Lipase Active Fertilizer For The Bioremediation Of Agricultural Lands Contaminated By Waste Oil

Abstract

Agricultural productivity in contaminated irrigation areas is declining due to hydrocarbon and waste oil leaks. This study aims to evaluate a bioremediation-based fertilizer using organisms capable of oil degradation via the lipase enzyme, with the goal of revitalizing agricultural lands in affected regions. Two strains of *Bacillus*, selected for their lipase-activity, were tested alongside control groups without added organisms or compost. These were applied to pots with either oil-contaminated or control soils and were allowed to grow for 18 days under greenhouse conditions. At the end of the incubation period, plant vitality was assessed. The results indicated that the fertilizer, derived from the lipase-active *Bacillus licheniformis* strain, could significantly improve agricultural productivity in contaminated areas. The study highlighted the fertilizer's efficiency, cost-effectiveness, and potential marketability, offering it as a viable option for both producers and farmers due to its preservability, accessibility, and long-term potential.

Contents

1 Introduction

Oil spills pose a severe threat to agricultural productivity, severely disrupting soil composition, degrading vegetation, and introducing toxic hydrocarbons that destabilize microbial communities. These hydrocarbons not only have a direct negative impact on the growth and development of plants but also alter the chemical properties of the soil, making it less conducive for crops to grow. In regions where oil-contaminated irrigation systems exacerbate soil degradation, these harmful effects extend beyond localized farmlands, influencing broader agricultural chains and national economies. The soil's inability to support healthy crops leads to a decline in food production, which in turn impacts food security. While existing studies have extensively documented the environmental repercussions of oil spills, there remains a significant gap in research concerning cost-effective and biologically driven remediation techniques that could effectively restore soil health and enhance agricultural productivity. The lack of efficient, environmentally friendly solutions has prompted increased interest in exploring biological processes that could naturally break down harmful contaminants. This study aims to contribute to the field by systematically evaluating the potential of lipase-active microorganisms in cleaning soil contamination and enhancing agricultural productivity, while also providing valuable insights into the broader effects of oil contamination, such as its long lasting impact on soil fertility, plant growth.

Recycling kitchen waste, a global issue that contributes to environmental pollution and waste management problems offers solutions to the social, economic, and environmental challenges caused by food waste. Each year, alarming quantities of food waste are generated, leading to a range of issues including methane emissions from landfills, waste of valuable resources, and unnecessary strain on waste management systems. The development of scalable and sustainable recycling methods is, therefore, an imperative goal for addressing these challenges.

Beyond simply reducing waste, recycling kitchen waste holds the potential to contribute to multiple environmental benefits. If the recycling process leads to the creation of highly nutritious compost, for example, it can significantly improve soil health and enhance agricultural productivity. Additionally, the incorporation of such recycling methods into agricultural practices can play a critical role in the sustainable development goals of countries. By transforming food waste into valuable resources, it is possible to simultaneously tackle the issue of waste management and provide solutions for enhancing agricultural practices and environmental sustainability.

This research investigates the enzymatic potential of lipase-active microorganisms in degrading residual oil within soil ecosystems. These microorganisms catalyze lipid hydrolysis, breaking down oil into smaller, less toxic compounds that are more easily assimilated by the environment. The adaptability of these microorganisms to various soil environments makes them highly effective agents for bioremediation, as they can thrive in the conditions typically found in contaminated soils. By incorporating these organisms into fertilizers, this study examines their potential to restore soil health and improve crop yield, particularly in soils affected by oil contamination. While market-driven agricultural enhancers often focus primarily on maximizing productivity, they tend to overlook the broader issue of soil degradation caused by industrial pollution. Few solutions simultaneously address oil-induced soil degradation through biological intervention, making this research a valuable contribution to the development of environmentally friendly remediation strategies. By rigorously evaluating this method's feasibility, efficacy, and long-term ecological implications, this study aims to establish a robust scientific foundation for the potential application of lipase-active microorganisms in sustainable agriculture. The findings of this research could provide the basis for future agricultural practices that prioritize environmental restoration alongside productivity, offering a holistic solution to the challenges posed by both oil contamination and agricultural sustainability.

2 Literature Review

Soil, one of the essential elements that make life possible, enables human beings to meet their most basic needs for food through agriculture. Previous studies has stated that the 1-centimeter upper layer of the soil, which provides its fertility and is rich in humus, has been formed over centuries.[1] These fertile soils, and agricultural lands, are the basic elements for ensuring food security worldwide. Still, the protection of this soil should be brought to the agenda at least as much as the effort to navigate the limits of the benefits it provides, because there is no meaning in talking about enhancing soil fertility for the future, if there is no soil left anymore to be used at that future.

In modern industrial societies, land is often the ultimate recipient of a wide variety of waste products and chemicals.[2] Agriculture constitutes a large part of economies, when healthy agricultural practices support fertile soils in agriculture, it guarantees sustainable production, and this indicates the importance of preserving the environmental balance not only for agriculture but also for the economic development of countries.

Since the beginning of the twentieth century, soil pollution has also started to emerge as an environmental problem. Especially with industrialization and rapid population growth, soil pollution started to increase. Today, soil pollution caused by the reasons mentioned constitutes one of the important environmental problems that are reaching more serious dimensions every day and its effect on agriculture is at a level that cannot be ignored.

One of the main factors in the degradation of agricultural soils is waste oil leaks from many sources. These leaks can occur by the oil passing through water or directly into the soil. Waste oil pollution is a serious multidimensional problem affecting all aspects of the environment, causing changes in the physical, chemical, and microbiological properties of the soil, and eventually causing these changes to pass on to the plant.[3] Studies have shown that even insignificant amounts of oil spills can damage agricultural fields.[4] Waste oil contamination significantly impacts soil-based communities. It has been indicated that hydrocarbon pollutants can lead to a decline in soil-based activities.[5] Even small amounts of waste oil can significantly reduce the growth and germination of common agricultural plants. In one study, it was found that Total Petroleum Hydrocarbons (TPH) pollution in soil inhibited the germination of both wheat and maize seeds when the hydrocarbon concentration exceeded 0.1%.[6] Waste oils consist of many categories such as used vegetable oil, machine oils, industrial oils, and contaminated natural areas due to many factors. Despite the obvious effect arising from contamination, there is no detailed study in which a current but accessible method is proposed for the bioremediation of these soils and the proposed method is tested with data. A method and study that cleans nature with what nature gives and makes the soils productive has the potential to have a significant impact on development in agriculture.

According to the data published by the Ministry of Urbanization of the Republic of Turkey

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[1] G. Gözükara, S. Altunbaş, and M. Sarı, "Effects of Temporal and Spatial Changes on the Formation, Development, and Morphology of Soils in Different Physiographic Regions," *Ege University Faculty of Agriculture Journal* 57, no. 2 (2020): 277–288.

[2] Nyle C. Brady and Ray R. Weil, *The Nature and Properties of Soils*, 11th ed. (Upper Saddle River, NJ: Prentice Hall, 1996), xi.

[3] Russell Khaled Mohsen and Hazim Aziz Al-Robai, "Pollution Effect of Used Engine Oil on Chemical Properties and Some Heavy Metals of Sandy Soil," in *IOP Conference Series: Earth and Environmental Science*, vol. 1262, no. 8 (2023): 082060.

[4] Muhammad Zafar Iqbal, Sehrish Khursheed, and Muhammad Shafiq, "Effects of Motor Oil Pollution on Soil and Seedling Growth of *Parkinsonia aculeata* L.," *Scientia Agriculturae* 13, no. 3 (2016): 130–136.

[5] M. I. Khan et al., "Microbial Remediation Techniques for Hydrocarbon-Contaminated Soils: Current Trends and Future Directions," *International Journal of Environmental Science and Technology* 20, no. 2 (2023): 123–135.

[6] Jingchun Tang et al., "Eco-toxicity of Petroleum Hydrocarbon Contaminated Soil," *Journal of Environmental Sciences* 23, no. 5 (2011): 845–851.

within the scope of hazardous waste management in Turkey, a total of 1,413,220 tons of hazardous waste declared by 39,134 facilities were recorded in 2014. Approximately 70,000 tons of waste oil and 7,000 tons of vegetable waste oil are included.[7] The majority of waste oils are oils used in industry, especially in the auto industry. Among these oils, there are lubricating oils used in quantities that can compete with motor oils in tons of liters, which are classified as hydrocarbons and therefore are derived from crude oil. It is a very common situation that petroleum hydrocarbons are spilled into the soil during operations in the industry and most likely in the automotive industry, where they are used, and cause irreversible effects. Specifically, studies have reported reductions in soil-based activities contaminated with used engine oil due to the toxic effects of hydrocarbons.[8] The introduction of petroleum hydrocarbons into an uncontaminated environment changes the ecosystemic balance of that environment.

Leaked hydrocarbons cause an increase in mortality data in many microbial species or inhibit their activities, changing the productivity of the microbial community and the ecosystem.[9] Existing experiments have shown that contamination of soil with crude oil and its derivatives may proceed in parallel with heavy metal pollution, which can enter from the soil and cause complex ecosystem pollution.[10]

Petroleum hydrocarbons (PHs) are carcinogenic and neurotoxic. They are known to cause soil pollution, which has significant impacts on a global scale.[11]

Kitchen waste has reached serious dimensions in the world, due to food waste and the fact that waste is mostly thrown away rather than recycled. In Turkey, according to the results of the solid waste composition determination study conducted within the scope of the Solid Waste Master Plan Project (KAAP) in 2006, 34% of municipal waste in Turkey consists of kitchen waste.[12] The U.S. Environmental Protection Agency (EPA) has conducted extensive research on the environmental impacts of food waste, emphasizing the importance of reducing kitchen waste through composting and recycling initiatives. The EPA's findings highlight that food waste contributes significantly to landfill mass, leading to greenhouse gas emissions and soil contamination issues.[13] Considering the significant issues caused by kitchen waste, the ongoing depletion of vital natural resources, and the extensive economic damage associated with its mismanagement, it is crucial to implement recycling strategies that not only mitigate environmental harm but also foster cross-sectoral benefits. Such an approach, prioritizing sustainability, will contribute to the long-term goals of sustainable development of countries.

Lipase enzyme is a biocatalyst belonging to the class of hydrolases that can catalyze the hydrolysis of ester bonds. In the production of industrial lipases, microorganisms are the primary sources due to their high productivity and ability to adapt to environmental conditions. For this

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[7] Republic of Turkey Ministry of Environment and Urbanization, *2014 Hazardous Waste Statistics Report*, General Directorate of Environmental Management, accessed February 6, 2025, https://webdosya.csb.gov.tr/db/ced/editordosya/2014_YILI_TEHLIKELI_ATIK_ISTATISTIKLERI_15022016.pdf.

