas Bacillus species are strong degraders of organophosphate pesticides. When these bacteria are introduced into contaminated soil and given appropriate conditions (moisture, pH, temperature, aeration), they colonise the soil and start the breakdown of pesticide residues.The main goal is to demonstrate and evaluate the ability of selected bacterial cultures to degrade pesticide residues in contaminated soil over a defined incubation period using simple laboratory techniques. During soil bioremediation with bacterial strains, this research followed these steps: Prepare Bacterial Inoculum, Prepare Soil Samples, Prepare Soil Moisture, Inoculate Soil Samples, Incubation, Monitoring and Sampling, Analysis, and Precautions(Fig4)

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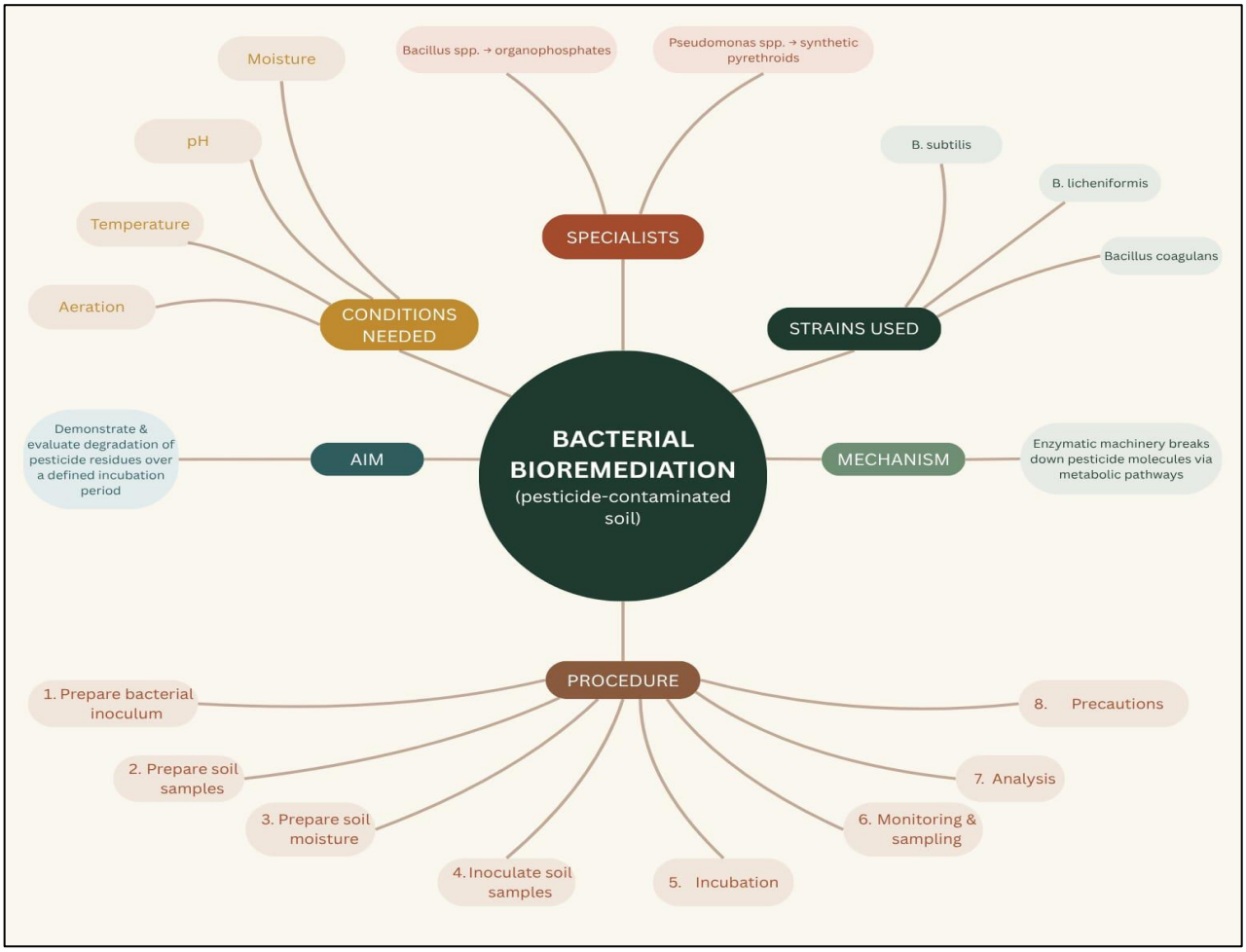

Fig4: Complete Outline of bacterial bioremediation

The main idea behind this is that plant extracts contain natural compounds (saponins, enzymes, and antimicrobial agents) that promote microbial activity and enhance organic matter decomposition in soil. Soap nuts, fenugreek, and moringa seeds are the plant extracts used in the bioremediation of pesticide-contaminated soil. These plant-based compounds function as biostimulants, hastening the natural biodegradation process.The primary goal is to investigate the soil's capacity for biodegradation utilising natural plant extracts (soap nuts, fenugreek, and moringa seeds) and evaluate how well they break down organic matter and enhance soil quality in a short amount of time.This research have followed these procedures while employing plant extracts for soil bioremediation. Sample preparation, application, upkeep, analysis, benefits, and troubleshooting(Fig5).

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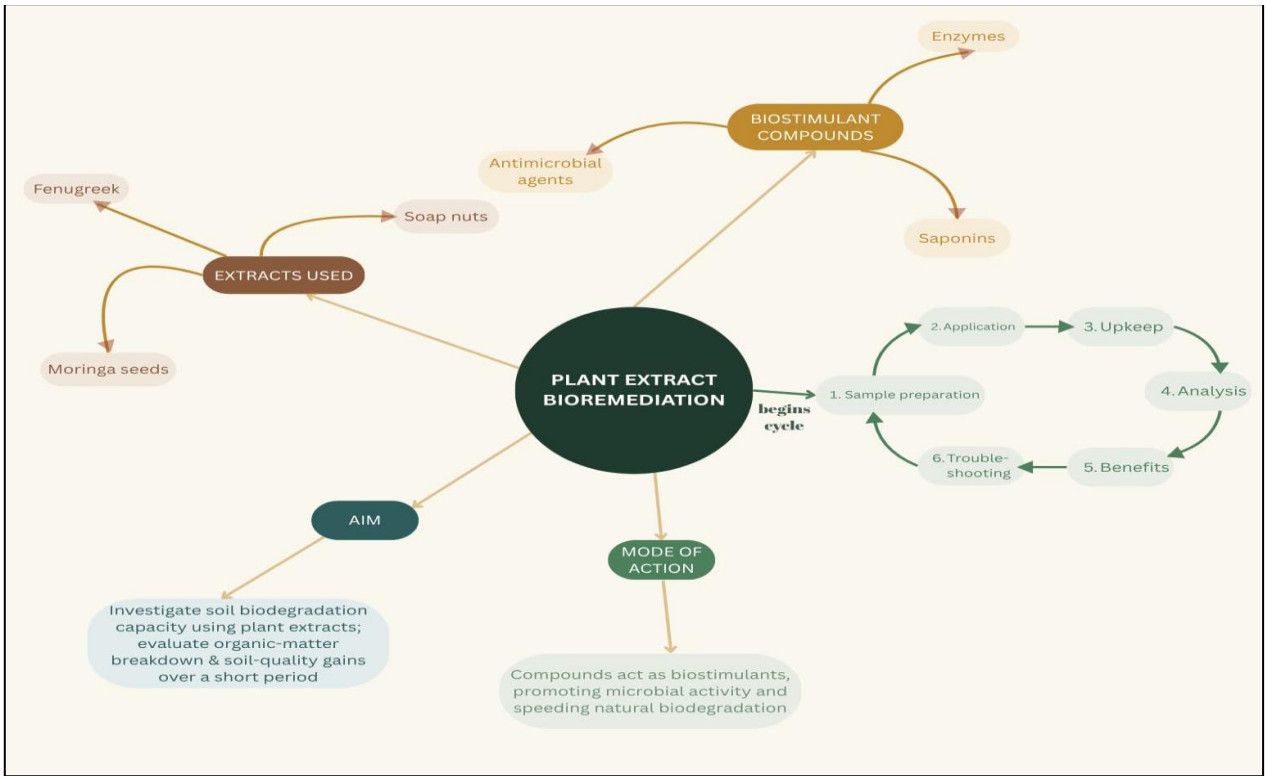

Fig5: Complete Outline of plant extracts bioremediation.

Finally, This paper will talk about how animal extracts were crucial to the bioremediation of soil tainted by pesticides. Utilised fish scales, egg shells, and cow dung as animal extracts.The primary principle underlying each species is discussed in Cow Dung:Naturally rich in beneficial microorganisms (bacteria, fungi, and actinomycetes); contains organic matter that improves soil structure; offers slow-release nutrients for microbial growth; contains phenolic compounds with antimicrobial qualities; is free of any dairy farm or agricultural area; and has already partially decomposed, making nutrients immediately available.Fish scales: Rich in chitin and collagen, a great source of carbon for microorganisms,It is free of fish markets and fisheries, biodegradable, non-toxic, contains amino acids that bacteria use to generate pesticide-degrading enzymes, and provides vital micronutrients (calcium, phosphorus, and minerals). Breakdown products also create an acidic microenvironment that boosts enzyme activity(Fig6).

Egg shells are rich in calcium (99% calcium carbonate), which promotes the synthesis of enzymes, somewhat alkaline, which regulates the pH of the soil for the best microbial activity; have a porous structure that offers a high surface area for microbial colonisation; contain trace minerals and proteins; and are free of any toxins.The primary goal of this is to include to remediate pesticide-contaminated soil by encouraging microbial biodegradation of organophosphate, pyrethroid, and organochlorine pesticides using readily available agricultural waste products (fish scales, egg shells, and cow dung).and This research followed these procedures when using animal extracts for soil bioremediation.Amendment preparation, soil preparation and contamination, upkeep and monitoring, safety measures, and troubleshooting.

