



Phytoremediation of Kerosene-Contaminated Soil Using *Vetivera Zizanioides* and *Ipomea Reptans Poir* with Bioaugmentation and Eggshell Biostimulant Addition

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ABSTRACT:

The use of large amounts of kerosene has the potential to cause environmental pollution. The greatest attention has been directed toward the environmental impacts of tanker accidents, pipeline leaks, and the use of kerosene as an asphalt extraction solvent. Spills of kerosene into the soil can damage ecosystems, threaten the survival of microorganisms, and affect the smell and taste of groundwater. The purpose of this study is to examine the ability of *Vetiveria zizanioides* and *Ipomea reptans Poir* plants to remediate kerosene-contaminated soils, and to analyze the effects of bioaugmentation and biostimulation on the reduction of kerosene contamination. The research was conducted on a laboratory scale using spiked soil. Phytoremediation was carried out with the addition of *Bacillus subtilis* bacteria at 10% v/v, and eggshell stimulants at 10% w/w. Analyses included testing Total Petroleum Hydrocarbon (TPH), Total Plate Count (TPC), and measuring plant height and growth rate. The best TPH elimination percentage was obtained in CS1, with the variation of *Vetiveria zizanioides* combined with *Bacillus subtilis*, achieving 70% removal on day 15 with a TPH degradation rate of 632 mg/kg/day. The integration of specific plants with bioaugmentation is highly effective for kerosene bioremediation; however, the application of biostimulants such as eggshells requires careful dosage optimization to avoid counterproductive effects.

Keywords: Fitoremediasi, *Ipomea reptans Poir*, Total Petroleum Hydrocarbon (TPH), *Vetiveria zizanioides*

INTRODUCTION

The use of large quantities of petroleum products has resulted in high levels of environmental pollution. The greatest attention has been paid to the environmental impact of crude oil spills due to refueling station tank leaks (Wahyudi et al., 2025), tanker and drilling rig accidents (Beyer, Trannum, Bakke, Hodson, & Collier, 2016), and the use of kerosene as an asphalt extraction solvent (Indragiri et al., 2025). Kerosene is a hydrocarbon derivative with a C_{15} carbon chain obtained from fractional petroleum distillation of light fractions. It is toxic, permeates easily into soil layers, and can contaminate groundwater sources. Spills of kerosene into the soil can harm the environment, threaten the survival of microorganisms, and affect the smell and taste of water in the soil (Handrianto, 2018).

The development of biological alternatives—bioremediation, bioaugmentation, composting, and rhizoremediation—is essential to achieve cost-effective soil decontamination while maintaining or improving soil quality. Careful selection of appropriate technology is a critical step for the successful recovery of contaminated sites (El Rasafi et al., 2023). Among these, phytoremediation has emerged as one of the most effective technologies by utilizing the innate properties of plants to restore contaminated soils (Park & Oh, 2023). Plants create a rhizospheric environment as their roots penetrate the soil, where they accumulate contaminants, break them down, and regulate their bioavailability. This process supports soil recovery and plays a key role in maintaining soil health and productivity (Pathak, Chauhan, Seth, Dubey, & Upadhyay, 2024). Unlike physical and chemical remediation techniques, phytoremediation requires fewer resources, including equipment, materials, energy, and labor, making it more efficient compared to alternatives such as natural attenuation (Aparicio et al., 2022). Bioaugmentation, which involves the decomposition of contaminants by microorganisms, and river self-purification, where rivers naturally cleanse themselves of pollutants, share similarities with phytoremediation in their reliance on natural mechanisms to remove contaminants (Oubohssaine & Dahmani, 2024).

The widespread use of petroleum derivatives, particularly kerosene, poses a persistent and significant threat to soil ecosystems worldwide. Kerosene contamination frequently results from leaking underground storage tanks, pipeline fractures, and its application as an industrial solvent. Upon release into the environment, its low viscosity and high mobility allow it to rapidly penetrate soil layers, threatening groundwater quality, disrupting soil microbiota, and altering soil physicochemical properties—ultimately jeopardizing ecosystem health and human safety. The complex hydrocarbon matrix of kerosene, comprising alkanes, cycloalkanes, and aromatic compounds, makes it resistant to natural attenuation and necessitates active intervention for effective remediation. This persistent contamination underscores the need for efficient, economical, and sustainable rehabilitation strategies for polluted soils.