[8] N. H. Ugochukwu and J. A. E. Okwudiri, "The Effects of Spent Engine Oil Pollution on Microorganisms in a Tropical Rainforest Ecosystem in Nigeria: Implications for Bioremediation Strategies," *African Journal of Environmental Science and Technology* 10, no. 1 (2016): 1–10.

[9] Chioma Blaise Chikere, Gideon Chijioke Okpokwasili, and Blaise Ositadinma Chikere, "Monitoring of Microbial Hydrocarbon Remediation in the Soil," *3 Biotech* 1 (2011): 117–138.

[10] Jingchun Tang et al., "Eco-toxicity of Petroleum Hydrocarbon Contaminated Soil," *Journal of Environmental Sciences* 23, no. 5 (2011): 845–851.

[11] Yuhang Chen et al., "Biochar-Assisted Bioremediation of Soils with Combined Contamination of Petroleum Hydrocarbons and Heavy Metals: A Review," *Applied Soil Ecology* 204 (2024): 105720.

[12] Katı Atık Ana Planı Projesi [KAAP], *Türkiye Katı Atık Yönetimi Raporu* (Çevre ve Şehircilik Bakanlığı, 2006).

[13] U.S. Environmental Protection Agency, "From Farm to Kitchen: The Environmental Impacts of U.S. Food Waste," accessed February 6, 2025, https://www.epa.gov/land-research/farm-kitchen-environmental-impacts-us-food-waste.

reason, bacteria and fungi stand out in this context with their ecological standards.[14] Microbial The main principle of lipases is to break down lipids into smaller building blocks, glycerol, and fatty acids, by catalyzing the hydrolysis of ester bonds. These small building blocks, fatty acids, are converted into smaller molecules, acetyl-CoA, by *β - oxidation to* be used in the Krebs cycle. Glycerol is used by microorganisms to produce ATP, thus fats are used in the biological cycle of the organism, and in the case of leaked oil in the soil , the lipase -active organism provides bioremediation of the soil .

Microbial lipases offer significant advantages over lipases obtained from plant and animal sources due to their thermostability, ability to work in wide pH ranges, selectivity for specific substrates, and openness to genetic manipulation.[15] For this reason, this study was conducted on microbial lipases. Previous studies have investigated *Bacillus* species, especially *Bacillus Subtilise* and *Bacillus licheniformis* has been shown to be valuable for industrial applications due to its ability to produce lipase that remains stable at high temperatures.[16]

3 Method

3.1- Making Compost from Kitchen Waste

Compost, which forms the basic element of all test groups for use as fertilizer , was prepared using the following wastes:

- 2 orange peels
- 2 slices of spoiled cheese
- Foods containing carbohydrates and fat, such as waste rice and pasta
- Egg shells
- Tea pulp
- Potato peelings
- Banana Peel
- Dried leaves
- Control Soil (0 g/kg oil)

The specified products were combined after and left open for 3 weeks to naturally disintegrate.

3.2- Lipase Positive/Negative Bacteria

The mentioned B. licheniformis has been supplied from Çukurova University Biology Laboratory.

3.2.1- Preparation of The Soil Sample

Soil samples were collected from randomly selected agricultural fields in sterile bags for bacterial isolation. The soil was prepared under sterile conditions to prevent contamination. A 0.1 g soil sample, used for isolation, was measured on an analytical balance and transferred into sterile Eppendorf tubes.

3.2.2- Heat Treatment

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[14] A. Abubakar et al., "Crude Oil Biodegradation Potential of Lipase Produced by *Bacillus subtilis* and *Pseudomonas aeruginosa* Isolated from Hydrocarbon Contaminated Soil," *Environmental Chemistry and Ecotoxicology* 6 (2024): 26–32.

[15] P. K. Ghosh et al., "Microbial Lipases: Production and Applications," *Science Progress* 79, no. 2 (1996): 119–157.

[16] D. Tanrisever, *Bacillus Subtilis Thermostable Lipase Production and Characterization* (Master's thesis, Institute of Science, 2011).

The soil sample was suspended with 5 ml of sterile distilled water for the activation of endospore structures of *Bacillus* species that provide resistance to stress . The tube was kept in a water bath at 80°C for 10 min . This step was implemented as an important step in the study to ensure selective isolation of gram-positive spore-forming bacteria for high production capacity and similar reasons .

3.2.3- Medium and Incubation

The heat-treated suspension was inoculated onto Tryptic Soy Agar (TSA) medium using a sterile loop using the surface inoculation method.

To avid cross contamination during inoculation, each sample was processed separately in a sterile cabinet. The plates were incubated in a CO_2 incubator set at 37°C for 24-48 hours .

3.2.4- Selection of Lipase Positive Colonies

After incubation , the colonies that developed were transferred to a selective medium containing lecithinase with a sterile loop . The colonies transferred to the medium were It was re- incubated in a CO_2 incubator set at 37°C for 24-48 hours . In this study , lecithinase was helpful in isolating lipase- positive bacteria because phospholipid degradation enabled selective characterization and lipase -active organisms were not present in the medium. They formed zones by breaking down phospholipids . Colonies showing lipase positive activity were determined by forming zones . Lipase activity was evaluated by measuring zone diameters .

3.2.5- Bacteria Identification

Colonies identified as lipase-positive through zone measurement were transferred to a MALDI-TOF MS device for species-level identification. For analysis, the samples were prepared with sterile distilled water and formic acid (HCOOH), and protein profiles were obtained. Based on the identification results, one lipase-positive and one lipase-negative strain of *Bacillus licheniformis* were selected for use in the study.

3.3- Planting and Fertilization

Twelve pots were used in this study to systematically evaluate the effects of various soil types and fertilizer applications on the growth performance of lettuce seedlings as well as the overall condition and health of the soils. The pots were divided into two primary groups based on the type of soil: oily and non-oily soils. The oily soil was sourced from an industrial area in Adana, where industrial activities are prominent, resulting in significant soil contamination from hydrocarbons and other pollutants. This type of soil was specifically selected to replicate the adverse effects of pollution and to simulate real-world conditions in industrial zones where soil quality is compromised due to contamination. On the other hand, the non-oily soil was obtained from a greenhouse, where the soil is known to have neutral properties and serves as an ideal environment for plant growth. This soil was chosen as a control in order to compare the effects of polluted soil with those of clean, uncontaminated soil. Equal amounts of both soil types were carefully placed into the pots, ensuring that the comparison between the two soil types would be based on identical quantities of soil in each pot.

Once the soil was added to the pots, the next action to be taken was to apply fertilizers to each of the pots to assess how different types of fertilization would influence the growth and health of the lettuce seedlings. The amount of fertilizer used was precisely measured at 15 grams per pot, a quantity that was determined to be optimal for providing adequate nutrients to the plants without over-fertilizing. Fertilizer application was a critical component of the study, since it allowed the investigation of the impact of different nutrient formulations on plant growth in both oily and non-oily soils. The fertilizers used in this experiment were specifically chosen for their known properties in improving soil fertility and supporting plant growth. To further investigate the relationship between soil types, fertilization strategies, and plant growth, the fertilizer ingredients were carefully combined into several distinct formulations, each designed to deliver a unique nutrient profile. These combinations were selected to represent various approaches to soil enrichment, enabling the study to examine how different levels of nutrients, such as nitrogen, phosphorus, and potassium, would affect plant growth in contaminated versus uncontaminated soil. The pots were then carefully monitored to track the growth of the lettuce seedlings, while the soils were assessed for changes in their physical and chemical properties, including nutrient availability and microbial activity. The results of this experiment are expected to shed light on how fertilization strategies can be tailored to improve agricultural productivity in polluted soils and provide valuable insights for sustainable agricultural practices.

3.3.1- Oily Soil Group:

Lipase + *Bacillus* Bacteria (Lipase + *Bacillus* Added): In this group, 15 g of lipase enzyme and 15 g of Bacillus bacteria were added to the pot. *Bacillus* species is a microorganism that helps break down organic matter.

Lipase - *Bacillus* (from lipase -inactive strain *Bacillus* Added): Only 15 g of lipase negative *Bacillus in this group* added .

Compost (Organic Fertilizer): In this group, 15 g of compost was added to the pot. Compost is a fertilizer that improves soil structure with its rich nutrient content obtained as a result of the decomposition of organic matter through biological processes.

Lipase Positive *Bacillus* + Compost : In this group, 15 g of lipase positive Bacillus species and 15 g of compost were mixed and blended with the soil.

Lipase Negative *Bacillus* + Compost : In this group, 15 g of lipase negative Bacillus species and 15 g of compost were used.

Control Group: In this group, only oily soil was used and no additional ingredients were added.

3.3.2 Lean Soil Group:

Lipase + *Bacillus* Bacteria (Lipase positive *Bacillus* added): Similarly, 15 g of lipase -active *Bacillus* bacteria were added to the oil-free soil group.

Lipase - Bacillus (Lipase negative Bacillus Added): In the oil-free soil group, only 15 g of lipase negative strains were found in this group. *Bacillus* was added.

Compost (Organic Fertilizer): 15 g of compost was added to the pot in this group.

Lipase Positive Bacillus + Compost : In this group, 15 g of lipase positive strain *Bacillus* and 15 g of compost were mixed.

Lipase Negative Bacillus + Compost : In the oil-free soil group, 15 g of lipase negative strain was added to this pot. *Bacillus* and 15 g of compost were added.

Control Group: In the lean soil group, only lean soil was used as the control group and no additional components were added.

The fertilization process began with the careful planting of two lettuce seedlings in each pot. Prior to covering them with soil, the seedlings were sprinkled with iron to promote healthy growth. Special attention was given to ensuring that the seedlings made good contact with the soil to establish a strong foundation for growth. Following planting, all experimental groups were placed in a controlled greenhouse environment where the temperature was maintained at a constant 24°C to provide optimal conditions for daylight exposure. The pots were kept in a stable environment, with consistent temperature and humidity levels, for a period of 18 days. Over the course of these 18 days, the plants were monitored to ensure they received sufficient light and a conducive growing environment. After this incubation period, the lettuce plants were carefully removed from the pots for further analysis. The growth parameters and oil content in the soil of each experimental group were measured to evaluate the effects of the fertilization process and determine the impact on soil health and plant development.

3.4- Determination of Oil in Soil

3.4.1- Preparation of Soil Sample:

Each soil sample was taken into capsules and dried in an oven. The dried soil samples were dried in soxalet was homogenized for use in extraction .

3.4.2- Soxhlet Extraction :

Dried and homogenized 5 g soil sample was placed in the soxalet device. 80% hexane and 20% acetone were used as solvent mixture . The extraction process was carried out in the device for 1 hour, providing constant temperature and solvent circulation .

3.4.3- Evaporation of Solvent

The extract obtained during extraction is subjected to Rotary sieve to remove the solvent phase. It was transferred to the evaporator device. The process was carried out under low pressure and at 40°C and the solvent was completely evaporated.