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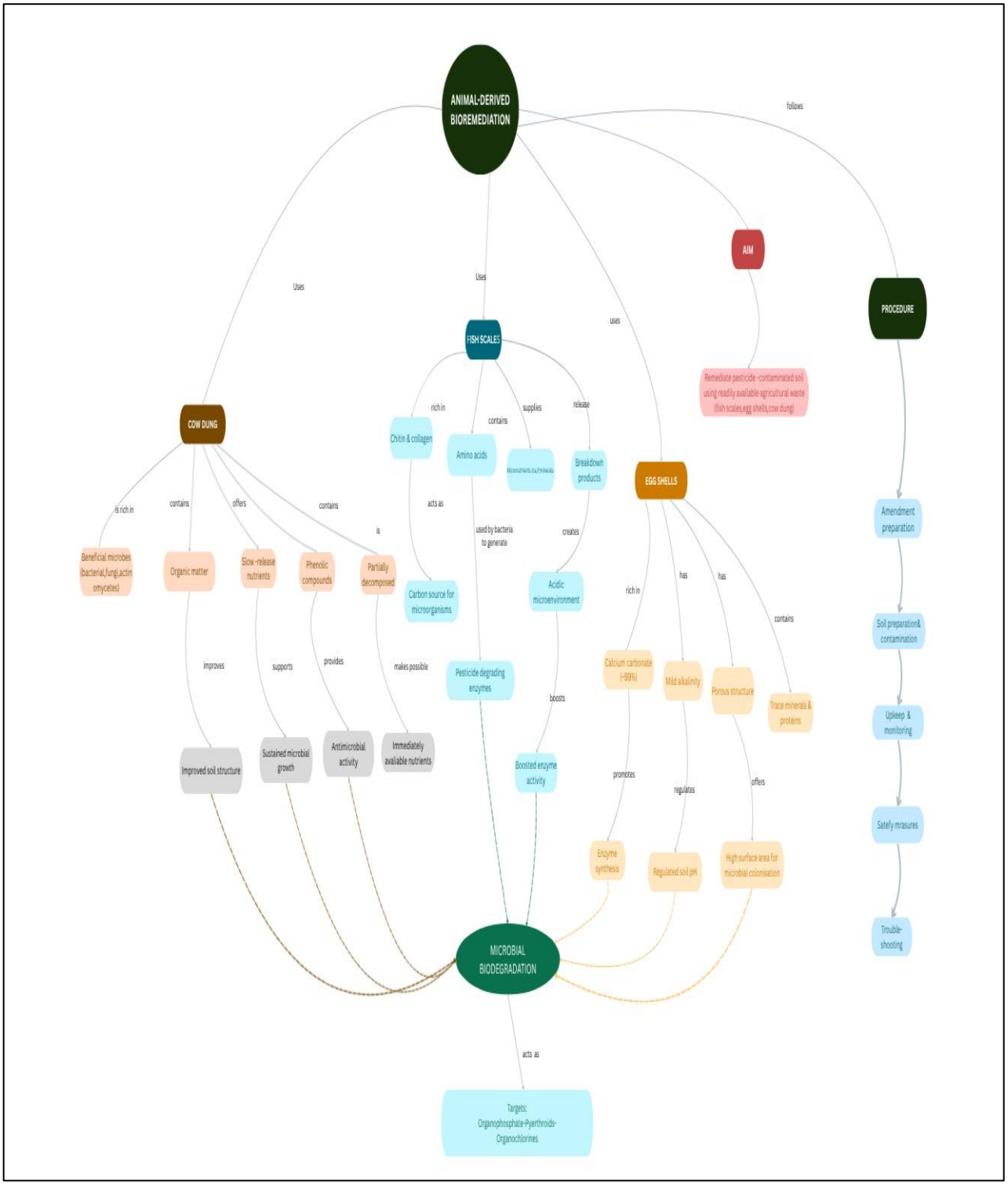

Fig6: Complete Outline of Animal derived Bioremediation.

This research article mainly explains about bioremediation of only pesticides contaminated soil using different classifications such as bacterial species, fungal species, plant and animal extracts, and mainly it also includes combinations of samples with in the classifications itself, such as in bacterial species there are individual, combinations of two, and combinations of all three such combinations results are tabulated and the main goal of this research is the different classification of species and extracts used in the same research, such as bacterial species, fungal species, animal extracts, and plant extracts, is what makes this paper novel.All these classifications have not been documented elsewhere until now. Additionally, conducted combined tests within these classifications to assess pesticide reduction in soil. These

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tests included pH measurement, chemical tests using copper sulfate (CuSO4) and sodium hydroxide (NaOH), microbial growth measurements, and organoleptic assessments (evaluating changes in soil color and smell).This research also discussed troubleshooting for each classification separately.

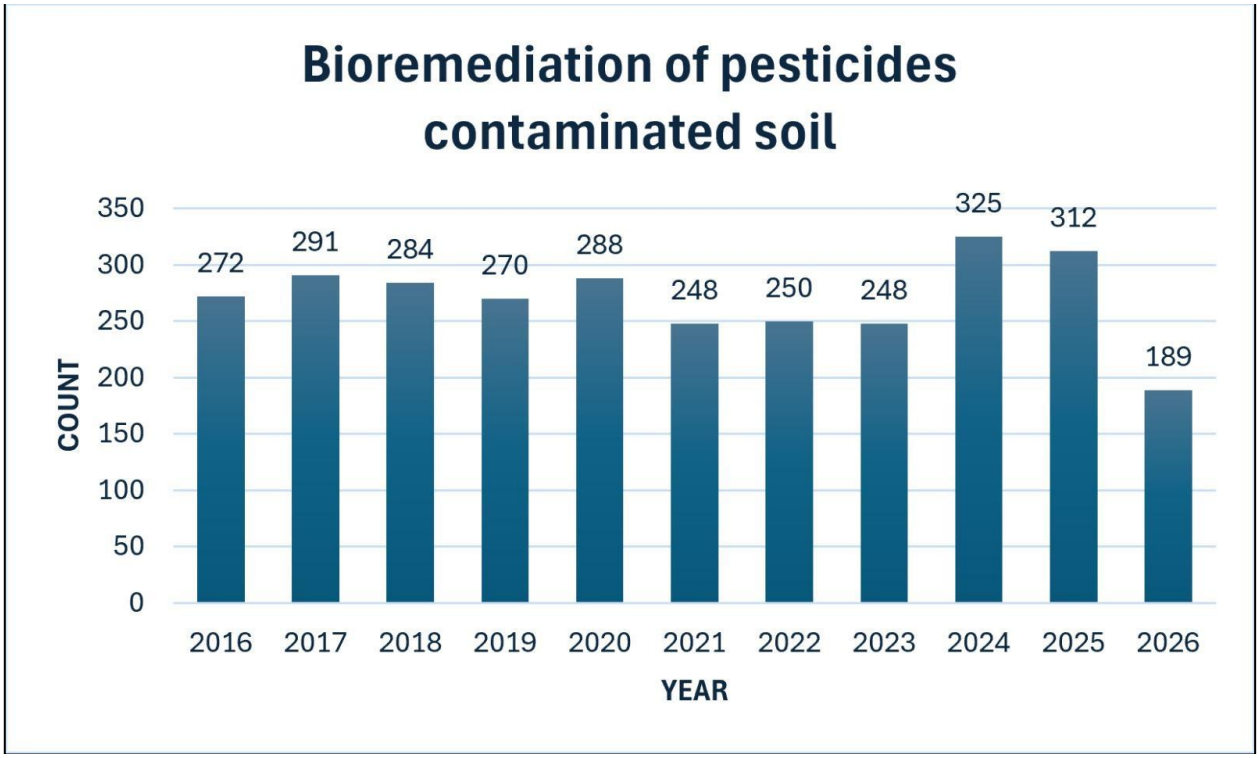

Fig 7: Bioremediation of pesticides contaminated soil

2.MATERIALS AND METHODS

2.1 PREPARATION OF SOIL

Since researchers collected the soil from a garden close to our university in Visakhapatnam, Andhra Pradesh, it is red soil. Then removed stones, debris, and roots from 25 kg of soil.Allowed it to dry for a full day at room temperature. The following day, we used a weighing machine to weigh 20 kg of soil, and then used sieves to filter the soil to get rid of tiny particles.Now, measure out 500 grams of soil into various containers, cover them with lids, and leave them at room temperature with a little water.

2.1.1Contamination of soil using pesticides

This research has employed an aqueous method based on water to contaminate soil with pesticides.For laboratory-scale bioremediation research, this water-based technique contaminates soil samples with chlorpyrifos at regulated concentrations(Raffa et al.,2021) This method, which uses distilled water and 20% Emulsifiable Concentrate (EC) chlorpyrifos, avoids organic solvents and is appropriate for undergraduate research in Indian institutions.Water-Based Method Advantages: No hazardous acetone solvent is required, lower toxicity risk during handling, simpler lab setup (no fume hood required), faster evaporation (24-48 hours with water only), cost-effective and accessible in all labs, and more sustainable/environmentally responsible(Fig8).

The 20% EC chlorpyrifos formulation is designed to disperse in water and form a stable emulsion. The pesticide is dispersed throughout the soil matrix by combining this emulsion with dried soil and letting it dry naturally(Farhan, et al.,2021).A thorough explanation of the contamination process is provided.The process starts with the necessary materials: distilled or deionised water (DM water), which is a standard lab supply; and chlorpyrifos 20% Emulsifiable Concentrate (EC), which is available from approved agricultural chemical suppliers in India. Hazard of chlorpyrifos: Organophosphate insecticide-cholinesterase inhibitor,principal routes of hazard: Skin absorption, concentrated spray inhalation, unintentional ingestion, and aqueous suspension hazards: minimal (emulsified form is less volatile than technical powder)(Amani et al., 2018).

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2.1.2 Safety Precautions for Water-Based Method

When working with concentrated chlorpyrifos 20% EC, gloves should always be worn.Work in an area with good ventilation, such as an open window or, if available, a fume hood.Steer clear of skin contact; if it does happen, wash right away for 15 minutes with soap and water.When making eye contact: Get medical help after rinsing with water for at least fifteen minutes.Do not consume; if accidentally swallowed, seek immediate medical attention (antidote: atropine).When handling pesticides, avoid eating, drinking, or smoking.After the procedure, thoroughly wash the hands.Properly dispose of aqueous pesticide waste by collecting it in a waste container for hazardous waste disposal rather than pouring it down the drain(Malik et al .,2024).

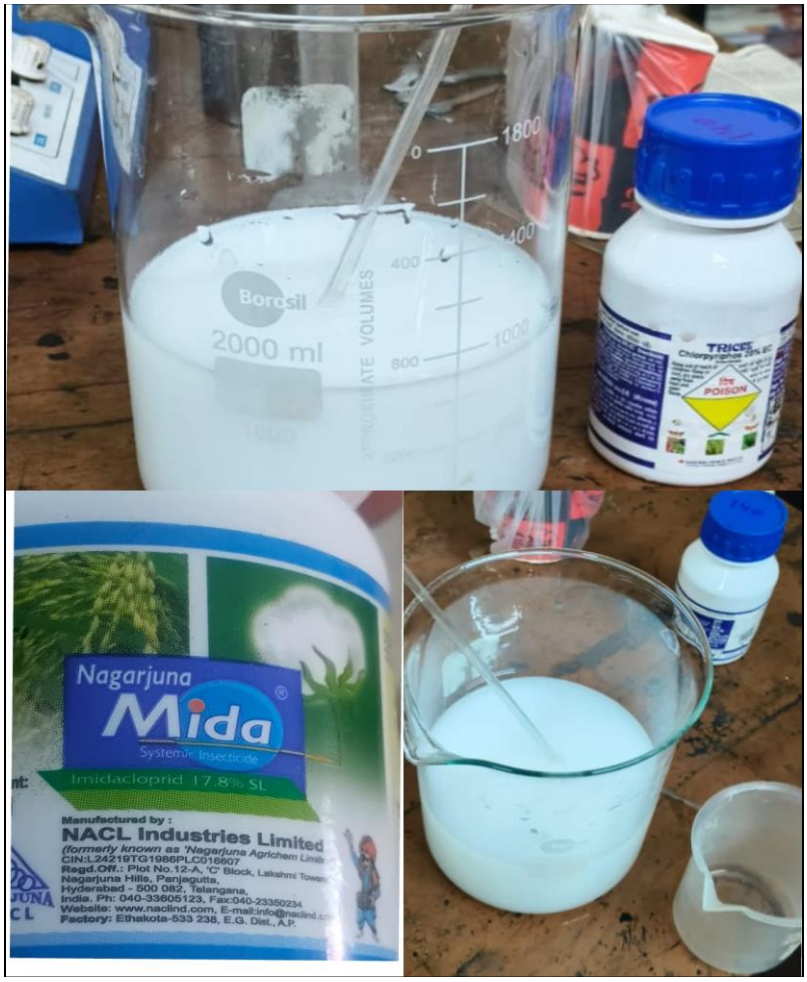

Fig8: Chlorpyrifos 20% EC Pesticide and Also Solution of Pesticides After Mixed With Water.