Conventional physico-chemical remediation methods—such as soil incineration, landfilling, and chemical washing—are often employed to treat hydrocarbon-contaminated sites. However, these methods are typically associated with exorbitant costs, high energy consumption, secondary pollution, and the destruction of soil structure and fertility. By contrast, bioremediation techniques harness natural biological processes to degrade contaminants, offering a more environmentally sound alternative. Within this domain, phytoremediation stands out as particularly promising. This approach employs plants and their associated rhizospheric microbes to extract, degrade, or immobilize contaminants, providing a solar-powered, cost-effective, and aesthetically viable solution that can restore soil functionality.

Vetiveria zizanioides (vetiver) has emerged as a prominent species in phytoremediation due to its deep root system, tolerance to extreme conditions, and ability to accelerate hydrocarbon degradation. Phytoremediation using *Vetiveria zizanioides* with the addition of bioaugmentation (*Staphylococcus gallinarum*, 10% v/v) and biostimulants achieved a degradation yield of 33.83% of Total Petroleum Hydrocarbons (TPH) (Saputri & Purwanti, 2024). Research Putri et al. (2023) demonstrated that *Ipomea reptans* Poir (water spinach) is capable of surviving in soils

contaminated with heavy metals at concentrations of up to 10 ppm. This plant exhibits broad adaptability to different environments, functions as a bioremediatory agent, and offers the practical benefit of being harvestable every 10–15 days (Kresna, Sukerta, & Suryana, 2016).

Bacillus subtilis is a bacterium known for its ability to reduce TPH and survive under contaminated conditions (Purwanti et al., 2024). According to Paramata (2023), bioaugmentation using *B. subtilis* reduced TPH in kerosene-contaminated soil by 62.62%. Similarly, Garcia & Purwanti (2022) reported that *B. subtilis* degraded hydrocarbons by 70% within 20 days. In addition, organic amendments such as eggshells can enhance soil fertility through various mechanisms. Eggshells are rich in calcium carbonate (CaCO₃), which increases the pH of acidic soils and creates more favorable conditions for plant growth in phytoremediation systems (Najamuddin, Sherman, & Muh. Ikbal Putera, 2024).

Substantial prior research has demonstrated the efficacy of different plant species in hydrocarbon remediation. Grass such as *Vetiveria zizanioides* are notable for their extensive, deep root systems and exceptional tolerance to abiotic stresses, including heavy metals and organic pollutants. For instance, Sinha et al. (2021) documented vetiver's capacity to absorb and facilitate the degradation of petroleum hydrocarbons. Conversely, *Ipomea reptans* Poir has been recognized for its rapid growth, high biomass production, and adaptability in contaminated environments. Putri et al. (2023) observed its tolerance to heavy metal stress, further supporting its phytoremediation potential. The effectiveness of phytoremediation can be significantly enhanced through bioaugmentation with pollutant-degrading microbes. Notably, *Bacillus subtilis* has been studied extensively: Garcia & Purwanti (2022) reported a 70% degradation of hydrocarbons within 20 days, while Paramata (2023) recorded a 62.62% reduction in TPH in kerosene-contaminated soil.

Despite these advancements, a notable research gap remains in exploring the synergistic integration of these components. While studies have examined either vetiver or water spinach separately, and others have tested *B. subtilis* independently, there is a lack of comparative analysis evaluating both plant species within the same experimental framework when combined with bacterial augmentation. Furthermore, the potential of biostimulation using waste-derived amendments, such as eggshell powder, to enhance microbial activity has not been adequately studied. Eggshells, rich in calcium carbonate, can improve soil pH and provide essential minerals. However, their specific interactions with hydrocarbon-degrading bacteria such as *B. subtilis*, and their influence on plant performance and overall TPH removal efficiency in kerosene-contaminated soils, remain poorly understood—representing a critical knowledge gap.

The urgency of this research is underscored by the continuous risk of kerosene spills, particularly in developing regions with aging infrastructure and limited regulatory oversight. Soil pollution directly impacts agricultural productivity, water security, and public health, necessitating immediate and practical remediation strategies. Additionally, within the framework of a global circular economy, there is a pressing need for technologies that are not only effective but also utilize waste materials, such as eggshells, to add value while reducing environmental footprints.

This study addresses that urgency by striving to optimize a remediation protocol that is both highly effective and sustainable, turning waste into a resource for environmental restoration.