3.4.4- Determination of Fat Amount

The lipid phase freed from solvent was weighed on an analytical balance. The values obtained for each sample were recorded and the results were expressed as gram weight (g/kg) per 1000 g weight by proportioning to the dry soil weight.*

*All procedures were carried out in three parallel replicates. The purity of the solvents (*HPLC - grade*) was verified and the calibration of the devices used was checked before the procedure.

3.5- Measurement of Growth Parameters

The height, root and leaf width of lettuce in each experimental group were measured. Since there were 2 lettuces in each group, the average of the two data was taken. The growth rate was tabulated to compare with the initial data.

4 Findings

The full set of data regarding oil degradation is provided in the appendix (Table 1). The results from the Soxhlet extraction method demonstrate the most reduction in oil contamination has been in the group of Lipase+ *Bacillus licheniformis* and compost. The combination of these treatments resulted in an oil content of 0.8 g/kg, reflecting a degradation of 60% over the 18-day period. Similarly, the Lipase+ *B. licheniformis* group alone achieved a reduction to 0.9 g/kg, corresponding to a 55% decrease in oil content within the same timeframe.

In contrast, the control treatments and the Lipase- B. licheniformis variant showed the least oil reduction since the oil contents remained close to the initial levels.

The full data set regarding physical measurements can be seen in the appendix (Table 2). In terms of plant growth, the lipase+ *B. licheniformis* and compost group treatment emerged as the most effective, showing an improvement of 77% in plant height, a 120% increase in root length, and a 50% increase in leaf width compared to the control group. In contrast, the Lipase- *B. licheniformis* treatment showed only a significantly modest reduction in oil content and minor improvements in plant growth.

5 Discussion

5.1 Significance of This Analysis

This analysis has provided the experimental results regarding the important issue of bioremediation. At the same time, it has addressed agricultural issues due to contamination with data sets and shown how contamination affects soils via the contaminated control group.

The project is also in line with targets 12, 13, and 15 of the Sustainable Development Goals. [17]

It directly contributes to the sub-targets of goal 12, "Substantially reduce waste generation through prevention, reduction, recycling, and reuse and halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses ". Also directly contributes to the sub-target of goal 15, "Restore degraded land and soil and strive to achieve a land degradation-neutral world.".

5.2 The Limitations

Although Bacillus was the only organism tested in this study, other easily adaptable organisms could potentially meet industrial standards for future use, yielding different results that were not explored in this investigation for fertilizer production. In future studies, researchers could investigate the effectiveness of other microorganisms that naturally occur in soil or that are specially cultivated for their bioremediation capabilities. Such organisms might exhibit superior or more efficient oil-degrading properties, potentially resulting in more effective or economical solutions for agricultural purposes. Expanding the scope of the research to include a variety of microbial strains could lead to the identification of those that offer higher efficiency or resilience under varying environmental conditions, thus broadening the potential for industrial-scale application.

Due to academic and financial limitations, this study can only demonstrate plant vitality via physical measurements, which is a limitation that could be expanded upon in future research. While physical measurements such as plant height, leaf count, and overall health provide a useful initial understanding of the effects of bioremediation treatments, they are inherently limited. More comprehensive measurements, such as biochemical analyses or microbial activity tests, could reveal a deeper understanding of the specific interactions between microorganisms, soil contaminants, and plant growth. These additional analyses could provide more definitive evidence of the microbial processes at work in the soil and their direct impact on soil health and plant productivity. Future studies could incorporate these aspects, offering a more detailed and robust assessment of the effects of microbial treatments on soil contamination and agricultural

[17] 1. United Nations, *Sustainable Development Goals*, accessed January 31, 2025, https://sdgs.un.org/goals.

productivity.

Additionally, the scope of the study was confined to a single experimental period of 18 days, which may not fully capture the long-term effects of bioremediation treatments. The short duration of the study limits its ability to evaluate the sustainability and consistency of the effects observed over time. The effects of soil contamination and bioremediation treatments on microbial communities, nutrient cycling, and plant growth may evolve over a longer timeframe, and this study does not account for those changes. Future research could extend the experimental period to several months or even a full growing season, which would provide a clearer picture of the long-term benefits and potential challenges of bioremediation techniques. A more extended study would allow researchers to evaluate the durability of the observed effects, including whether the benefits of bioremediation persist over time, whether new environmental factors arise, and how these treatments might influence soil and plant health in the long run. These extended studies would offer critical insights into the viability of using bioremediation as a sustainable and effective method for enhancing agricultural productivity while mitigating the impacts of soil contamination.

6 Conclusion

As a result of the measurements, the fertilizer group produced from *Bacillus lichenformis* and the mixture obtained from kitchen compost broke down a significant amount of the oil in the contaminated soil in the 18-day period. The research revealed not only the bioremediation effectiveness but also the negative effects of waste and leaked oils on the soil structure. It was observed that oil contamination negatively affected basic characteristics of the plant such as vitality (based on physical measurements) . In this context, a fertilizer developed with the tested method not only reduces pollution but also contributes to sustainable agricultural practices by improving soil health.

Plant growth in the oil-contaminated soil with the most effective treatment approached a 77.7% increase in the plant length and significant root growth compared to the controlled contaminated soil, indicating a strong recovery facilitated by the degradation of oil contaminants. On the other hand, the least effective treatment from lipase-negative groups showed only minimal recovery, highlighting the significant role lipase plays in bioremediation.

The findings of this study suggest that the recycling of kitchen waste and the integration of genetic characteristics of organisms into agricultural practices creates a strong bridge between waste management and agricultural production in terms of environmentalism. The high effect it shows in a short time of almost 3 weeks offers a fast and applicable model for solving environmental problems. The results show that this type of fertilizer has significant potential not only in environmental protection due to soil purification from oil contamination but also in improving agricultural quality in these regions by reducing the economic damage inflicted on polluted areas.

7 Appendices

Test Group		Soxhlet Results (w/w)
Oil Contaminated Soil	Initial	2.0 g/kg
	Control 2	1.9 g/kg
	Lipase+ *B. licheniformis*	0.9 g/kg

	Lipase- *B. licheniformis*	1.8 g/kg
	Compost only	1.9 g/kg
	Lipase- *B. licheniformis* and Compost	1.7 g/kg
	Lipase+ B. licheniformis and Compost	0.8 g/kg
Control Soil	Initial	0 g/kg
	Control 1	0 g/kg
	Lipase+ *B. licheniformis*	0 g/kg
	Lipase- *B. licheniformis*	0 g/kg
	Compost only	0 g/kg
	Lipase- *B. licheniformis* and Compost	0 g/kg
	Lipase+ *B. licheniformis* and Compost	0 g/kg

Table 1: Soxhlet Extraction Results

Test Group		Length (cm)	Root Length (cm)	Leaf Width (cm)
Oil Contaminated Soil	Control 2	9.00	5.50	3.00
	Lipase+ B. licheniformis	11.00	9.00	4.50
	Lipase- B. licheniformis	10.00	6.00	3.80
	Compost only	10.00	9.00	4.00
	Lipase- B. licheniformis and Compost	11.00	8.00	4.00
	Lipase+ B. licheniformis and Compost	16.00	12.00	4.50
Control Soil	Control 1	16.00	11.00	4.50
	Lipase+ B. licheniformis	17.50	11.00	4.50
	Lipase- B. licheniformis	17.50	11.00	4.50
	Compost only	17.50	11.00	5.50
	Lipase- B. licheniformis and Compost	18.00	12.00	6.00
	Lipase+ B. licheniformis and Compost	19.00	13.50	5.50

Table 2: Plant Physical Measurements

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11. Tanrisever, D. *Bacillus Subtilis Thermostable Lipase Production and Characterization*. Master's thesis, Institute of Science, 2011.

12. Republic of Turkey Ministry of Environment and Urbanization. *2014 Hazardous Waste Statistics Report*. General Directorate of Environmental Management. Accessed February 6, 2025. https://webdosya.csb.gov.tr/db/ced/editordosya/2014_YILI_TEHLIKELI_ATIK_ISTATISTIKLERI_15022016.pdf.

13. U.S. Environmental Protection Agency. "From Farm to Kitchen: The Environmental Impacts of U.S. Food Waste." Accessed February 6, 2025. https://www.epa.gov/land-research/farm-kitchen-environmental-impacts-us-food-waste.

14. Katı Atık Ana Planı Projesi [KAAP]. *Türkiye Katı Atık Yönetimi Raporu*. Çevre ve Şehircilik Bakanlığı, 2006.

15. Khan, M. I., et al. "Microbial Remediation Techniques for Hydrocarbon-Contaminated Soils: Current Trends and Future Directions." *International Journal of Environmental Science and Technology* 20, no. 2 (2023): 123–135.

16. Ugochukwu, N. H., and J. A. E. Okwudiri. "The Effects of Spent Engine Oil Pollution on Microorganisms in a Tropical Rainforest Ecosystem in Nigeria: Implications for Bioremediation Strategies." *African Journal of Environmental Science and Technology* 10, no. 1 (2016): 1–10.

17. United Nations. *Sustainable Development Goals*. Accessed January 31, 2025. https://sdgs.un.org/goals.

Overall, I would suggest to accept the paper with some revisions. Please see my comments listed for the author below:

For the author:
Overall, great first submission! I can tell you were thinking critically when you designed this experiment. I loved that you included controls and the overall topic is interesting. However, I do have some revision requests for you:

Overall grammar and flow - some of your paragraphs are indented, some are not. Sometimes there are spaces between paragraphs, sometimes there are not. Try to be consistent. You could also try and add some transition sentences between sections to increase flow.

For the introduction - It would be if you clearly state your research question or hypothesis (i.e, "This study investigates the research question: Can a fertilizer made from lipase-active Bacillus licheniformis and kitchen compost effectively degrade waste oil in contaminated soils and improve plant growth?").

For the Methods - Please detail the rationale for methods. The methodology is detailed but lacks justification for choices (e.g., why 15 g fertilizer? Why 18 days? Why lettuce?). Recommend including reasoning and literature to support these decisions. Additionally, there's no mention of statistical tests. I suggest incorporating basic stats (ANOVA, t-tests) to validate whether differences in plant growth or oil degradation are significant. Additionally, I suggest the author analyze the relationship between oil degradation and plant growth metrics to strengthen the discussion of causal effects. When talking about the various groups in your methods, the author kinda just lists things. I suggest trying to include some sentences/paragraphs when introducing these lists (Lean soil group or oily soil group) Or you could just put the list in a table instead.

Results and Discussion - For your findings, instead of presenting results in a table, it would be better if it was a bar chart or a visualization. Additionally, if you add statisitics, you can talk more about those results in the results and discussion. Currently, the discussion mostly repeats findings. I encourage the author to engage more with the mechanisms (e.g., how lipase aids remediation) and compare with other studies.