2.1.3Procedure for soil contamination

Procedure includes the following steps

2.1.3.1 Precontamination preparation

a) soil preparation

Gather clean, uncontaminated soil (ideally sandy loam), sieve it through a 2–3 mm mesh screen to get rid of rocks, roots, and debris, dry it at room temperature for a full day, and then let it cool to room temperature in a sealed container. Weigh the required soil mass on an analytical balance.

b) Calculate Pesticide Volume Required:

Used this formula to calculate the exact volume of 20% EC chlorpyrifos:

Volume of 20% EC (mL) = [Target concentration (mg/kg) × Soil mass (kg) × 1,000] / [200,000 mg/L]

Examples:

• For 50 mg/kg in 1 kg soil: (50 × 1) / 200 = 0.25mL ≈ 0.25mL

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As 20%EC is low concentration pesticide preferred 100mg/kg .so by using formula (100x1)/200=0.5ml per kg. add along with water that is for one kg 50ml
it means for one kg of soil mix 0.5ml of pesticide +50ml of distilled water.

c) Prepare Aqueous Pesticide Suspension:
Put on all protective gear, such as a lab coat, gloves, and safety glasses. Fill a sanitized 250 mL beaker with 50 mL of distilled water. Calculate the volume of 20% EC chlorpyrifos using a micropipette or syringe. To create a stable milky-white emulsion, add the chlorpyrifos dropwise to the water and stir constantly with a glass rod for two to three minutes. Because it is hydrophobic, the pesticide will not dissolve in water; instead, it will disperse into tiny droplets. The solution should be homogeneous, with no visible oil layer separating.

d)Mix with Soil
The weighed amount of pre-dried soil should be transferred to a clean, dry glass jar (500 mL or 1 L).Apply the pesticide emulsion to the soil. Close the jar lid firmly. Shake vigorously for three to four minutes, or until in two to three minutes. Visual inspection: Each soil particle should appear uniformly moist (slightly darker/damper than dry soil).

e) Drying
On fresh, absorbent paper or cloth, spread the damp, contaminated soil.
Place in a warm, well-ventilated area (an open lab bench, near a window, or in a 30-40°C oven if available).In 24 to 48 hours, water will naturally evaporate. Gently stir the soil every 12 hours to promote even drying.Within 24 to 48 hours, the soil should resume its dry, flowing texture. Transfer to a labeled glass jar for storage once it is completely dry (no moisture to the touch).

Table1: It shows drying time according to temperature

Method	Temperature	Drying Time
Room temperature (20-25°C)	20-25°C	36-48 hours
Sunny room (with breeze)	25-30°C	24-36 hours
Low oven (30-40°C)	30-40°C	12-18 hours

2.1.4 POST-CONTAMINATION HANDLING & VERIFICATION
Storage:
kept in steel gas jars with tight-fitting lids, 'CHLORPYRIFOS CONTAMINATED SOIL -[X] mg/kg', the preparation date, and the preparer's name should be clearly labeled.Store in a cool, dark, well-ventilated area at room temperature (18–25°C).Under typical storage conditions, chlorpyrifos remains stable in soil for four to twelve weeks.For optimal outcomes, use prepared contaminated soil within eight weeksSuhara et al 20.
2.1.5Quality control:
The soil should be uniform, free-flowing, and entirely dry.There is no obvious moisture or clumping.No smell (all of the water has evaporated).The color should be uniform (no wet patches or variations).
2.1.6 Tests for confirming pesticides
Researchers have taken a full day for drying soil .The first thing did after 24 hours was to check the soil's odour, which is somewhat headache-inducing and smells like a highly concentrated pesticide if smelled longer than two minutes.This was verified by an odour test.Then checked the pH now, and it is nearly 6.and the soil's color is darker than usual.and took pictures for each of these tests.The final test have been conducted to confirm pesticides was with chemicals such as copper sulfate (CuSO4) and sodium hydroxide (NaOH).Add 20 milliliters of acetone to 2 grams of pesticide-contaminated soil using a weighing machine.After 15 minutes of constant mixing, filter them through filter paper.Now add two drops of CuSO4 solution and two drops of NaOH solution to the two milliliters of solution in the test. Now, take a picture immediately after adding the solution, then wait 15 minutes before taking another picture for comparison.The presence of pesticides is indicated by the solution turning from blue to brown(Fig9).

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Fig9: Pesticides Conformation Test Results Before and after

2.1.7 Troubleshooting

Troubleshooting primarily entails identifying various issues

1) The soil becomes wet/clumpy even after 48 hours, which could be due to high humidity and poor ventilation. This can be solved by spreading out in a sunny, breezy spot or baking at 35 to 40 degrees Celsius for 6 to 12 hours.

2)Soil heterogeneity (uneven color/clumps), which could be due to insufficient mixing during application. This can be fixed by going back to a moist state, remixing for five minutes, and then letting it dry once more.

3)Emulsion separates (oil layer visible); this could be due to insufficient stirring or mixing with soil for too long; the solution is to discard and remake the suspension; it must be used within 30 minutes of preparation.

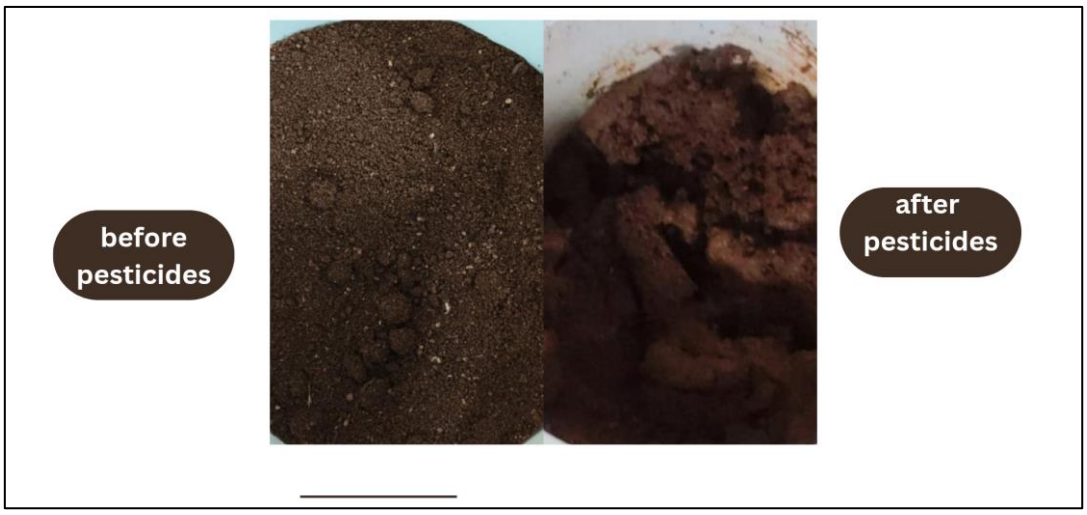

Fig 10: Soil color and texture before and after adding pesticides

2 .1.8 DATA RECORDING & DOCUMENTATION

We have kept a thorough lab notebook with the information below.

Date and time of contamination, Chlorpyrifos 20% EC source: supplier, lot number, batch number, soil source, sterilisation method (oven or air-dry), drying date, soil mass used (in grams), target contamination concentration (mg/kg), calculated volume of 20% EC used (in mL), volume of distilled water used (in mL), emulsion quality (appearance, homogeneity), soil mixing duration and appearance, drying conditions (temperature, location, duration), physical inspection results, and storage jar label.

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2.1.9 AQUEOUS PESTICIDE WASTE:

Any leftover pesticide-water suspension should be collected in a labelled, sealed container and disposed of through the institution's hazardous waste disposal program. Do not pour the mixture down the washbasin or into the drain as this could contaminate the environment.

2.2Bioremediation of pesticides contaminated soil using bacterial species

2.2.1 Culturing of bacterial species

Then carried out numerous methodical procedures for subculturing bacteria onto agar plates from a laboratory stock culture(Dhakal et al., 2025). Agar plates that have been preheated to 37°C are the materials and tools Then Sterile cotton swabs, sterile glass container or plastic spreaders, a marker pen or labelling tape, a 10% sodium hypochlorite bleach solution for waste decontamination, and an inoculation loop made of metal nichrome or disposable sterile plastic (Suhara et al.,2011,Struthers et al.,1998). Biological Safety Cabinet (BSC), Bunsen burner or biosafety cabinet flame source (if open-flame method used), incubator (set to species-appropriate temperature, usually 37 °C for human pathogens), vortex mixer, microwave or autoclave (media sterilisation) and refrigerator/freezer (stock culture storage: 80 °C glycerol stocks).

2.2.2 MEDIA PREPARATION AND STOCK CULTURE

These are the requirements used to prepare media in our laboratory.Take 200ml of distilled water, along with LB broth and agar. both LB broth and agar because LB agar was not available in the lab. For 200ml of distilled water, use a weighing machine to weigh 5g of LB broth and 2g of Agar.Then, autoclave for 15-20 minutes at 121 °C, 15 psi (liquid cycle). Use the autoclave indicator tape to confirm(Baczynski et al.,2010)(Fig13).

Allow the medium to cool in a water bath to 45-50 °C before pouring to avoid thermal injury to the additives and excessive condensation.Fill each 90 mm Petri dish in the BSC with about 20–25 mL.Swirl gently to remove bubbles. Let it solidify at room temperature for half an hour.Invert plates and store in sealed bags at 4°for up to 4 weeks. Label with medium type and date of preparation.Take the cryovial from the -80°C freezer. Verify the label's passage number, strain designation, and organism identity.Move to the BSC or work area on dry ice.Before inserting the vial cap into the BSC, flame or UV-sterilize its exterior.Gently scrape the top layer of the frozen glycerol stock with a sterile loop.Inoculate directly onto a new agar plate.Within two minutes of opening, return frozen stock to -80 °C.Here, strains like *Bacillus coagulans, Bacillus licheniformis*, and *Bacillus subtilis* have been employed.

Three days later, the cultures had spread widely.and kept records and documentation prior to transferring it into the soil.The inoculation date and time,Passage number, strain designation, and organism name,Inoculum source (stock number, location, last subcultured date),expiration date, lot number, and medium type,Conditions of incubation (temperature, duration, atmosphere)The operator and supervisor's names(Fig12).