The novelty of this research lies in its holistic and comparative approach to bioremediation. It innovatively combines two distinct plant species with different remediation mechanisms—a deep-rooted grass and a fast-growing broadleaf plant—with the bioaugmentation capacity of *B. subtilis* and the biostimulant potential of eggshell powder, an agricultural byproduct. This multi-faceted investigation seeks to unravel the complex interactions between plants, bacteria, and a biostimulant in kerosene-contaminated soils. By directly comparing the performance of vetiver and water spinach under identical enhanced conditions, the study aims to identify the most effective and synergistic combination, providing novel insights into tailored remediation strategies.

The primary objective of this study is to rigorously evaluate and compare the effectiveness of *Vetiveria zizanioides* and *Ipomea reptans Poir* in remediating kerosene-contaminated soil, both independently and when enhanced with bioaugmentation (*Bacillus subtilis*) and biostimulation (eggshell powder). Specifically, the research seeks to quantify the efficiency of TPH removal, monitor microbial population dynamics (Total Plate Count), and assess plant growth responses under different treatments. The anticipated contributions are twofold: practically, this study aims to deliver a validated, sustainable, and cost-effective soil remediation protocol applicable to real-world scenarios; academically, it will enrich the fields of phytoremediation and bioremediation by elucidating the synergistic mechanisms between plants, bacteria, and organic amendments, thereby guiding future innovations in environmental restoration technologies.

RESEARCH METHOD

Plant Preparation

The *Ipomea reptans Poir* (land kale) plants used in this study were obtained from seedlings grown on rockwool for one week. Meanwhile, the *Vetiveria zizanioides* plants were treated with vitamin B1 by soaking their roots for five minutes in a solution prepared with one tablespoon of vitamin B1 mixed into five liters of water. The application of vitamin B1 aimed to minimize plant stress during transport and to accelerate the growth of roots, stems, and leaves. After preparation, both *I. reptans* and *V. zizanioides* were planted in the sample medium within the reactor and placed in a shaded area with sufficient exposure to sunlight.

Bacterial Rejuvenation

Cultures of two bacterial isolates were prepared by growing them on oblique agar containing Nutrient Agar (NA; Merck, USA). Using the scratching method, the isolates were incubated at 37°C for 24 hours. This procedure was performed to rejuvenate and prepare the bacteria for use in the experimental process.

Phytoremediation Process

The phytoremediation experiment was conducted for 15 days using *Ipomea reptans Poir* and *Vetiveria zizanioides*, with the addition of 10% v/v *Bacillus subtilis* and a 10% w/w eggshell stimulant. The bioremediation was carried out in glass reactors with dimensions of 27 × 17 × 23

cm (Saputri & Purwanti, 2024). The soil samples used were spiked soils prepared by mixing Lumajang sand with kerosene at a concentration of 5% v/v bulk density.

RESULTS AND DISCUSSION

Total Petroleum Hydrocarbon (TPH)

The mechanism of elimination of organic matter in the form of *Total Petroleum Hydrocarbon* (TPH) kerosene can be caused by the ability of bacteria and the absorption of plant roots in kerosene-contaminated soil. The TPH allowance on kerosene-contaminated soil can be seen in **Figure 1**.