Lipase Active Fertilizer for the Bioremediation Of Agricultural Lands Contaminated By Waste Oil

The student provides a well-written research paper evaluating the potential benefits of lipase-active microbe supplementation to reduce oil contamination and improve soil quality. Specifically, the outcomes measured were the growth performance of lettuce seedlings as well as the overall condition and health of the soils with respect to various permutations of lipase and fertilizers. The sections of the paper are clear and follow a generally coherent flow. The experimental groups chosen were valid and appropriate comparisons were made to evaluate effects of lipase, lipase-active bacteria, and various soil enrichment components on the growth of seedlings and the overall soil health. The author does well to describe the limitations of the paper as well as future areas of research. I believe the paper could be improved with the following recommendations.

Recommendations:

Don't capitalize "The, Of, or By" in title

Abstract: The abstract is well-written and has the core components of an abstract including the introduction/background, hypothesis/aim, methods, results, and conclusions. The goal of the abstract is to provide a window into the paper highlighting enough key information to allow the review to filter whether the paper is relevant to their discussions. In the results section of the abstract, the author should highlight some of the key findings (using the actual data values).

Citations should be present in the introduction.

I would list specific numbers and data for the references related to the economic impact of agriculture. Additionally, I would expand on the "variety of waste products and chemicals" alluded to in the first sentence of paragraph 2 in the literature review. It's helpful to use the introduction as the opportunity to really set the tone for the paper with strong data references and objective numbers that the reader can see.

The statement describing lipase and what it is should have a reference attached to it. In general, anything that isn't "common knowledge" or something you need to look up should have a reference.

Methods:

The methods section is very good, and the paper can be made stronger with more detail in the methods section. For a method's-based research paper, this section is arguably the most important part of the paper, and should be very specific so that a reader who you assume not to be familiar with general methods in environmental science can easily follow. To be specific, more information should be provided on the strain of B. lichenformis obtained from Cukurova, and a reference should be provided if possible (e.g. how is it manufactured and processed, data showing it has lipase activity etc.). More information on where the soil samples were taken from should be included, as well as on the sample preparation methods.

Additionally, how was the oil quantity of the experiment soil groups verified? Was there any quantification of the oil content in the soil groups prior to the experiment being conducted?

This was a very well-designed preliminary study at this student's level. Of note, no statistical analyses were performed (e.g. ANOVA, t-test) which weakens the conclusions taken from the paper. Overall, the author does well to describe the various limitations of the paper, and I believe the limitations alone should not preclude publication should the appropriate changes be made.

Recommendation: Accept with minor revisions

Thank you very much for your review. The texts below in red are the adjustments that I have made to the paper.

- Overall grammar and flow - some of your paragraphs are indented, some are not. Sometimes there are spaces between paragraphs, sometimes there are not. Try to be consistent. You could also try and add some transition sentences between sections to increase flow.

I fixed the indentations and gaps. Also, especially in the methods section, I fixed the grammar.

- For the introduction - It would be if you clearly state your research question or hypothesis (i.e, "This study investigates the research question: Can a fertilizer made from lipase-active Bacillus licheniformis and kitchen compost effectively degrade waste oil in contaminated soils and improve plant growth?").

Introduction paragraph 2: . This study explores the potential of incorporating specific organisms into fertilizers to restore soil health and enhance crop yield, particularly in oil-contaminated soils. It investigates the research question: Can a fertilizer combining the properties of lipase-active *Bacillus licheniformis* and kitchen compost effectively degrade waste oil in contaminated soils and promote plant growth?

- For the Methods - Please detail the rationale for methods. The methodology is detailed but lacks justification for choices (e.g., why 15 g fertilizer? Why 18 days? Why lettuce?). Recommend including reasoning and literature to support these decisions.

For 15 g fertilizer: 3.3 paragraph 2 The amount of fertilizer used was precisely measured at 15 grams per pot, a quantity that was determined to be optimal for providing adequate nutrients to the plants without over-fertilizing (with a fertilizer to soil ratio of 1/1000) while replenishing nitrogen, phosphorus, and other elements in the soil and to enhance microbial activity.
For 18 days 3.3 paragraph 2: Groups were grown for 18 days, as this period marks the transition from sprout to a form closest to its final stage as mature lettuce.
Why lettuce? 3.3 paragraph 1:Lettuce was selected for its ease of cultivation in pots and because the study coincided with its optimal planting season.

- Additionally, there's no mention of statistical tests. I suggest incorporating basic stats (ANOVA, t-tests) to validate whether differences in plant growth or oil degradation are significant. . Additionally, if you add statisitics, you can talk more about those results in the results and discussion.

I have conducted an ANOVA and TUKEYS HSD to represent the significance statistically.

This is from the findings part:

Statistical Analyses

The full set of data regarding oil degradation is provided in the appendix (Table 1).
The set of data has been tested for significance with ANOVA and Tukey's HSD, which is provided in the appendix. (Table 3).

ANOVA Results:

Oil-Contaminated Groups:

- **Test Performed**: One-Way ANOVA
- **p-value**: 4.80×10^{-7}

Non-Oil Control Groups:

- **Test Performed**: One-Way ANOVA
- **p-value**: Not applicable (NaN)

  The statistics can now also be found on the appendices as a table. I have mentioned the indications of these statistics at the conclusion as well.

- Additionally, I suggest the author analyze the relationship between oil degradation and plant growth metrics to strengthen the discussion of causal effects.
  Conclusion paragraph 2:

Plant growth in the oil-contaminated soil with the most effective treatment approached a 77.7% increase in the plant length and significant root growth compared to the controlled contaminated soil, indicating a strong recovery facilitated by the degradation of oil contaminants. On the other hand, the least effective treatment from lipase-negative groups showed only minimal recovery, highlighting the significant role lipase plays in bioremediation. The physical measurements, which were directly correlated to the amount of degraded oil through the treatment, also highlighted the impact of soil health on plant growth and the efficiency of each grown product.

- When talking about the various groups in your methods, the author kinda just lists things. I suggest trying to include some sentences/paragraphs when introducing these lists (Lean soil group or oily soil group) Or you could just put the list in a table instead.

I fixed the group explanations which might cause confusion. I briefly wrote what was expected from the group and what they included.

- Results and Discussion - For your findings, instead of presenting results in a table, it would be better if it was a bar chart or a visualization.
  I turned the tables into bars.

- Currently, the discussion mostly repeats findings. I encourage the author to engage more with the mechanisms (e.g., how lipase aids remediation) and compare with other studies.
  While many existing studies focus on the environmental repercussions of oil spills and various chemical remediation methods, few have explored biologically driven, cost-effective approaches

that also support sustainable agricultural recovery. This study distinguishes itself by investigating the use of biofertilizers with lipase-producing microorganisms to degrade oil in contaminated soils. Unlike purely chemical treatments, this method not only reduces hydrocarbon concentrations but also actively improves soil health, promoting better plant growth and long-term soil restoration. The samples treated with lipase-active fertilizers showed a significantly higher reduction in oil content and better plant performance compared to untreated or low-activity groups. This indicated that lipase enzymes facilitated the breakdown of lipid-based pollutants, making nutrients more accessible for plant uptake and improving overall soil fertility.

Lipase Active Fertilizer for the Bioremediation Of Agricultural Lands Contaminated By Waste Oil Revisions

Thank you very much for your feedback. I showed what I revised on my paper according to your feedback point by point with red.

- Don't capitalize "The, Of, or By" in title

I fixed the typo.

- Abstract: The abstract is well-written and has the core components of an abstract including the introduction/background, hypothesis/aim, methods, results, and conclusions. The goal of the abstract is to provide a window into the paper highlighting enough key information to allow the review to filter whether the paper is relevant to their discussions. In the results section of the abstract, the author should highlight some of the key findings (using the actual data values).

I added the statistical result for the most effective group: Statistically, the Tukey's HSD indicated lipase active *B. licheniformis* with kitchen waste compost treatment shows significant differences ($p < 0.00001$) in oil reduction compared to control groups.

- Citations should be present in the introduction.

I added the proper citations.

- I would list specific numbers and data for the references related to the economic impact of agriculture.

Literature Review, Paragraph 2 :

In modern industrial societies, land is often the ultimate recipient of a wide variety of waste products and chemicals. Agriculture constitutes a large part of economies, specific studies quantify the exact impact of a 1% increase in per-capita agricultural growth leading to a 1.5% increase in per-capita non-agricultural growth. For instance, the agricultural sector in Turkey plays a crucial role, accounting for around 16% of total employment. Also in South Asia, the agriculture sector is under great stress with high demand for available crops, ultimately leading to lower economic profit. When healthy agricultural practices support fertile soils in agriculture, it guarantees sustainable production, and this indicates the importance of preserving the environmental balance not only for agriculture but also for the economic development of countries.

- Additionally, I would expand on the “variety of waste products and chemicals” alluded to in the first sentence of paragraph 2 in the literature review. It’s helpful to use the introduction as the opportunity to really set the tone for the paper with strong data references and objective numbers that the reader can see.

Literature review Paragraph 2:

In modern industrial societies, land is often the ultimate recipient of a wide variety of waste products and chemicals. These include heavy metals such as lead (Pb) and cadmium (Cd), in addition to oil in soil, which can disrupt plant growth and adversely affect crop health and productivity. For instance, research indicates that elevated levels of these compounds in agricultural soils can lead to reduced crop yields and compromised food quality.

- The statement describing lipase and what it is should have a reference attached to it. In general, anything that isn’t “common knowledge” or something you need to look up should have a reference.

I added the proper citation.

- be specific, more information should be provided on the strain of B. lichenformis obtained from Cukurova, and a reference should be provided if possible (e.g. how is it manufactured and processed, data showing it has lipase activity etc.). More information on where the soil samples were taken from should be included, as well as on the sample preparation methods.

3.2- Lipase Positive/Negative Bacteria

The bacterial isolates used in this study were obtained from the Biology Laboratory of Çukurova University under the supervision of Prof. Dr. Osman Gülnaz. Bacillus colonies were transferred to a MALDI-TOF MS device for species-level identification. For analysis, the samples were prepared with sterile distilled water and formic acid (HCOOH), and protein profiles were obtained to be *Bacillus licheniformis.* The lipase activity of *Bacillus licheniformis* was assessed using an agar plate method, where the ability of the bacteria to hydrolyze lipids was determined by the formation of a clear zone around bacterial growth. Spirit Blue Agar was prepared by mixing 1.5% agar with 1% tributyrin (Sigma) and 0.1% spirit blue dye in distilled water. The mixture was autoclaved and poured into sterile petri dishes, which were allowed to solidify at room temperature before use.