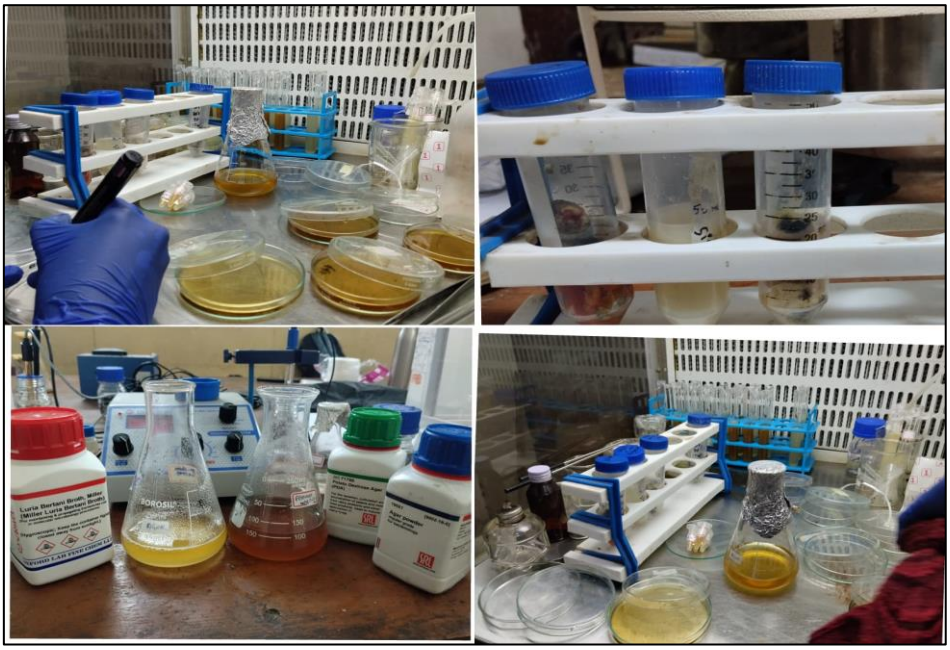

Fig11: Sample, Media and laboratory environment

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2.2.3 Transfer of bacteria to soil

Researchers have transferred bacterial species using the saline water method Then added 20 millilitres of saline water to 500 grams of soil after transferring three to four colonies of each bacterial species.Then choose various ratios of bacteria to soil based on combinations.Take seven distinct soil containers.B1-B7(B-bacteria),(B1-*bacilluscoagulans*),(B2-*b.licheniformis*),(B3-*B.subtilis*),(B4-b.c+b.l), to (B5-b.l+b.s), (B6-b.s+b.c),(B7-b.c+b.l+b.s).Equal amounts of particular strains are present in each container.Once a certain quantity of bacteria has been added, thoroughly mix the soil with a spatula and cover the container with a cloth or container plate. Take a picture and document the observations, including colour, moisture, and scent(Laura et al.,2013).Keep a certain distance between the containers and place them in a specific location to prevent contamination.In parallel with this, Then created containers filled with plant, animal, and fungal extracts.The next step is then thoroughly described.

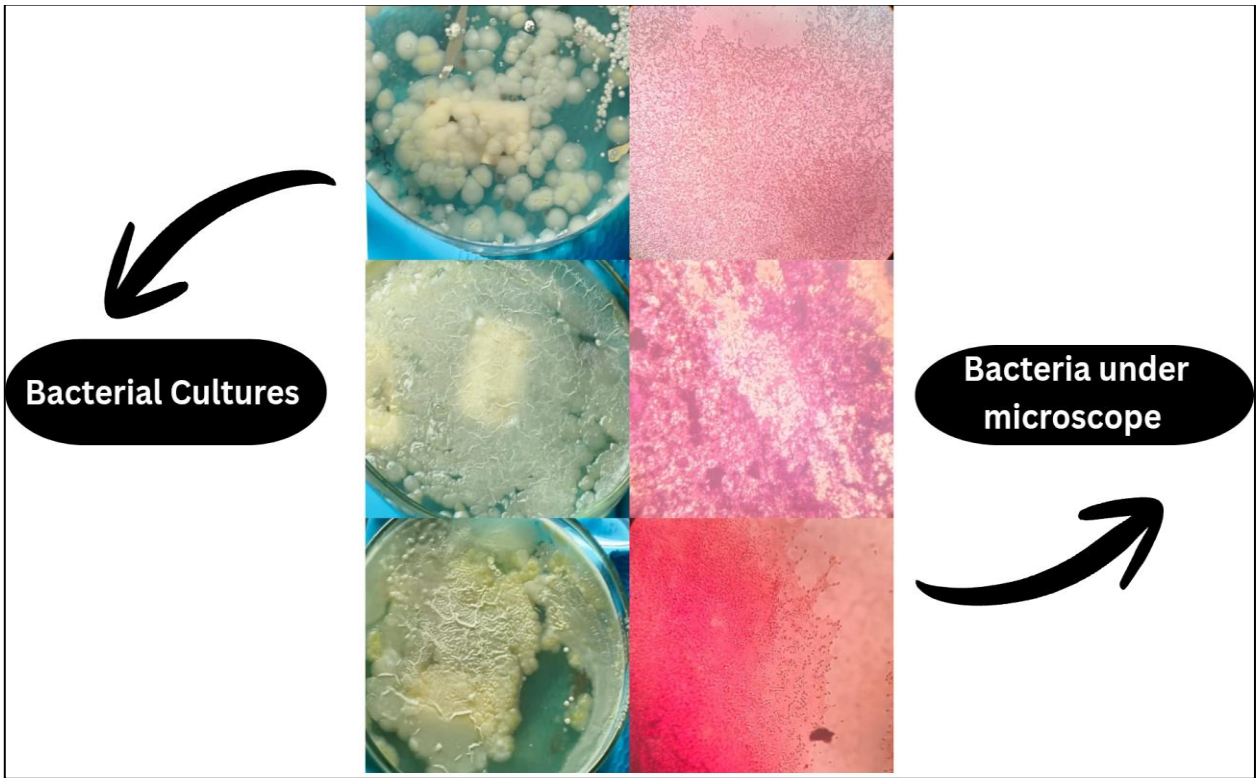

Fig12: Bacterial species cultures and bacterial strains under microscope.

2.3. Bioremediation of pesticides contaminated soil using Fungal species

2.3.1 Culturing fungi species

In this research researchers used spoiled bread, tomatoes, and yeast and put them in a container before cultivating fungi. Utilising commercially available Potato Dextrose Agar (PDA) powder to cultivate fungi in a lab environment(Mohapatra et al., 2022). Due to its nutrient-rich composition of potato extract and dextrose, PDA is the most popular general-purpose fungal culture medium that promotes the growth of a wide variety of fungi(Vaksma et al., 2023).

2.3.2 Preparation of Commercial PDA Medium

2.3.2.1 Weigh and dissolve the powder

Using an analytical balance, precisely weigh 39 g of commercial PDA powder.

Transfer to a 2-liter Erlenmeyer flask with about 800 millilitres of distilled water in it.Swirl by hand or gently stir over low-medium heat on a magnetic stir plate until the powder is totally dissolved and the mixture turns clearamber.Now Adjust volume:Top up the flask to exactly 1 L with distilled water. Mixwell(Fig13).

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Fig13: LB media and PDA media

2.3.2.2 Check and adjust pH

The target pH is 5.6 0.2. Most commercial PDA powders are pre-buffered and should fall within this range. If necessary, adjust with 1N HCl to lower pH or 1N NaOH to raise pH. Check after each addition.

2.3.2.3 Sterilization by Autoclaving

Use foil, a cotton plug, or a vented cap to cover the flask loosely. Do not seal tightly.

Put the flask inside the autoclave.Next, autoclave for 15 to 20 minutes at 121 °C and 15 psi (liquid cycle). Use the autoclave indicator tape to confirm.Allow the flask to cool in the autoclave to 80°C before transferring it to a 50°55°C water bath or monitoring it by hand until it is hot but comfortable to hold. This is the optimal pouring temperature.

2.3.2.4 Aseptic Plate Pouring

After using 70% ethanol to clean the laminar flow hood's surface, let it air dry for fifteen minutes. Use an ethanol spray and let the fumes settle if you're using a still-air box.Place sterile, unopened Petri dishes in stacks of four to five in the hood.

Don't completely remove the Petri dish lid; only lift it just enough to pour. In a single, smooth motion, pour 20–25 mL of hot molten PDA into each 90 mm plate.Gently swirl each plate to distribute the agar evenly around the edges.To let surface condensation escape, leave lids slightly open for three to five minutes before closing them.Allow plates to solidify undisturbed on a level surface for 15-20 minutes.

2.3.2.5 Plate Inspection and Storage

After it has solidified, check each plate for:Agar surface uniformity (no cracks, bubbles, or uneven edges)contamination indicators (cloudiness, particulates)

To stop condensation from dripping onto the agar surface, flip every plate (agar side up).Store inverted at 4°C in sterile bags with labels(Tripathi et al.,2013).

2.3.2.6 Inoculation of Fungal Specimens.

To reduce condensation, take the necessary plates out of the 4°C refrigerator and let them come to room temperature for half an hour before inoculation.

Put specific names on the bottom of each plate.

Now inoculate petri dishes with particular spoilt tomatoes, bread, and yeast.

After three days of cultivation, obtained some colonies, which under a microscope revealed to be *Botrytis cinerea, Alternaria alternate, Saccharomyces cerevisiae, Rhizopus tolonifera, and Penicillium chrysogenum*(Fig14).

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Fig14: Fungi Samples and cultures.

2.3.2.7 Transfer of fungi to soil

We have used the same saline method with some modifications according to low salt conditions.and we have transferred to soil. And again taken 7 different containers like F1-F7. In which (F1- Tomato cultures),(F2-Bread cultures),(F3-yeast),(F4- comb of Tomato+bread),(F5- bread+ yeast),(F6- Yeast+Tomato),(F7- Bread, Tomato,yeast)with equal concentration of same amount. Then closed the lid and kept it in a safe place and continued further animal and plant extracts so we could do further steps all at a time.Here tomato contains -*Botrytis cinerea,alternaria alternate*.bread-*penicillium chrysogenum*.Yeast-*saccharomyces cerevisiae*.*Rhizopus stolonifer*(Fig15).