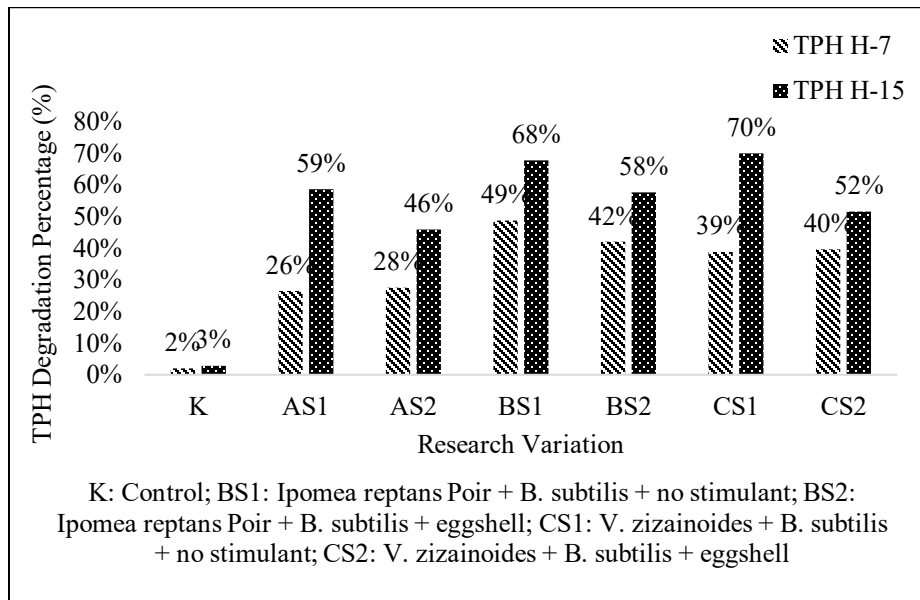


Figure 1. Percentage of TPH Degradation in Soil

The best percentage of elimination was CS1 with a variation of *Vetivera Zizainoides* and *Bacillus subtilis* bacteria on H-15 of 70% with the result of a TPH degradation rate of 632 mg/kg/day according to **Figure 2**. The results of the degradation rate of CSI variation were able to obtain an optimal TPH value <0.001% on day 20.

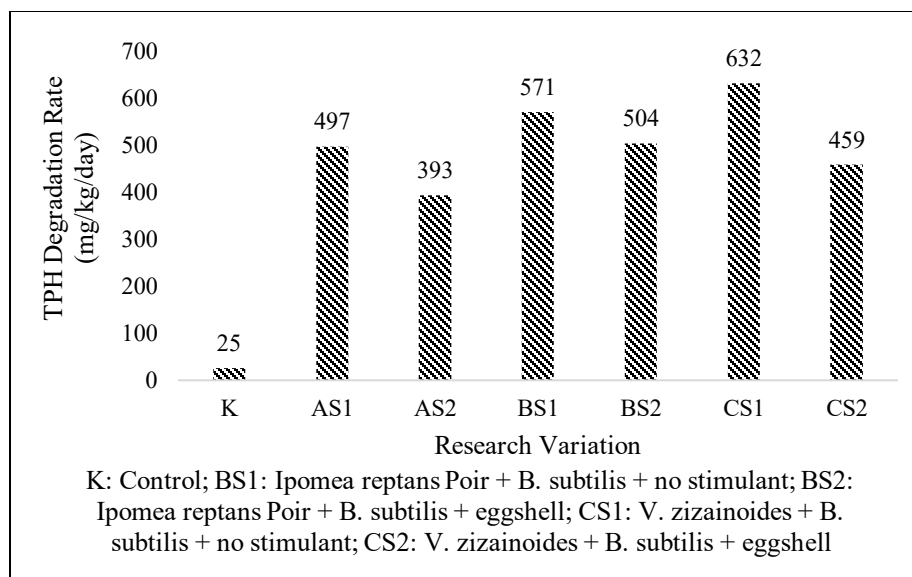


Figure 2. TPH Degradation Rate in Soil

This was followed by BS1 treatment (*I. reptans* + *B. subtilis* without stimulants) with a TPH degradation percentage on H-15 of 68% and a degradation rate of 571 mg/kg/day. Then BS2 (*I. reptans* + *B. subtilis* + eggshell) had a TPH degradation percentage of 58% and a TPH degradation rate of 504 mg/kg/day. Meanwhile, a decrease in efficiency was seen in treatment with biostimulants such as CS2 (459 mg/kg/day) and AS2 (393 mg/kg/day). This suggests that the presence of plants and bacteria has a major role in bioremediation, while the effectiveness of eggshells as biostimulants depends on biological interactions and soil conditions. In addition, these results confirm that *Vetiveria zizanioides* plants have a deep root system and are able to increase soil aeration and support microbial activity, such as *B. subtilis*, in degrading hydrocarbon compounds (Sinha et al., 2021; Oyedeji et al., 2022). *B. subtilis* itself is known to produce biosurfactants and important enzymes such as oxygenase that facilitate TPH degradation (Elumalai et al., 2021; Rahman et al., 2022).

The occurrence of an increase in the percentage of TPH elimination is influenced by enzymes produced by bacteria to break down the initial intracellular organic pollutants. The enzymes used are in the form of oxygenase enzymes that carry out oxidative and activate processes as well as the incorporation of oxygen. This process will convert organic pollutants into intermediates from intermediate metabolism such as the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from precursor metabolites such as Acetyl Co-A and pyruvate until it finally produces H₂O and CO₂ (Paramata & Purwanti, 2022). Bacterial addition *Bacillus subtilis* As hydrocarbonoclastic bacteria, both have the enzymes needed to break down the carbon source from N-alkane kerosene into simpler compounds so that the energy obtained is also more to reduce kerosin levels. The amount of carbon in the reactor also affects the process of reducing TPH where the availability of energy sources for bacteria is very abundant so that the bacterial process does not need to compete in using carbon sources.