A culture of *Bacillus licheniformis* was streaked onto the surface of the prepared agar plate using a sterile inoculating loop. The plates were incubated at 30°C for 24-48 hours to allow bacterial growth and lipase secretion. After the incubation period, the plates were visually examined for the formation of a clear zone around the bacterial growth. This clear zone indicates the hydrolysis of tributyrin by lipase enzymes, which break down the lipids into fatty acids and glycerol, causing the surrounding medium to become transparent.

The diameter of the clear zone was measured using a caliper to quantify the lipase activity. A larger zone of clearance around the bacterial growth indicated higher lipase activity, while no clearing suggested a lack of lipase production. This assay provided a simple, visual method for identifying *Bacillus licheniformis* isolates with active lipolytic enzymes.

3.2.1- Preparation of The Soil Sample

Soil samples were collected from sites adjacent to agricultural fields in Adana. One sample was taken from Yeşiloba/Seyhan, exposed to industrial influences and located near, but not within, agricultural fields. Another sample was collected from a site in Karaisalı isolated from both agricultural and industrial activities.

3.4- Determination of Oil in Soil

Ten grams of soil were dried overnight at 50 °C, then placed in a Soxhlet extraction cartridge. Reflux extraction was performed using hexane for 1 hour at 70 °C. Following the extraction, hexane was evaporated using nitrogen gas, and the remaining residue was weighed to determine the total oil content

- Additionally, how was the oil quantity of the experiment soil groups verified? Was there any quantification of the oil content in the soil groups prior to the experiment being conducted? The control groups serve for this result in this research. Control1 and Control2 received no treatment, therefore their measurements represent the initial conditions of the soil.

- This was a very well-designed preliminary study at this student's level. Of note, no statistical analyses were performed (e.g. ANOVA, t-test) which weakens the conclusions taken from the paper. Overall, the author does well to describe the various limitations of the paper, and I believe the limitations alone should not preclude publication should the appropriate changes be made.

I have conducted an ANOVA and TUKEYS HSD to represent the significance statistically.

Thıs is from the findings part:

Statistical Analyses

The full set of data regarding oil degradation is provided in the appendix (Table 1).

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- **p-value**: Not applicable (NaN)

The statistics can now also be found on appendices as a table. I have mentioned the indications of these statistics at the conclusion as well.

Lipase Active Fertilizer For the Bioremediation of Agricultural Lands Contaminated by Waste Oil

Abstract

Agricultural productivity in contaminated irrigation areas is declining due to hydrocarbon and waste oil leaks. This study aims to evaluate a bioremediation-based fertilizer using organisms capable of oil degradation via the lipase enzyme, to revitalize agricultural lands in affected regions. Two strains of *Bacillus*, selected for their lipase activity, were tested alongside control groups without added organisms or compost. These were applied to pots with either oil-contaminated or control soils and were allowed to grow for 18 days under greenhouse conditions. At the end of the incubation period, plant vitality was assessed. The results indicated that the fertilizer, derived from the lipase-active *Bacillus licheniformis*, could significantly improve agricultural productivity in contaminated areas. Statistically, Tukey's HSD indicated that lipase activity was significant. *B. licheniformis* with kitchen waste compost treatment shows significant differences (p< 10^ -6) in oil reduction compared to control groups. The study highlighted the fertilizer's efficiency, cost-effectiveness, and potential marketability, offering it as a viable option for both producers and farmers due to its preservability, accessibility, and long-term potential.

1 Introduction

Oil spills pose a severe threat to agricultural productivity, severely disrupting soil composition, degrading vegetation, and introducing toxic hydrocarbons that destabilize microbial communities. These hydrocarbons not only have a direct negative impact on the growth and development of plants but also alter the chemical properties of the soil, making it less conducive for crops to grow. [1]In regions where oil-contaminated irrigation systems exacerbate soil degradation, these harmful effects extend beyond localized farmlands, influencing broader agricultural chains and national economies. [2]The soil's inability to support healthy crops leads to a decline in food production, which in turn impacts food security. While existing studies have extensively documented the environmental repercussions of oil spills, there remains a significant gap in research concerning cost-effective and biologically driven remediation techniques that could effectively restore soil health and enhance agricultural productivity.[3] The lack of efficient, environmentally friendly solutions has prompted increased interest in exploring biological processes that could naturally break down harmful contaminants. This study aims to contribute to the field by systematically evaluating the potential of lipase-active microorganisms in cleaning soil contamination and enhancing agricultural productivity, while also providing valuable insights into the broader effects of oil contamination, such as its long-lasting impact on soil fertility, plant growth.

Recycling kitchen waste, a global issue that contributes to environmental pollution and waste

[1] Kanthi Masakorala et al., "Effects of Petroleum Hydrocarbon Contaminated Soil on Germination, Metabolism and Early Growth of Green Gram, *Vigna radiata* L.," *Bulletin of Environmental Contamination and Toxicology* 91, no. 2 (2013): 224–30, https://doi.org/10.1007/s00128-013-1042-3.

[2] Nusrat Yaqoob et al., "The Effects of Agriculture Productivity, Land Intensification, on Sustainable Economic Growth: A Panel Analysis from Bangladesh, India, and Pakistan Economies," *Environmental Science and Pollution Research International* 30, no. 55 (2023): 116440–48.

[3] F. P. Carvalho and M. F. Garcia, "Long-Term Environmental Impact of Oil Spills: A Review," *Environmental Pollution* 246 (2019): 170-183, https://doi.org/10.1016/j.envpol.2018.11.089.

management problems, offers solutions to the social, economic, and environmental challenges caused by food waste. Each year, alarming quantities of food waste are generated, leading to a range of issues, including methane emissions from landfills, waste of valuable resources, and unnecessary strain on waste management systems.[4] The development of scalable and sustainable recycling methods is, therefore, an imperative goal for addressing these challenges. Beyond simply reducing waste, recycling kitchen waste holds the potential to contribute to multiple environmental benefits. If the recycling process leads to the creation of highly nutritious compost, for example, it can significantly improve soil health and enhance agricultural productivity. Additionally, the incorporation of such recycling methods into agricultural practices can play a critical role in the sustainable development goals of countries. By transforming food waste into valuable resources, it is possible to simultaneously tackle the issue of waste management and provide solutions for enhancing agricultural practices and environmental sustainability.

This research investigates the enzymatic potential of lipase-active microorganisms in degrading residual oil within soil ecosystems. These microorganisms catalyze lipid hydrolysis, breaking down oil into smaller, less toxic compounds that are more easily assimilated by the environment. The adaptability of these microorganisms to various soil environments makes them highly effective agents for bioremediation, as they can thrive in the conditions typically found in contaminated areas. This study explores the potential of incorporating specific organisms into fertilizers to restore soil health and enhance crop yield, particularly in oil-contaminated soils. It investigates the research question: Can a fertilizer combining the properties of lipase-active *Bacillus licheniformis* and kitchen compost effectively degrade waste oil in contaminated soils and promote plant growth?

While market-driven agricultural enhancers often focus primarily on maximizing productivity, they tend to overlook the broader issue of soil degradation caused by industrial pollution. Few solutions simultaneously address oil-induced soil degradation through biological intervention, making this research a valuable contribution to the development of environmentally friendly remediation strategies. By rigorously evaluating this method's feasibility, efficacy, and long-term ecological implications, this study aims to establish a robust scientific foundation for the potential application of lipase-active microorganisms in sustainable agriculture. The findings of this research could provide the basis for future agricultural practices that prioritize environmental restoration alongside productivity, offering a holistic solution to the challenges posed by both oil contamination and agricultural sustainability.

2 Literature Review

Soil, one of the essential elements that make life possible, enables human beings to meet their most basic needs for food through agriculture. Previous studies have stated that the 1-centimeter upper layer of the soil, which provides its fertility and is rich in humus, has been formed over centuries.[5] These fertile soils and agricultural lands are the basic elements for ensuring food security worldwide. Still, the protection of this soil should be brought to the agenda at least as much as the effort to navigate the limits of the benefits it provides, because there is no meaning in talking about enhancing soil fertility for the future if there is no soil left anymore to be used at that future.

In modern industrial societies, land is often the ultimate recipient of a wide variety of waste

[4] United Nations Environment Programme (UNEP), "Food Waste Index Report 2024," United Nations Environment Programme, 2024, https://www.unep.org/resources/report/food-waste-index-report-2024.
[5] G. Gözükara, S. Altunbaş, and M. Sarı, "Effects of Temporal and Spatial Changes on the Formation, Development, and Morphology of Soils in Different Physiographic Regions," *Ege University Faculty of Agriculture Journal* 57, no. 2 (2020): 277–288.

products and chemicals.[6] These include heavy metals such as lead (Pb) and cadmium (Cd), in addition to oil in soil, which can disrupt plant growth and adversely affect crop health and productivity. For instance, research indicates that elevated levels of these compounds in agricultural soils can lead to reduced crop yields and compromised food quality.[7] Additionally, oil contamination in soil can disrupt plant growth. Agriculture constitutes a large part of economies, specific studies quantify the exact impact of a 1% increase in per-capita agricultural growth leading to a 1.5% increase in per-capita non-agricultural growth. For instance, the agricultural sector in Turkey plays a crucial role, accounting for around 16% of total employment.[8] Also in South Asia, the agriculture sector is under great stress with high demand for available crops, ultimately leading to lower economic profit.[9] When healthy agricultural practices support fertile soils in agriculture, it guarantees sustainable production, and this indicates the importance of preserving the environmental balance not only for agriculture but also for the economic development of countries. Since the beginning of the twentieth century, soil pollution has also started to emerge as an environmental problem. Especially with industrialization and rapid population growth, soil pollution started to increase. Today, soil pollution caused by the reasons mentioned constitutes one of the important environmental problems that are reaching more serious dimensions every day, and its effect on agriculture is at a level that cannot be ignored. One of the main factors in the degradation of agricultural soils is waste oil leaks from many sources. These leaks can occur by the oil passing through water or directly into the soil. Waste oil pollution is a serious multidimensional problem affecting all aspects of the environment, causing changes in the physical, chemical, and microbiological properties of the soil, and eventually causing these changes to pass on to the plant.[10] Studies have shown that even insignificant amounts of oil spills can damage agricultural fields.[11] Waste oil contamination significantly impacts soil-based communities. It has been indicated that hydrocarbon pollutants can lead to a decline in soil-based activities.[12] Even small amounts of waste oil can significantly reduce the growth and germination of common agricultural plants. In one study, it was found that Total Petroleum Hydrocarbons (TPH) pollution in soil inhibited the germination of both wheat and maize seeds when the hydrocarbon concentration exceeded 0.1%.[13] Waste oils consist of many categories, such as used vegetable oil, machine oils, industrial oils, and contaminated natural areas, due to many factors. Despite the obvious effect arising from contamination, there is no detailed study in which a current but accessible method is proposed for the bioremediation of these soils and the proposed method is tested with data. A method and study that cleans nature with what nature gives and makes the soils productive has the potential to have a significant impact on development in agriculture. According to the data published by the Ministry of Urbanization of the Republic of Turkey within the scope of hazardous waste management in Turkey, a total of 1,413,220 tons of hazardous waste declared by 39,134 facilities were recorded in 2014.