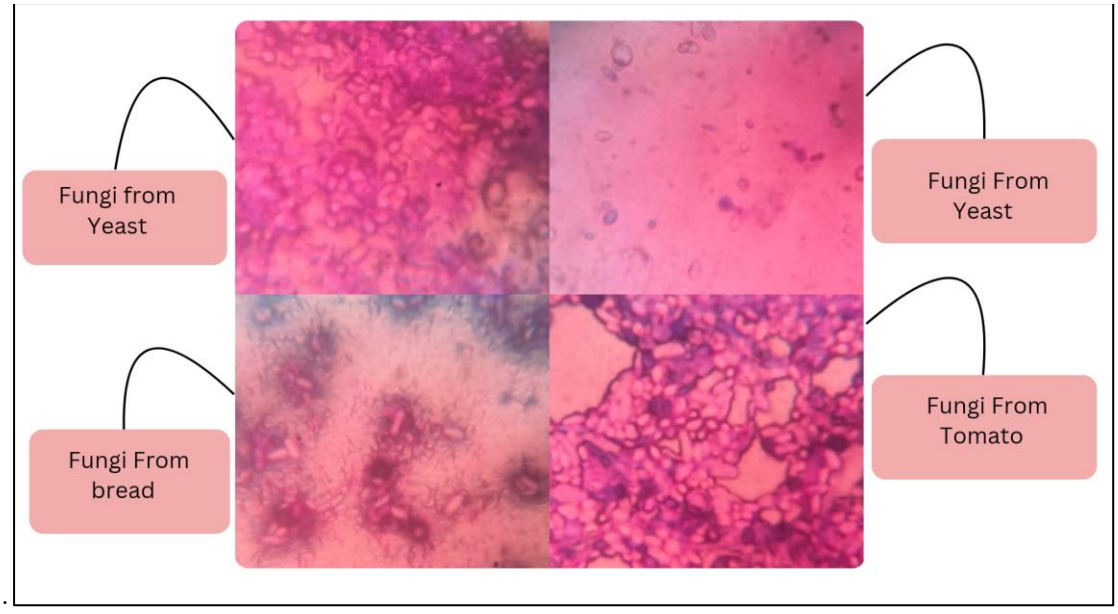

Fig 15: Fungi under microscope

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2.4 Bioremediation of soil using plant extracts

Using plant extracts from soap nuts, fenugreek, and moringa seeds, evaluate the bioremediation potential of pesticide-contaminated soil and determine which extract or combination is most effective in breaking down pesticide residues within four weeks.

The idea behind bioremediation of pesticide-contaminated soil using plant extracts is that pesticides are broken down into hazardous molecules by enzymes and helpful bacteria. Among the plant extracts are: All three plants have peroxidases and laccases, which are enzymes that break the molecular bonds of pesticides. Polyphenol Oxidase (Moringa): An effective enzyme for breaking down organic pollutants and Antimicrobial Compounds (Soap Nuts): Encourage the growth of helpful soil microorganisms that break down pesticides Proteins called phytochelatins (found in fenugreek) bind and absorb pesticide residues. Soap nuts, or saponins, help make insecticides more bioavailable for breakdown**.**

2.4.1 PREPARATION OF PLANT EXTRACTS

The soap nuts are involved in step A. Preparing the extract involves bringing the soap nuts from the market, chopping them into little pieces, soaking them in 350 milli liters of distilled water for 2.5 hours at room temperature, and stirring them every 30 minutes to maximize saponin extraction. Filter through filter paper into a clean beaker after 2.5 hours. Gather transparent liquid (yellow indicates the presence of saponins)(Betancur-Corredor, et al., 2015). The next step in the preparation of B-fenugreek seed extract involves gathering 70 grams of fenugreek seeds from the local market, soaking them in 350 milli liters of distilled water for more than 45 minutes, turning them into a paste with a mortar and pestle, letting them settle for 30 minutes, filtering the paste with filter paper, and collecting a clear, yellowish-brown liquid (Fig16).

Step C- Moringa extract preparation- First, collect drum sticks from the local vegetable market and separate the seeds from the drum sticks. On the first day, the seeds are sun dried and hot air woven for 1-2 minutes. The seeds are then turned into fine powder using a mortar-pestle and sieve it for fine powder. The powder is weighed and mixed with 350 ml distilled water and let it settle for 30 minutes. Collect the extract after carefully filtering the liquid with filter paper.
In the final step, three combinations of extracts of different concentrations are prepared: fenugreek extract + moringa extract, soap nut extract + fenugreek extract, Soap nuts +moringa, and fenugreek + moringa extract + soap nut extract.

2.4.2 EXTRACT APPLICATION TO SOIL

The used soil that had been maintained for 24 hours with insecticide which is mixed thoroughly before addition of samples. Add soap nut extract, moringa extract, and fenugreek extract to pots 1, 2, and 3. The next three pots will contain a combination of two extracts (soap nuts + moringa, fenugreek extract + moringa extract, soap nut extract + fenugreek extract) and a combination of three extracts of calculated amounts of each pot contaminating cleaned and pesticide-contaminated soil(Chia et al., 2024). For two to three minutes, combine the extracts with the soil. Place all pots at room temperature (20–25 degrees Celsius) in a well-ventilated area without firmly sealing the pots or containers. Put a label on each and note the pH, date, time, and initial smell(Bartucca et al., 2022).

The sample's initial pH values are 7 for soap nuts, 6 for fenugreek, 6 for moringa seeds, 7 for the combination of p1+p2, 6 for p2+p3, 6 for p2+p1, and 6 for the final combination of all three, i.e., p1+p2+p3. The insecticide is first seen to be potent.

A day before the bioremediation process, the soil is artificially polluted with 20% of the pesticide chlorpyrifos (CP) in laboratory-scale quantities. For example, seven pots of soil polluted by pesticides are removed. Each of the seven pots should contain 500 grams of soil. Clearly label the following:let here (P-Plant extracts) P1-Soap Nut Extract; P2-Fenugreek Extract; P3- Moringa Extract; P4- Soap Nuts + Moringa; P5- Fenugreek Extract + Moringa Extract; P6- Soap Nuts Extract + Fenugreek Extract; and P7- Fenugreek + Moringa Extract + Soap Nut Extract. The soap nut extract, fenugreek extract, and moringa seeds extract as p1, p2, and p3. The labels for their combinations are p1+p2, p2+p3, and p3+p1.According to the order, the final mixture of all blended extract samples is referred to as p1+p2+p3. Recording the initial pH using pH strips, visual colour observation, odour evaluation, and date-stamped photos of every pot.

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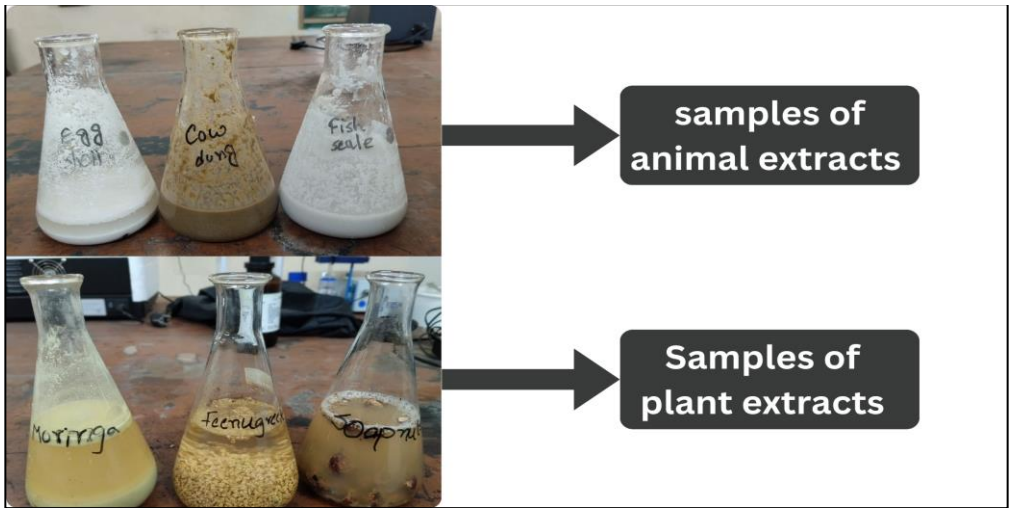

Fig 16: Samples of plants and animal extracts.

2.4.3 Bioremediation of pesticide contaminated soil by animal extracts

The animal waste extracts are used for the bioremediation of pesticide contaminated soil. The animal waste materials include the cow dung, fish scales, and egg shells. Cow dung was used as a microbial source, while fish scale powder served as a nutrient and carbon source, and eggshells helped maintain suitable soil pH and supplied nutrients.

2.5 PREPARATION OF ANIMAL EXTRACTS

Fresh cow dung was obtained from a nearby animal house, weighed, and mixed with distilled water(Randhawa et al.,2011). Fish scales were purchased from the market, thoroughly cleaned, and dried in the sun for a day before being heated to 80 to 110 degrees Celsius in a hot air oven. They were then ground into a powder using a mortar and pestle, sieved for fine powder, and stored. Egg shells were gathered from our university hostel kitchen, cleaned, washed, and risen before being heated for 35 minutes at 80 to 110 degrees Celsius . Then place the cleaned and dried egg shells in a bag, break them with a hammer, and then use a mortar-pestle, and sieve to turn them into fine powder, which we then keep in the appropriate glass container at room temperature in our lab. These three extracts are first combined with distilled water and filtered in a 1:2 ratio on the soil. The combinations are as follows: here(A=animal extracts), (A1-cow dung),(A2-egg shells),(A3-fishscales),(A4-CD+ES),(A5-ES+FS),(A6-FS+CD),(A7-CD+FS+ES).The supplies utilized include little bowls or cups for mixing, spoons for stirring, a strainer for filtering, aluminium foil for covering containers, newspaper or kraft paper, thermometer (or leave it out if the room temperature is steady) weighing scale (ideally with 0.1g accuracy) 500 mL beakers and small bowls or cups for mixing Glass rods, Petri plates, pH paper, strips, spatulas, and an incubator (or use a warm room corner).

2.5.1 APPLICATION OF EXTRACTS TO THE SOIL

Before adding the filtered animal extracts to the 24 hours the artificially pesticide-contaminated soil, which is divided into seven equal-sized pots of 500 grams each. All seven samples of animal waste extracts are added to these pots in the following order of calculated concentrations: A1, A2, A3, A1+A2, A2+A3, A3+A1, and finally A1+A2+A3. Then using a spatula to mix the soil before and after the addition of the extracts for two to three minutes, noting the date and label photos of each one.

3. RESULT AND DISCUSSION

After transferring all of the bacterial and fungal species, animal extracts, and plant extracts into containers have positioned two combos and one control container in addition to these. All bacterial and fungal species are equally concentrated in that one combination.Another combination consists of the same concentration of all plant and animal extracts.All of the containers were placed in a dark, room-temperature environment. Every two days, we came to the lab and sprayed a little water into each container, mixed it with a spatula, and took pictures of each classification and slightly placed cap. Then continued this until 30 days, and on the final day, have tested using pH strips and the CuSO4 test and tabulated the results in tabular form. We have chosen to calculate the percentage of degradation using the odour score provided below.

$\%\ \text{Degradation} = [(\text{Score}_0 - \text{Score}_t) / \text{Score}_0] \times 100$

here; Score_0 -Initial Score,

Score_t-Score At Time t.Based on the table below have calculated scores.

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Table2: Represents scores based on color and odour

Score	Color	odour	Texture
4	Dark stained	Strong pesticide smell	Oily, hard
3	Slightly darker	Strong	Slightly oily
2	Reddish-brown	Moderate	Crumbly, some residue
1	Light brown/tan	Weak	Mostly crumbly
0	Normal soil color	No smell	Fully crumbly, no residue

Note: This research will state that the degradation percentage may not be 100% accurate because we have calculated these based on smell and color . Therefore, even if we calculate the degradation percentage and get 100%, we have only mentioned 90% because this research fully considers other factors and does not rely solely on the degradation percentage to estimate the concentration of pesticides.