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[6] Nyle C. Brady and Ray R. Weil, *The Nature and Properties of Soils*, 11th ed. (Upper Saddle River, NJ: Prentice Hall, 1996), xi.
[7] Rafique Akram, Zafar Iqbal, Muhammad Ijaz, and Muhammad Rizwan, "Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants," *MDPI Agriculture* 13, no. 6 (2023): 1521, https://www.mdpi.com/2073-4395/13/6/1521.
[8] Büşra Sağlam and Caner Korkmaz, "The Relationship between Agricultural Production, Agricultural Exports and Economic Growth in Türkiye: Cointegration and Causality Analysis," *Sosyal Bilimler Dergisi* 11, no. 1 (2023): 115–35, https://dergipark.org.tr/tr/pub/comuybd/article/1453376.
[9] Yaqoob et al., "The Effects of Agriculture Productivity," 116440–48.
[10] Russell Khaled Mohsen and Hazim Aziz Al-Robai, "Pollution Effect of Used Engine Oil on Chemical Properties and Some Heavy Metals of Sandy Soil," in *IOP Conference Series: Earth and Environmental Science*, vol. 1262, no. 8 (2023): 082060.
[11] Muhammad Zafar Iqbal, Sehrish Khursheed, and Muhammad Shafiq, "Effects of Motor Oil Pollution on Soil and Seedling Growth of *Parkinsonia aculeata* L.," *Scientia Agriculturae* 13, no. 3 (2016): 130–136.
[12] M. I. Khan et al., "Microbial Remediation Techniques for Hydrocarbon-Contaminated Soils: Current Trends and Future Directions," *International Journal of Environmental Science and Technology* 20, no. 2 (2023): 123–135.
[13] Jingchun Tang et al., "Eco-toxicity of Petroleum Hydrocarbon Contaminated Soil," *Journal of Environmental Sciences* 23, no. 5 (2011): 845–851.

Approximately 70,000 tons of waste oil and 7,000 tons of vegetable waste oil are included.[14] The majority of waste oils are oils used in industry, especially in the auto industry. Among these oils, there are lubricating oils used in quantities that can compete with motor oils in tons of liters, which are classified as hydrocarbons and therefore are derived from crude oil. It is a very common situation that petroleum hydrocarbons are spilled into the soil during operations in the industry and most likely in the automotive industry, where they are used, and cause irreversible effects. Specifically, studies have reported reductions in soil-based activities contaminated with used engine oil due to the toxic effects of hydrocarbons.[15] The introduction of petroleum hydrocarbons into an uncontaminated environment changes the ecosystemic balance of that environment. Leaked hydrocarbons cause an increase in mortality data in many microbial species or inhibit their activities, changing the productivity of the microbial community and the ecosystem.[16] Existing experiments have shown that contamination of soil with crude oil and its derivatives may proceed in parallel with heavy metal pollution, which can enter from the soil and cause complex ecosystem pollution.[17] Petroleum hydrocarbons (PHs) are carcinogenic and neurotoxic. They are known to cause soil pollution, which has significant impacts on a global scale.[18]

Kitchen waste has reached serious dimensions in the world, due to food waste and the fact that waste is mostly thrown away rather than recycled. In Turkey, according to the results of the solid waste composition determination study conducted within the scope of the Solid Waste Master Plan Project (KAAP) in 2006, 34% of municipal waste in Turkey consists of kitchen waste.[19] The U.S. Environmental Protection Agency (EPA) has conducted extensive research on the environmental impacts of food waste, emphasizing the importance of reducing kitchen waste through composting and recycling initiatives. The EPA's findings highlight that food waste contributes significantly to landfill mass, leading to greenhouse gas emissions and soil contamination issues.[20] Considering the significant issues caused by kitchen waste, the ongoing depletion of vital natural resources, and the extensive economic damage associated with its mismanagement, it is crucial to implement recycling strategies that not only mitigate environmental harm but also foster cross-sectoral benefits. Such an approach, prioritizing sustainability, will contribute to the long-term goals of sustainable development of countries.

Lipase enzyme is a biocatalyst belonging to the class of hydrolases that can catalyze the hydrolysis of ester bonds. In the production of industrial lipases, microorganisms are the primary sources due to their high productivity and ability to adapt to environmental conditions. For this reason, bacteria and fungi stand out in this context with their ecological standards.[21] Microbial The main principle of lipases is to break down lipids into smaller building blocks, glycerol, and fatty acids, by catalyzing the hydrolysis of ester bonds. These small building blocks, fatty acids, are

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[14] Republic of Turkey Ministry of Environment and Urbanization, *2014 Hazardous Waste Statistics Report*, General Directorate of Environmental Management, accessed February 6, 2025, https://webdosya.csb.gov.tr/db/ced/editordosya/2014_YILI_TEHLIKELI_ATIK_ISTATISTIKLERI_15022016.pdf.

[15] N. H. Ugochukwu and J. A. E. Okwudiri, "The Effects of Spent Engine Oil Pollution on Microorganisms in a Tropical Rainforest Ecosystem in Nigeria: Implications for Bioremediation Strategies," *African Journal of Environmental Science and Technology* 10, no. 1 (2016): 1–10.

[16] Chioma Blaise Chikere, Gideon Chijioke Okpokwasili, and Blaise Ositadinma Chikere, "Monitoring of Microbial Hydrocarbon Remediation in the Soil," *3 Biotech* 1 (2011): 117–138.

[17] Jingchun Tang et al., "Eco-toxicity of Petroleum Hydrocarbon Contaminated Soil," *Journal of Environmental Sciences* 23, no. 5 (2011): 845–851.

[18] Yuhang Chen et al., "Biochar-Assisted Bioremediation of Soils with Combined Contamination of Petroleum Hydrocarbons and Heavy Metals: A Review," *Applied Soil Ecology* 204 (2024): 105720.

[19] Katı Atık Ana Planı Projesi [KAAP], *Türkiye Katı Atık Yönetimi Raporu* (Çevre ve Şehircilik Bakanlığı, 2006).

[20] U.S. Environmental Protection Agency, "From Farm to Kitchen: The Environmental Impacts of U.S. Food Waste," accessed February 6, 2025, https://www.epa.gov/land-research/farm-kitchen-environmental-impacts-us-food-waste.

[21] A. Abubakar et al., "Crude Oil Biodegradation Potential of Lipase Produced by *Bacillus subtilis* and *Pseudomonas aeruginosa* Isolated from Hydrocarbon Contaminated Soil," *Environmental Chemistry and Ecotoxicology* 6 (2024): 26–32.

converted into smaller molecules, acetyl-CoA, by *β - oxidation to* be used in the Krebs cycle. Glycerol is used by microorganisms to produce ATP, thus fats are used in the biological cycle of the organism, and in the case of leaked oil in the soil , the lipase -active organism provides bioremediation of the soil.[22] Microbial lipases offer significant advantages over lipases obtained from plant and animal sources due to their thermostability, ability to work in wide pH ranges, selectivity for specific substrates, and openness to genetic manipulation.[23] For this reason, this study was conducted on microbial lipases. Previous studies have investigated *Bacillus* species, especially *Bacillus Subtilis* and *Bacillus licheniformis,* has been shown to be valuable for industrial applications due to its ability to produce lipase that remains stable at high temperatures.[24]

3 Method

3.1- Making Compost from Kitchen Waste

Compost, which forms the basic element of all test groups for use as fertilizer , was prepared using the following wastes:

- 2 orange peels
- 2 slices of spoiled cheese
- Foods containing carbohydrates and fat, such as waste rice and pasta
- Egg shells
- Tea pulp
- Potato peelings
- Banana Peel
- Dried leaves
- Control Soil (0 g/kg oil)

The specified products were combined after and left open for 3 weeks to naturally disintegrate.

3.2- Lipase Positive/Negative Bacteria

The bacterial isolates used in this study were obtained from the Biology Laboratory of Çukurova University under the supervision of Prof. Dr. Osman Gülnaz. Bacillus colonies were transferred to a MALDI-TOF MS device for species-level identification. For analysis, the samples were prepared with sterile distilled water and formic acid (HCOOH), and protein profiles were obtained to be *Bacillus licheniformis.* The lipase activity of *Bacillus licheniformis* was assessed using an agar plate method, where the ability of the bacteria to hydrolyze lipids was determined by the formation of a clear zone around bacterial growth. Spirit Blue Agar was prepared by mixing 1.5% agar with 1% tributyrin (Sigma) and 0.1% spirit blue dye in distilled water. The mixture was autoclaved and poured into sterile petri dishes, which were allowed to solidify at room temperature before use.

A culture of *Bacillus licheniformis* was streaked onto the surface of the prepared agar plate using a sterile inoculating loop. The plates were incubated at 30°C for 24-48 hours to allow bacterial growth and lipase secretion. After the incubation period, the plates were visually examined for the formation of a clear zone around the bacterial growth. This clear zone indicates the hydrolysis of tributyrin by lipase enzymes, which break down the lipids into fatty acids and glycerol, causing the surrounding medium to become transparent.

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[22] Pratyoosh Shukla, Enespa, Ramesh Singh, and P. K. Arora, "Microbial Lipases and Their Industrial Applications: A Comprehensive Review," *Microbial Cell Factories* 19, no. 169 (2020): https://doi.org/10.1186/s12934-020-01428-8.
[23] P. K. Ghosh et al., "Microbial Lipases: Production and Applications," *Science Progress* 79, no. 2 (1996): 119–157.
[24] D. Tanrisever, *Bacillus Subtilis Thermostable Lipase Production and Characterization* (Master's thesis, Institute of Science, 2011).

The diameter of the clear zone was measured using a caliper to quantify the lipase activity. A larger zone of clearance around the bacterial growth indicated higher lipase activity, while no clearing suggested a lack of lipase production. This assay provided a simple, visual method for identifying *Bacillus licheniformis* isolates with active lipolytic enzymes.

3.2.1- Preparation of The Soil Sample

Soil samples were collected from sites adjacent to agricultural fields in Adana. One sample was taken from Yeşiloba/Seyhan, exposed to industrial influences and located near, but not within, agricultural fields. Another sample was collected from a site in Karaisalı isolated from both agricultural and industrial activities. They were collected in sterile bags for bacterial isolation. The soil was prepared under sterile conditions to prevent contamination. A 0.1 g soil sample, used for isolation, was measured on an analytical balance and transferred into sterile Eppendorf tubes.