3.1 Results for the classification of bacteria in Pesticides degradation:

B5 (*B. licheniformis* + *B. subtilis*): approximately 90% degradation; no pesticide (verified). The best performer has the largest pH shift (6→12) and no undesired colour change, indicating a thorough and clean breakdown. Therefore, B5 is verified as the best container for pesticide degradation.Next, B1 *(B. coagulans*), B3 (*B. subtilis*), and B4 (B.c+B.l) are tied at about 75% degradation, with no pesticide present (confirmed). Strong, dependable degraders; B3 is marginally the "cleanest" of the three (no colour shift, no visible side byproducts).On paper, B7 (B.c+B.l+B.s combo) showed about 75% degradation, but the pesticide test yielded a "cannot determine" result. Although the numbers appear good, further testing is necessary before you can rely on this outcome.B6 (B.s+B.c) – ~75% degradation score, but the pesticide test indicates that it is present.A high degradation score shouldn't coexist with pesticide detection, which is contradictory. Instead of marking this as a true strong performer, mark it as an anomaly (possible test interference, false positive, or scoring error).B2 *(B. licheniformis*) has the lowest degradation rate at approximately 60%, with pesticide only partially removed ("semi"). The set's weakest degrader.

Table 3: Shows the Results for the classification of bacteria in Pesticides degradation

S.NO	BACTERIA	Initial pH	Final pH	color change	odour (pesticides smell)	Pesticides Test confirming whether pesticide present or absent (CuSO4+ NaOH)	Score	%of Degradation [(Score$_0$ − Score$_t$) / Score$_0$] × 100
1.	(B1)*B.Coagulans*	7	8	Yes(darker than usual)	low	Absent	1	~75%
2.	(B2)*B.licheniformis*	7	7	No change	low	Semi	0	~60%
3.	B3(*B.subtilis)*	7	9	No Change	low	Absent	1	~75%
4.	B4(B.c+B	7	8	Yes(little	low	Absent	1	~75%

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	.l)			darker)				
5.	B5(B.l+B.s)	6	12	No	low	Absent	0	~90%
6.	B6(B.s+B.c)	7	9	Yes	No	Present	1	~75%
7.	B7(B.c+B.s+B.l)	8	9	Yes	low	cannot determine	1	~75%

The graph given below shows Initial and final pH of bacterial samples for the majority of samples, the final pH tracks closely to the initial pH, typically remaining in a mildly alkaline range (6–9) with no discernible drift toward acidic or strongly alkaline conditions.The most significant pH shifts toward alkalinity are seen in samples 1 and 6, which should be noted if you're connecting this to a specific bacterial strain (e.g., if these correspond to eggshell-amended soils, since eggshell/CaCO3 amendments are known to push pH alkaline).
The fact that Sample 3 remains unchanged may indicate a well-buffered sample or minimal microbial/chemical activity affecting pH.
No samples became acidic (<7 remained mild, nothing below 6), indicating that the bacterial activity or amendments used are not producing significant acidic byproducts.

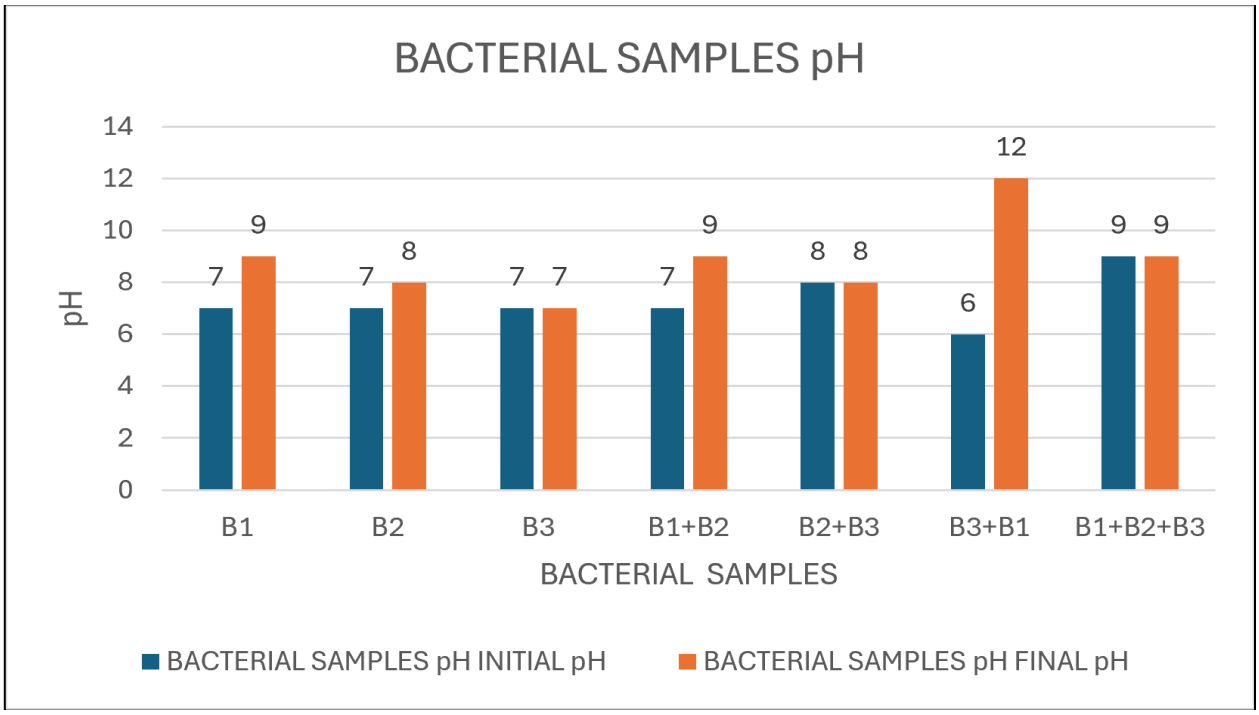

Fig 17: Represent Initial pH and final pH of bacterial samples

3.2 Results for the classification of Fungi in Pesticides degradation

F3 (yeast), F4 (T+B), F5 (B+Y), and F7 (T+B+Y) are tied at about 90% degradation, all pesticides are confirmed to be absent, and there is no odour or undesired colour change. These are your cleanest and most dependable degraders, with high breakdown, no pesticide residue, and no visible side effects.F6 (Y+T) - ~90% degradation on paper, but pesticide test confirms presence, with a lingering low odour. A top-tier score that differs from the confirmatory test is the same contradiction pattern as B6 in your bacterial table. Rather than blindly trusting, flag for retesting.F2 (Bread Mould) - ~86% degradation, pesticide-free (confirmed), with colour change and low odour. Solid performer, only slightly behind the top tier.F1 (Tomato) -~86% degradation, but pesticide test shows present, with dark colour change and detectable odour. The lowest-confidence results in the set have a high degradation number but multiple signs of pesticide residue or breakdown byproducts remain.

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Table4:Shows the Results for the classification of Fungal in Pesticides degradation

S.NO	FUNGI	Initial pH	Final pH	color change	Odour (Pesticides smell)	Pesticides Test confirming whether pesticide present or absent (CuSO4+ NaOH)	Score	%of Degradation [(Score$_0$ − Score$_t$) / Score$_0$] × 100
1.	F1Tomato	7	4	Yes (dark)	very low(1)	Present	1	~86%
2.	F2(Bread)	7	5	Yes	very low	Absent	1	~86%
3.	F3(yeast)	7	7	No	No	Absent	0	~90%
4.	F4(T+B)	7	4	No	No	Absent	0	~90%
5.	F5(B+Y)	6	8	No	No	Absent	0	~90%
6.	F6(Y+T)	6	9	No	low	Present	0	~90%
7.	F7(T+Y)	7	5	No	No	Absent	0	~90%

The graph given below shows Initial and final pH of fungal samples. In almost every sample, the final pH is at or above the initial pH, indicating a consistent alkaline shift across fungal and combination treatments that is more uniform than the bacterial sample chart. which indicates that the pH changes in treatment samples are probably caused by microbial or amendment activity rather than measurement drift or environmental factors.The strongest alkaline push is seen in F3+F1 and F1 alone, indicating that F3 and F1, either separately or together, may be more metabolically active or generate more alkaline byproducts than F2.The outlier is F1+F2, which is the only combination where pH decreased. This is intriguing because F1 by itself increased significantly (+2), but when combined with F2, it reverses. May show an antagonistic relationship between F1 and F2 cultures or a change in the production of more acidic metabolites when they are mixed together.

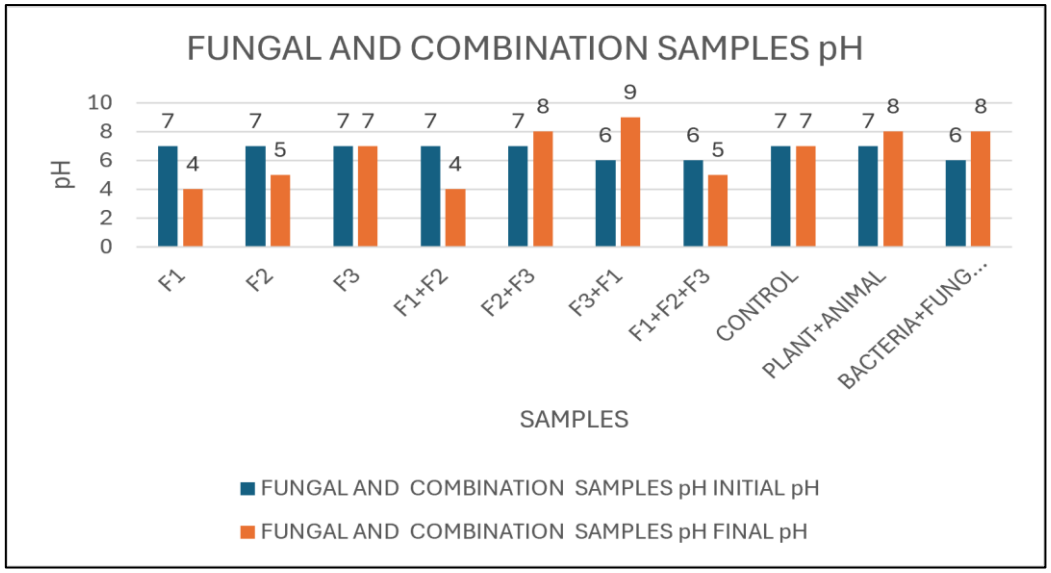

Fig18: Initial pH and final pH of Fungal samples

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3.3 Results for the plants extracts in Pesticides degradation:

P6 (Fenugreek + Soap Nuts): ~90% degradation, no colour change, low odour, and no pesticide presence (fully confirmed). With the highest degradation and a clear "absent" confirmation, this is your only clean top-tier result. The best performer in this set overall.P2 (Moringa seeds): There is about 90% degradation on paper, but the pesticide test indicates that it is present. There is also a strong moringa smell, which could be hiding or affecting the smell-based reading. The same red-flag pattern as in B6 and F1/F6-a high score contradicted by the confirmatory test. Re-verification is necessary because the smell of moringa may be interfering with the odour-based evaluation.P1 (soap nuts) and P3 (fenugreek) are tied at about 75% degradation, both of which have semi-complete pesticide removal. Consistent, moderate performers-real reduction confirmed but not entirely cleared.P5 (Moringa + Fenugreek) has a semi-degradable rate of approximately 75%. Similar to P1/P3, there is no discernible synergy benefit when these two are combined.P4 (Soap nuts + Moringa) and P7 (Fenugreek + Soap nuts + Moringa, partial data) have about 75% degradation but pesticide is still present. The weakest confirmed results were that the combinations did not outperform their individual components, and pesticide was still detectable.

Table 5:Shows the Results for the plant extracts in Pesticides degradation.

S.NO	Plant extracts	Initial pH	Final pH	Color change	Odour(Pesticides)	Pesticides Test confirming whether pesticide present or absent (CuSO4+ NaOH)	score	%of Degradation [(Score$_0$ − Score$_t$) / Score$_0$] × 100
1.	P1(Soap nuts)	7	9	Yes	No	Semi	1	~75%
2.	P2(moringa seeds)	6	8	No	yes(moringa smell)(0)	Present	0	~90%
3.	P3(Fenugreek)	6	6	Yes	No	Semi	1	~75%
4.	P4(S.n+M.s)	6	9	Yes	No(0)	Present	1	~75%
5.	P5(M.s+F.g)	6	8	Yes	No(0)	Semi	1	~75%
6.	P6(F.g+S.n)	7	8	No	low	Absent	0	~90%
7.	P7(F.g+S.n+M.s)	6	7	Yes	No(0)	Present	1	~75%

The graph given below shows Initial and final pH of plant extracts.All plant extracts and combinations exhibit a consistent, uniform alkaline shift, with final pH ≥ initial pH in every sample. Out of the three charts, this one has the

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cleanest trend.The only sample with zero pH change is fenugreek alone, indicating that it has little buffering or alkalising activity on its own (consistent with its saponin/enzyme profile being weaker than soap nuts).
Soap nuts seem to be the main cause of alkaline shift; they cause a significant shift on their own (+2) and the largest shift of all when combined with moringa (+3), indicating that soap nuts and moringa seeds in particular have a synergistic effect.
Compare Moringa Seeds alone (+2) vs. Fenugreek+Moringa (+1) and Soap Nuts alone (+2) vs. Soap Nuts+Fenugreek (+1) to see how adding fenugreek tends to lessen the effect. This indicates that fenugreek is diluting rather than enhancing the other extracts, which is consistent with its poor standalone performance.When you combine this with your fungal and bacterial data, the two most notable synergistic pairs are soap nuts + moringa (+3) and F3+F1 (+3). If you're creating a "most effective combination" section in the article, you should flag both of them together.

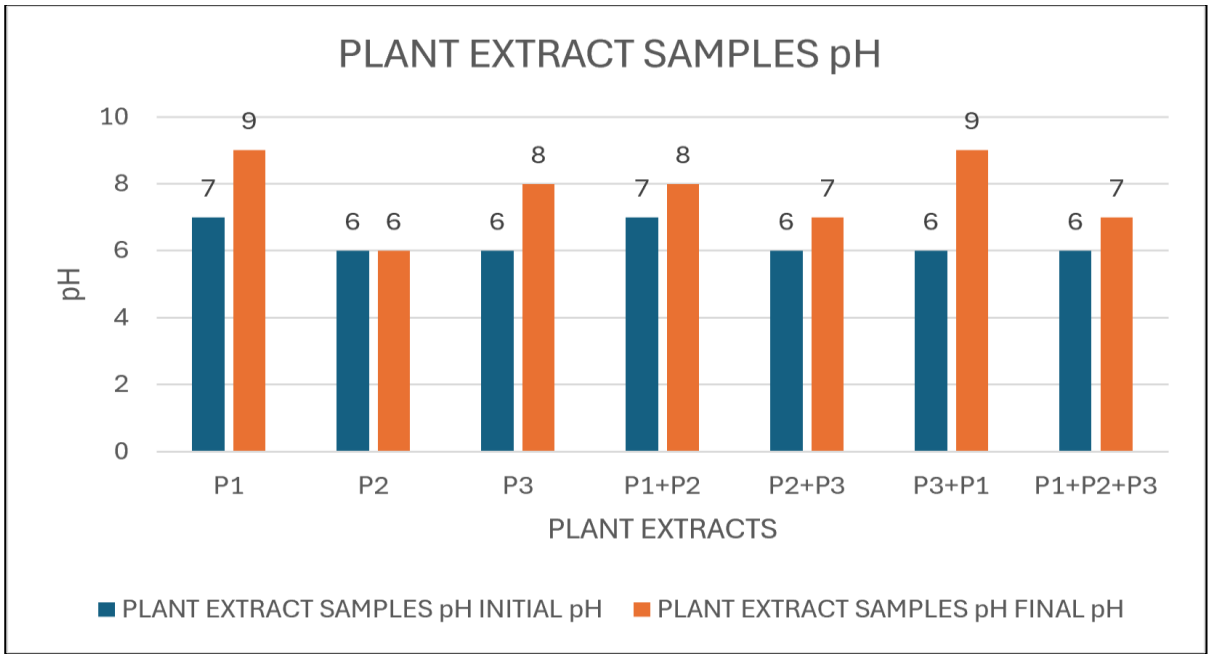

Fig19: Initial pH and final pH of plant extracts samples

3.4 Results for the animal extracts in Pesticides degradation:
A1 (cow dung) and A2 (egg shells) are tied at about 90% degradation, and there is
no pesticide present (confirmed). These are your cleanest top performers, with high degradation and a genuine confirmed-absence result. A1 does smell like dung, which is to be expected given the source, but that has no bearing on the pesticide confirmation(Olawale et al.,2020) .A7 (Cow dung + Egg shells + Fish scales) - ~75% degradation and pesticide-free (confirmed). This triple combination doesn't demonstrate synergy because its percentage is lower than that of A1/A2 alone; rather, it is a step down from the two individual components.On paper, A3 (fish scales), A5 (egg shells + fish scales), and Combo (B+F) show about 90% degradation, but all three indicate the presence of pesticides. This is the same pattern of contradiction that appears in your other tables: a high score that isn't supported by the test. All of these flagged results seem to have fish scales as a common theme, which merits further investigation (it may interfere with the CuSO4+NaOH reaction, e.g., via residual calcium/protein content).Fish scales plus cow dung (A6): ~86% degradation, pesticide present, partial score (0.5). Unconfirmed and in the middle.

A4 (Cow dung + egg shells): pesticide present, about 75% degradation. less deterioration and unverified.Control: about 75% degradation when pesticide is present and there is a noticeable pesticide odour. This is your reference/baseline; if it still exhibits significant "degradation" by score, it should be checked again because a true control should exhibit minimal breakdown; if it scores similarly to actual treatments, it might be a sign that the scoring parameter (color/odour) isn't entirely specific to microbial/enzymatic degradation.

Plant + Animal Combo (P+A): ~50% degradation, pesticide present. This is your most internally consistent, if unimpressive, data point. It's the weakest result in the entire table, and the low score and "present" confirmation actually agree with each other.

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Table 6:Shows the Results for the animal extracts in Pesticides degradation.

S.NO	ANIMAL	Initial pH	Final pH	color change	odour (Pesticides smell)	Pesticides Test confirming whether pesticide present or absent (CuSO4+ NaOH)	Score	%of Degradation [($Score_0 - Score_t$) / $Score_0$] × 100
1.	A1(Cow dung)	7	9	Yes	Dung smell	Absent	1	~90%
2.	A2(Egg shells)	7	8	No	No	Absent	0	~90%
3.	A3(Fish scales)	8	8	No	No	Present	0	~90%
4.	A4(C.d+E.s)	7	6	Yes	No	Present	1	~75%
5.	A5(E.s+F.s)	8	7	No	No	Present	0	~90%
6.	A6(F.s+C.d)	6	9	Yes	No	Present	0.5	~86%
7.	A7(C.d+E.s+F.s)	9	8	Yes	yes(dung smell)	Absent	1	~75%
8.	Control	6	6	No	Pesticides smell	Present	1	~75%
9.	Combo(B+F)	7	8	No	No	Present	0	~90%
10.	Combo(P+A)	6	8	Yes	yes(dung smell)	Present	2	~50%

The graph given below shows Initial and final pH of animal samples. The triple combo and the majority of single-extract samples (A1, A2) either remain stable or shift toward alkalinity.

When two-way combinations (A1+A2, A2+A3) are combined, the pH tends to decrease, indicating some acidifying interaction that may result from microbial/enzymatic activity or buffering effects between extracts.

The outlier is A3+A1, which exhibits a significant increase from 6 to 9, the opposite pattern of the other two-way combinations. Since it deviates from the pattern, it is worthwhile to double-check that data point or the circumstances for that specific run.

The only sample with zero net change is A3 alone, indicating that it is comparatively pH-stable by itself.

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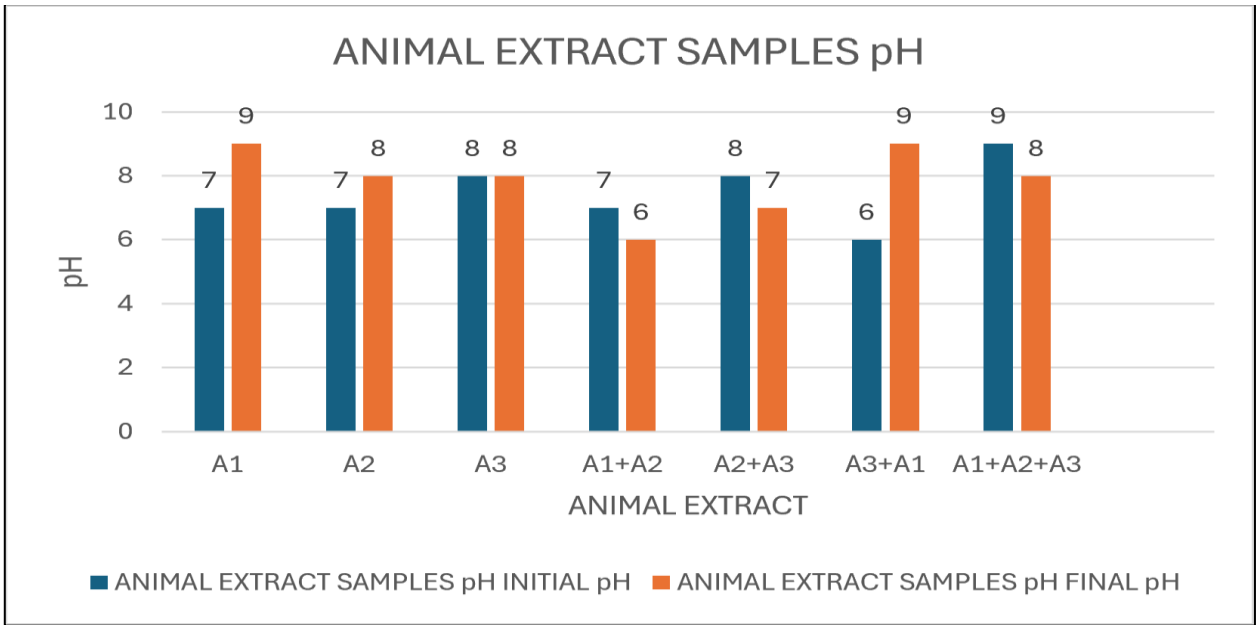

Fig20: Initial pH and final pH of animal extracts samples.