3.2.5- Bacteria Identification

Colonies were transferred to a MALDI-TOF MS device for species-level identification. For analysis, the samples were prepared with sterile distilled water and formic acid (HCOOH), and protein profiles were obtained. Based on the identification results, one lipase-positive and one lipase-negative strain of *Bacillus licheniformis* were selected for use in the study.

3.3- Planting and Fertilization

Twelve pots were used in this study to systematically evaluate the effects of various soil types and fertilizer applications on the growth performance of lettuce seedlings as well as the overall condition and health of the soils. Lettuce was selected for its ease of cultivation in pots and because the study coincided with its optimal planting season. The pots were divided into two primary groups based on the type of soil: oily and non-oily soils. The oily soil was sourced from an industrial area in Adana, where industrial activities are prominent, resulting in significant soil contamination from hydrocarbons and other pollutants. This type of soil was specifically selected to replicate the adverse effects of pollution and to simulate real-world conditions in industrial zones where soil quality is compromised due to contamination. On the other hand, the non-oily soil was obtained from a greenhouse, where the soil is known to have neutral properties and serves as an ideal environment for plant growth. This soil was chosen as a control to compare the effects of polluted soil with those of clean, uncontaminated soil. Equal amounts of both soil types were carefully placed into the pots, ensuring that the comparison between the two soil types would be based on identical quantities of soil in each pot.

Once the soil was added to the pots, the next action to be taken was to apply fertilizers to each of the pots to assess how different types of fertilization would influence the growth and health of the lettuce seedlings. The amount of fertilizer used was precisely measured at 15 grams per pot, a quantity that was determined to be optimal for providing adequate nutrients to the plants without over-fertilizing (with a fertilizer to soil ratio of 1/1000) while replenishing nitrogen, phosphorus, and other elements in the soil and to enhance microbial activity. Fertilizer application was a critical component of the study, since it allowed the investigation of the impact of different nutrient formulations on plant growth in both oily and non-oily soils. The fertilizers used in this experiment were specifically chosen for their known properties in improving soil fertility and supporting plant growth. To further investigate the relationship between soil types, fertilization strategies, and plant growth, the fertilizer ingredients were carefully combined into several distinct formulations, each designed to deliver a unique nutrient profile. These combinations were selected to represent various approaches to soil enrichment, enabling the study to examine how different levels of nutrients, such as nitrogen, phosphorus, and potassium, would affect plant growth in contaminated versus uncontaminated soil. The pots were then carefully monitored to track the growth of the lettuce seedlings, while the soils were assessed for changes in their physical and chemical properties, including nutrient availability and microbial activity. Groups were grown for 18 days, as this period marks the transition from sprout to a form closest to its final stage as mature lettuce. The results of this experiment are expected to shed light on how fertilization strategies can be tailored to improve agricultural productivity in polluted soils and provide valuable insights for sustainable agricultural practices.

3.3.1- Oily Soil Group:

Lipase Positive *Bacillus*: In this group, 15 g of lipase-active *Bacillus* bacteria fertilizer was added to the pot. This treatment did not include an additional compost, which was expected to supplement plant growth by providing nutrient availability.

Lipase Negative *Bacillus*: Only 15 g of lipase-negative *Bacillus* was added. This group was not expected to contribute to the oil degradation.

Kitchen Compost: In this group, 15 g of compost was added to the pot. Compost fertilizer was expected to improve soil structure with its rich nutrient content obtained as a result of the decomposition of organic matter through biological processes.

Lipase Positive *Bacillus* + Compost: In this group, 15 g of lipase-positive Bacillus species and 15 g of compost were mixed and blended with the soil. It was expected to improve soil nutrients while degrading oil.

Lipase Negative *Bacillus* + Compost: In this group, 15 g of lipase-negative Bacillus species and 15 g of compost were used. No change in oil amount was expected.

Control Group: In this group, only non-oily soil was used, and no additional ingredients were added. (0g/kg of oil amount of the initial soil was detected before the group formation)

3.3.2 Lean Soil Group:

Lipase Positive *Bacillus* Bacteria (Lipase positive *Bacillus* added): Similarly, 15 g of lipase-active *Bacillus* bacteria fertilizer was added to the oil-free soil group.

Lipase Negative Bacillus (Lipase negative Bacillus Added): In the oil-free soil group, only 15 g of lipase-negative fertilizer was added.

Kitchen Compost 15 g of compost was added to the pot in this group.

Lipase Positive Bacillus + Compost: In this group, 15 g of lipase-positive *Bacillus* fertilizer and 15 g of compost were mixed.

Lipase Negative Bacillus + Compost: 15 g of lipase-negative fertilizer and 15 g of the compost were added to this pot.

Control Group: In the lean soil group, only lean soil was used as the control group, and no additional components were added.

The fertilization process began with the careful planting of two lettuce seedlings in each pot. Prior to covering them with soil, the seedlings were sprinkled with iron to promote healthy growth. Special attention was given to ensuring that the seedlings made good contact with the soil to establish a strong foundation for growth. Following planting, all experimental groups were placed in a controlled greenhouse environment where the temperature was maintained at a constant 24°C to provide optimal conditions for daylight exposure. The pots were kept in a stable environment, with consistent temperature and humidity levels, for a period of 18 days. Over these 18 days, the plants were monitored to ensure they received sufficient light and a conducive growing environment. After this incubation period, the lettuce plants were carefully removed from the pots for further analysis. The growth parameters and oil content in the soil of each experimental group were measured to evaluate the effects of the fertilization process and determine the impact on soil health and plant development.

3.4- Determination of Oil in Soil

Ten grams of soil were dried overnight at 50 °C, then placed in a Soxhlet extraction cartridge. Reflux extraction was performed using hexane for 1 hour at 70 °C. Following the extraction, hexane was evaporated using nitrogen gas, and the remaining residue was weighed to determine the total oil content

3.5- Measurement of Growth Parameters

The height, root, and leaf width of lettuce in each experimental group were measured. Since there were 2 lettuce in each group, the average of the two data points was taken 3 times each. The growth rate was tabulated to compare with the initial data.

3.6- Statistical Analysis

Significance was measured by ANOVA and Tukey's HSD tests. Statistical analyses, including one-way ANOVA and Tukey's HSD post hoc tests, were performed using Python 3.10 with the SciPy and statsmodels libraries. ANOVA was conducted using the *scipy.stats.f_oneway( )* function, and Tukey's HSD was implemented via *statsmodels.stats.multicomp.pairwise_tukeyhsd( ).* All data was analyzed in Jupyter Notebook.

4 Findings

The results from the Soxhlet extraction method demonstrate the greatest reduction in oil contamination has been in the group of Lipase+ *Bacillus licheniformis* and compost. The combination of these treatments resulted in an oil content of 0.8 g/kg, indicating a 60% degradation over the 18-day period. Similarly, the Lipase+ *B. licheniformis* group alone achieved a reduction to 0.9 g/kg, corresponding to a 55% decrease in oil content within the same timeframe. In contrast, the control treatments and the Lipase- B. licheniformis variant showed the least oil reduction since the oil contents remained close to the initial levels. The full data set regarding physical measurements can be seen in the appendix (Figure 1). In terms of plant growth, the lipase-active *B. licheniformis* and compost group treatment emerged as the most effective, showing an improvement of 77% in plant height, a 120% increase in root length, and a 50% increase in leaf width compared to the control group. In contrast, the Lipase- *B. licheniformis* treatment showed only a significantly modest reduction in oil content and minor improvements in plant growth.

Statistical Analyses

The full set of data regarding oil degradation is provided in the appendix (Figure 2).
The set of data has been tested for significance with ANOVA and Tukey's HSD, which is provided in the appendix. (Table 1).

ANOVA Results:

There was a significant effect of treatment on oil degradation levels in contaminated soil ($F_{(6,14)} = 148.03$, $p < 0.0001$), as determined by a one-way ANOVA. In the non-oil control groups**,** the p-value was not applicable (NaN).

5 Discussion

5.1 Significance of This Analysis

This analysis has provided the experimental results regarding the important issue of bioremediation. At the same time, it has addressed agricultural issues due to contamination with data sets and shown how contamination affects soils via the contaminated control group. The project is also in line with targets 12, 13, and 15 of the Sustainable Development Goals. [25] It directly contributes to the sub-targets of goal 12, "Substantially reduce waste generation through prevention, reduction, recycling, and reuse and halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses. " Also directly contributes to the sub-target of goal 15, "Restore degraded land and soil and strive to achieve a land degradation-neutral world.".

While many existing studies focus on the environmental repercussions of oil spills and various chemical remediation methods, few have explored biologically driven, cost-effective approaches that also support sustainable agricultural recovery. This study distinguishes itself by investigating the use of biofertilizers with lipase-producing microorganisms to degrade oil in contaminated soils. Unlike purely chemical treatments, this method not only reduces hydrocarbon concentrations but also actively improves soil health, promoting better plant growth and long-term soil restoration. The samples treated with lipase-active fertilizers showed a significantly higher reduction in oil content and better plant performance compared to untreated or low-activity groups. This indicated that lipase enzymes facilitated the breakdown of lipid-based pollutants, making nutrients more accessible for plant uptake and improving overall soil fertility.

5.2 The Limitations

Although Bacillus was the only organism tested in this study, other easily adaptable organisms could potentially meet industrial standards for future use, yielding different results that were not explored in this investigation for fertilizer production. In future studies, researchers could investigate the effectiveness of other microorganisms that naturally occur in soil or that are specially cultivated for their bioremediation capabilities. Such organisms might exhibit superior or more efficient oil-degrading properties, potentially resulting in more effective or economical solutions for agricultural purposes. Expanding the scope of the research to include a variety of microbial strains could lead to the identification of those that offer higher efficiency or resilience under varying environmental conditions, thus broadening the potential for industrial-scale application..Due to academic and financial limitations, this study can only demonstrate plant vitality via physical measurements, which is a limitation that could be expanded upon in future research. While physical measurements such as plant height, leaf count, and overall health provide a useful initial understanding of the effects of bioremediation treatments, they are inherently limited. More comprehensive measurements, such as biochemical analyses or microbial activity tests, could reveal a deeper understanding of the specific interactions between microorganisms, soil contaminants, and plant growth. These additional analyses could provide more definitive evidence of the microbial processes at work in the soil and their direct impact on soil health and plant productivity. Future studies could incorporate these aspects, offering a more detailed and robust assessment of the effects of microbial treatments on soil contamination and agricultural

[25]1. United Nations, *Sustainable Development Goals*, accessed January 31, 2025, https://sdgs.un.org/goals.

productivity. Additionally, the scope of the study was confined to a single experimental period of 18 days, which may not fully capture the long-term effects of bioremediation treatments. The short duration of the study limits its ability to evaluate the sustainability and consistency of the effects observed over time. The effects of soil contamination and bioremediation treatments on microbial communities, nutrient cycling, and plant growth may evolve over a longer timeframe, and this study does not account for those changes. Future research could extend the experimental period to several months or even a full growing season, which would provide a clearer picture of the long-term benefits and potential challenges of bioremediation techniques. A more extended study would allow researchers to evaluate the durability of the observed effects, including whether the benefits of bioremediation persist over time, whether new environmental factors arise, and how these treatments might influence soil and plant health in the long run. These extended studies would offer critical insights into the viability of using bioremediation as a sustainable and effective method for enhancing agricultural productivity while mitigating the impacts of soil contamination.

6 Conclusion

As a result of the measurements, the fertilizer group produced from *Bacillus lichenformis* and the mixture obtained from kitchen compost broke down a significant amount of the oil in the contaminated soil in the 18-day period. The research revealed not only the bioremediation effectiveness but also the negative effects of waste and leaked oils on the soil structure. It was observed that oil contamination negatively affected basic characteristics of the plant such as vitality (based on physical measurements) . In this context, a fertilizer developed with the tested method not only reduces pollution but also contributes to sustainable agricultural practices by improving soil health.

Plant growth in the oil-contaminated soil with the most effective treatment approached a 77.7% increase in the plant length and significant root growth compared to the controlled contaminated soil, indicating a strong recovery facilitated by the degradation of oil contaminants. On the other hand, the least effective treatment from lipase-negative groups showed only minimal recovery, highlighting the significant role lipase plays in bioremediation.

The physical measurements, which were directly correlated to the amount of degraded oil through the treatment, also highlighted the impact of soil health on plant growth and the efficiency of each grown product. The findings of this study suggest that the recycling of kitchen waste and the integration of genetic characteristics of organisms into agricultural practices create a strong bridge between waste management and agricultural production in terms of environmentalism. The high effect it shows in a short time of almost 3 weeks offers a fast and applicable model for solving environmental problems. The results show that this type of fertilizer has significant potential not only in environmental protection due to soil purification from oil contamination but also in improving agricultural quality in these regions by reducing the economic damage inflicted on polluted areas.

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8 Acknowledgements

I would like to express my sincere gratitude to Prof. Dr. Osman Gülnaz from the Faculty of Education, Department of Mathematics and Science Education, Çukurova University, for his invaluable support and guidance throughout this research. His provision of the isolates and expert insight into microbiological procedures significantly contributed to this study.

9 Appendices

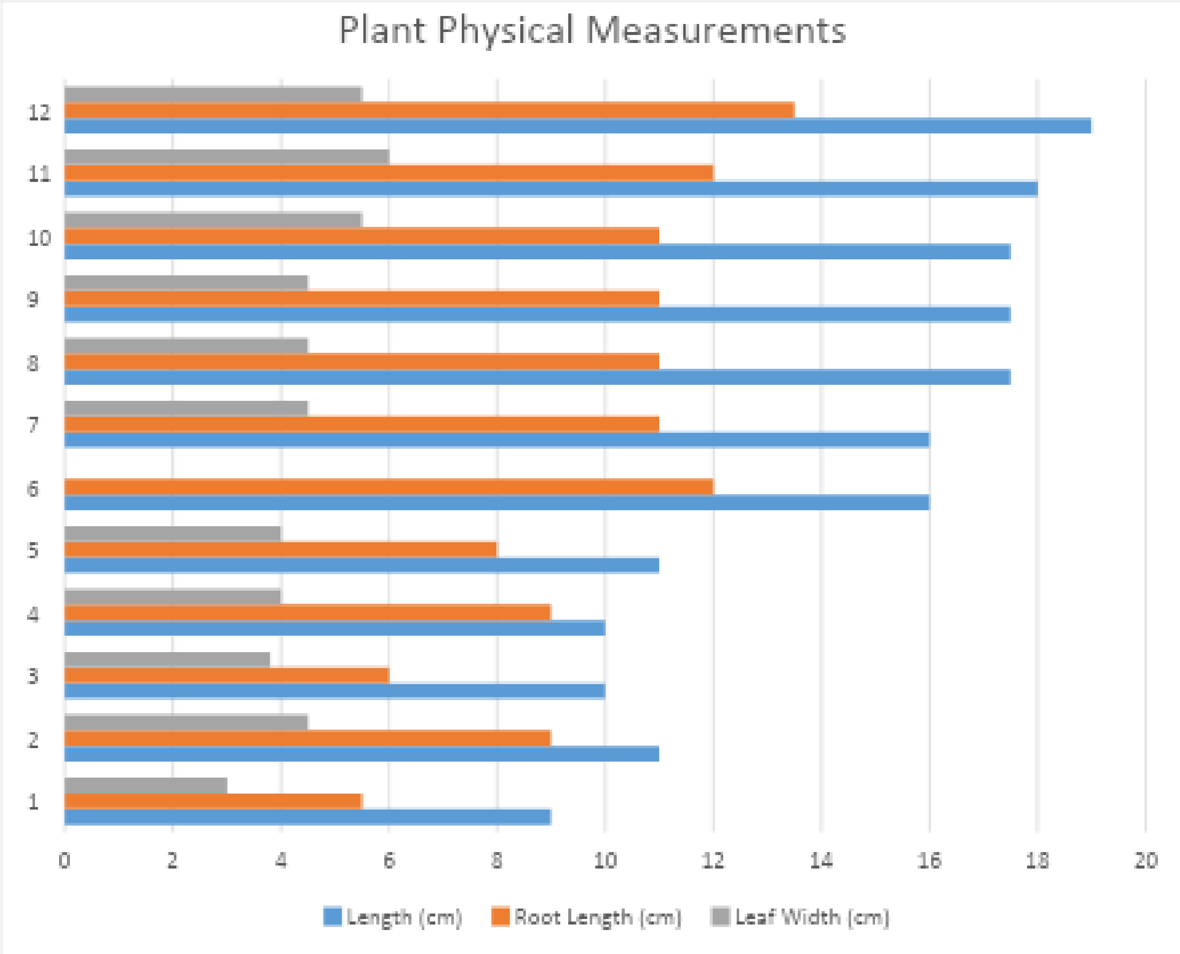

Figure 1: Plant Physical Measurements This figure displays the length (cm) of each group, measured in three different ways: width, overall length, and root length.

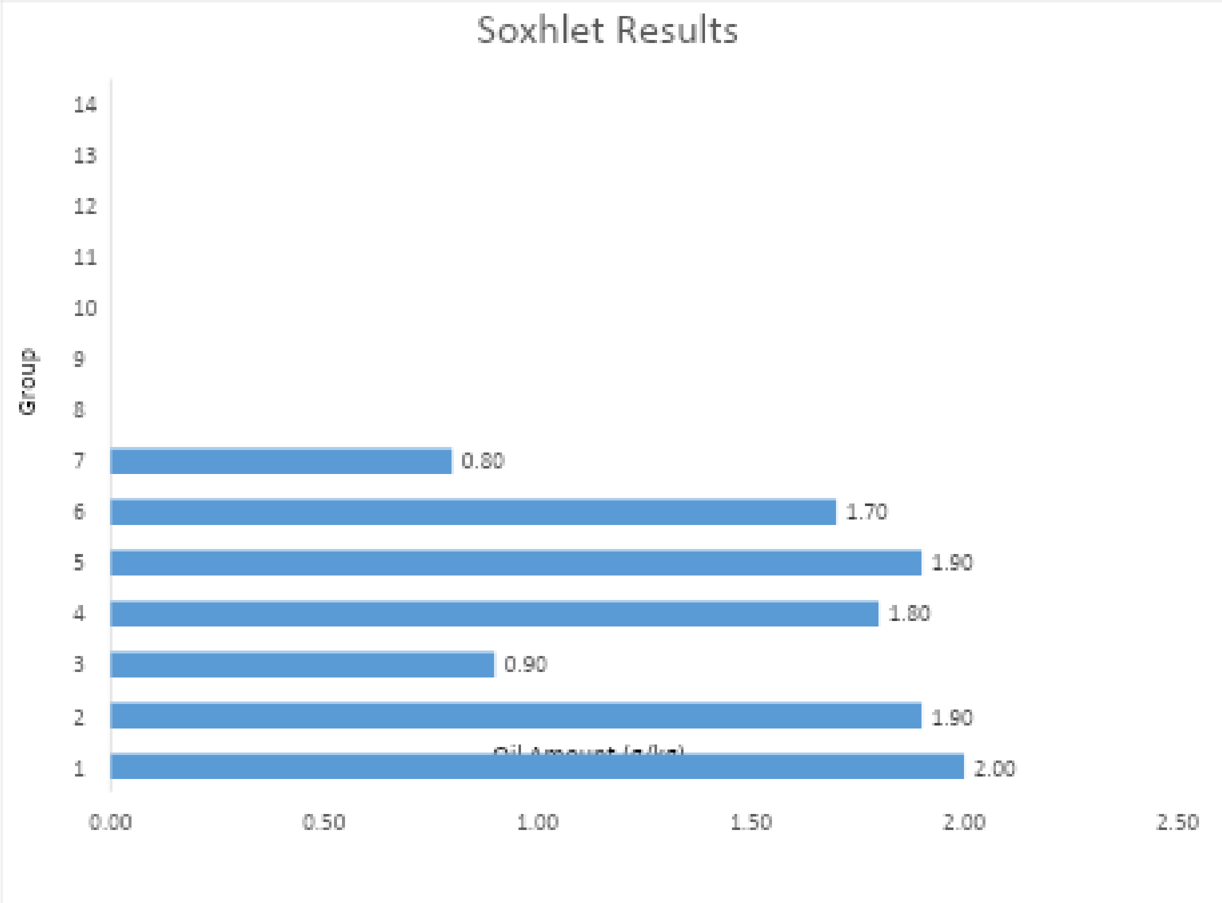

Figure 2: Final Soxhlet Oil Amount Measurements This figure displays the results of the initial and final oil amounts for each group.

Comparison	Mean Diff (g/kg)	p-value (10^x format)	Significance
Lip+ B. lich + Compost vs Control2	–1.0	1.056 × 10^–7	Yes
Lip+ B. lich vs Control2	–0.9	1.056 × 10^–7	Yes
Lip+ B. lich + Compost vs Compost only	–1.1	1.056 × 10^–7	Yes
Lip+ B. lich vs Compost only	–1.0	1.056 × 10^–7	Yes
Lip+ B. lich vs Lip– B. lich	–0.9	1.056 × 10^–7	Yes
Lip+ B. lich vs Lip– B. lich + Compost	–1.0	1.056 × 10^–7	Yes
All others (non-significant ones)*	Various	> 0.05	No

Table 1: Tukey's HSD Statistics

*No statistically significant differences were observed between the Control2, Lipase– B. licheniformis, Lipase– B. licheniformis + Compost, and Compost-only groups ($p > 0.05$), suggesting that these treatments did not significantly affect oil reduction.