The diagram below demonstrates All of the samples are included in the picture of containers with pesticides added on the first and last days of the experiment.

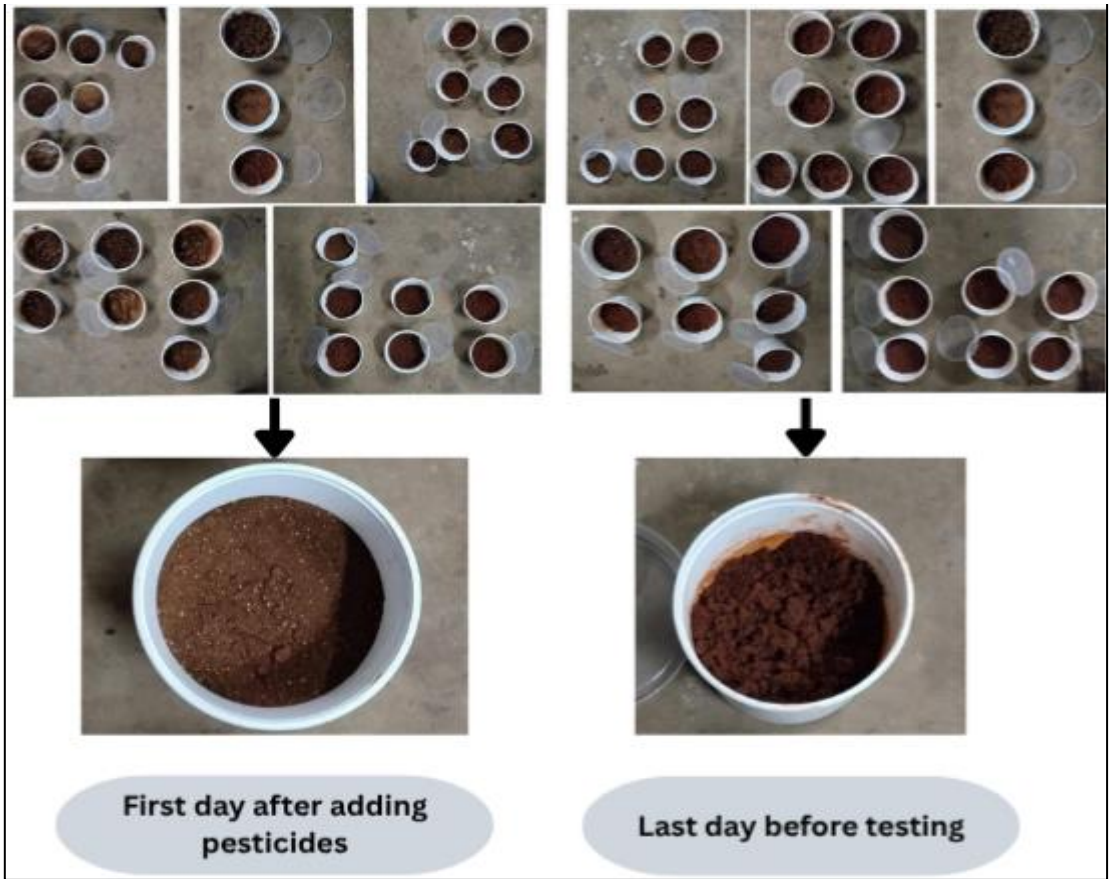

Fig15: Containers Difference in first day and last day after adding pesticides.

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CONCLUSION

This study demonstrated that bioremediation with biological agents such as bacteria, fungi, plant extracts, and animal-derived components is an effective, low-cost, and environmentally sustainable method for treating chlorpyrifos pesticide-contaminated soil.The best performing treatments in all four classifications achieved approximately 90% pesticide degradation with confirmed absence of residual pesticide: *B. licheniformis* + *B. subtilis* (B5) among bacterial strains, yeast-based combinations (F3, F4, F5, F7) among fungal strains, fenugreek + soap nuts (P6) among plant extracts, and cow dung and eggshells (A1, A2) among animal-derived extracts. With individual strains and some extracts outperforming their combinations, the results verified that all four classifications are viable bioremediation agents, suggesting that greater complexity does not always equate to better degradation.

Samples B6, F1, F6, and P2 had high apparent degradation scores based on colour and odour, but confirmatory chemical testing revealed residual pesticide.

To the best of our knowledge, this is the first study to look at intra-classification combinations as well as bacterial, fungal, plant, and animal-based bioremediation of pesticide-contaminated soil within a single comparative framework. The findings support the viability of using readily available, low-cost agricultural and household waste materials, such as eggshells, fish scales, and cow dung, as long-term alternatives to chemical remediation methods, particularly in resource-constrained settings like undergraduate research labs in India.

REFERENCES

1. Aljabri, M. (2025). Recent advances in pesticide bioremediation: integrating microbial, phytoremediation, and biotechnological strategies - a comprehensive review. Environmental Pollutants and Bioavailability, 37(1). https://doi.org/10.1080/26395940.2025.2554173
2. Amani, F., Safari Sinegani, A. A., Ebrahimi, F. and Nazarian, S. (2018). Biodegradation of Chlorpyrifos and Diazinon Organophosphates by Two Bacteria Isolated from Contaminated Agricultural Soils. Journal of Microbial Biology, 7(28), 27-39. doi: 10.22108/bjm.2017.105946.1079.
3. Baczynski, T. P., Pleissner, D., & Grotenhuis, T. (2010). Anaerobic biodegradation of organochlorine pesticides in contaminated soil - significance of temperature and availability. Chemosphere, 78(1), 22–28. https://doi.org/10.1016/j.chemosphere.2009.09.058
4. Bartucca, M. L., Cerri, M., Del Buono, D., & Forni, C. (2022). Use of Biostimulants as a New Approach for the Improvement of Phytoremediation Performance-A Review. Plants (Basel, Switzerland), 11(15), 1946. https://doi.org/10.3390/plants11151946
5. Betancur-Corredor, B., Pino, N. J., Cardona, S., & Peñuela, G. A. (2015). Evaluation of biostimulation and Tween 80 addition for the bioremediation of long-term DDT-contaminated soil. Journal of environmental sciences (China), 28, 101–109. https://doi.org/10.1016/j.jes.2014.06.044.
6. Chia, X. K., Hadibarata, T., Kristanti, R. A., Jusoh, M. N. H., Tan, I. S., & Foo, H. C. Y. (2024). The function of microbial enzymes in breaking down soil contaminated with pesticides: a review. Bioprocess and biosystems engineering, 47(5), 597–620. https://doi.org/10.1007/s00449-024-02978-6.
7. Dhakal, G., Thapa Magar, S., & Fujino, T. (2025). Pesticide Degradation by Soil Bacteria: Mechanisms, Bioremediation Strategies, and Implications for Sustainable Agriculture. Environments, 12(12), 492. https://doi.org/10.3390/environments12120492.
8. Farhan, M., Ahmad, M., Kanwal, A., Butt, Z. A., Khan, Q. F., Raza, S. A., ... & Wahid, A. (2021). Biodegradation of chlorpyrifos using isolates from contaminated agricultural soil, its kinetic studies. Scientific reports, 11(1), 10320.
9. Laura, M., Sanchez-Salinas, E., Dantn Gonzalez, E., & Luisa, M. (2013). Pesticide Biodegradation: Mechanisms, Genetics and Strategies to Enhance the Process. In Biodegradation - Life of Science. InTech. https://doi.org/10.5772/56098
10. Malik, D. K., Singh, V., Agnihotri, R., & Rathi, M. (2024). Bioremediation of chlorpyrifos-contaminated soil by exopolysaccharide, surfactant and biofilm synthesising plant growth promoting rhizobacteria. Biosci Biotechnol Res Asia, 21(4), 1409-1414.
11. Matúš, P., Littera, P., Farkas, B., & Urík, M. (2023). Review on Performance of Aspergillus and Penicillium Species in Biodegradation of Organochlorine and Organophosphorus Pesticides. Microorganisms, 11(6), 1485. https://doi.org/10.3390/microorganisms11061485
12. Mohapatra, D., Rath, S. K., & Mohapatra, P. K. (2022). Soil Fungi for Bioremediation of Pesticide Toxicants: A Perspective. Geomicrobiology Journal, 39(3–5), 352–372. https://doi.org/10.1080/01490451.2021.2019855.

DOI: 10.17148/IARJSET.2026.13910

13. Olawale, O., Obayomi, K. S., Dahunsi, S. O., & Folarin, O. (2020). Bioremediation of artificially contaminated soil with petroleum using animal waste: cow and poultry dung. Cogent Engineering, 7(1). https://doi.org/10.1080/23311916.2020.1721409.
14. Perumbakkam, S., Hess, T. F., & Crawford, R. L. (2006). A bioremediation approach using natural transformation in pure-culture and mixed-population biofilms. Biodegradation, 17(6), 545–557. https://doi.org/10.1007/s10532-005-9025-7
15. Raffa, C. M., & Chiampo, F. (2021). Bioremediation of Agricultural Soils Polluted with Pesticides: A Review. Bioengineering, 8(7), 92. https://doi.org/10.3390/bioengineering8070092.
16. Randhawa, G. K., & Kullar, J. S. (2011). Bioremediation of pharmaceuticals, pesticides, and petrochemicals with gomeya/cow dung. ISRN pharmacology, 2011, 362459. https://doi.org/10.5402/2011/362459
17. Randika, J. L. P. C., Bandara, P. K. G. S. S., Soysa, H. S. M., Ruwandeepika, H. A. D., & Gunatilake, S. K. (2022). Bioremediation of pesticide-contaminated soil: a review on the indispensable role of soil bacteria. Journal of Agricultural Sciences–Sri Lanka, 17(1).
18. Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., ... & Thukral, A. K. (2019). Worldwide pesticide usage and its impacts on ecosystems. SN applied sciences, 1(11), 1446.
19. Struthers, J. K., Jayachandran, K., & Moorman, T. B. (1998). Biodegradation of atrazine by Agrobacterium radiobacter J14a and use of this strain in bioremediation of contaminated soil. Applied and environmental microbiology, 64(9), 3368–3375. https://doi.org/10.1128/AEM.64.9.3368-3375.1998
20. Suhara, H., Adachi, A., Kamei, I., & Maekawa, N. (2011). Degradation of chlorinated pesticide DDT by litter-decomposing basidiomycetes. Biodegradation, 22(6), 1075–1086. https://doi.org/10.1007/s10532-011-9464-2
21. Tripathi, P., Singh, P. C., Mishra, A., Chauhan, P. S., Dwivedi, S., Bais, R. T., & Tripathi, R. D. (2013). Trichoderma: a potential bioremediator for environmental clean up. Clean Technologies and Environmental Policy, 15(4), 541-550.
22. Vaksmaa, A., Guerrero-Cruz, S., Ghosh, P., Zeghal, E., Hernando-Morales, V., & Niemann, H. (2023). Role of fungi in bioremediation of emerging pollutants. Frontiers in Marine Science, 10, 1070905.
23. Wang, X., Piao, X., Chen, J., Hu, J., Xu, F., & Tao, S. (2006). Organochlorine pesticides in soil profiles from Tianjin, China. Chemosphere, 64(9), 1514–1520. https://doi.org/10.1016/j.chemosphere.2005.12.052