The addition of eggshells as a biostimulant is intended to improve the chemical condition of the soil. However, decreased effectiveness in treatment with eggshells (BS2, CS2) compared to without stimulants (BS1, CS1) suggests that an excessively high pH increase may inhibit the activity of certain microbes (Duarte et al., 2020). Several studies show that eggshells are effective as soil amendments, but the dosage and time of application must be adjusted to environmental conditions (Rukmini et al., 2020; Putra et al., 2021).

Number of Bacterial Colonies

The role of microorganisms in the bioremediation of hydrocarbon-contaminated soils is crucial, especially bacteria that have the ability to degrade complex organic compounds such as *Total Petroleum Hydrocarbon* (TPH). Graphs of the number of bacterial colonies (Log CFU/ml) in the various treatments (**Figure 3**) show that there was an increase in the bacterial population over time, especially on day 15 (H-15), with the highest variation achieved by the BS2 treatment (*Ipomoea reptans Poir + Bacillus subtilis + eggshell*) of 6.3 Log CFU/ml on D-7, followed by CS2 (*Vetiveria zizanioides + B. subtilis + egg shell*) of 6.2 Log CFU/ml on D-7, and BS1 (*I. reptans + B. subtilis* without stimulant) %6 Log CFU/ml on D-7.

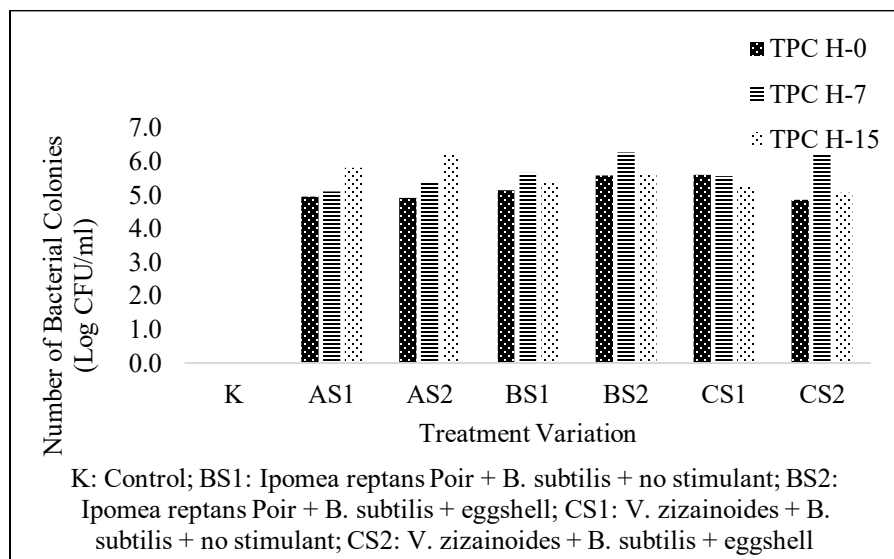


Figure 3. Number of Bacterial Colonies in the Soil

The increase in the number of bacterial colonies from day 0 to day 15 reflects the growth and biological activity that supports the bioremediation process. The presence of *B. subtilis* as a bioaugmentation agent is known to have the ability to produce biosurfactants and enzymes such as mono-oxygenase alkanes and dehydrogenase, which are effective in breaking down hydrocarbon chains (Rahman et al., 2022; Elumalai et al., 2021). The higher number of colonies in the treatment with eggshells (BS2 and CS2) suggests that eggshells as biostimulants are able to improve soil conditions, especially by increasing pH and providing calcium carbonate as a source of minerals (Rukmini et al., 2020; Duarte et al., 2020).

The number of bacterial colonies and TPH degradation clearly appear to have a positive correlation and correlation when comparing this graph with the rate of TPH degradation (**Figure 1**). The CS1 and BS1 treatments that showed the highest TPH degradation also showed high bacterial populations although not optimally. This suggests that the effectiveness of TPH degradation is determined not only by the number of colonies, but also by the metabolic activity of microorganisms as well as interactions with plants (Azubuike et al., 2022). The roots of plants such as *V. zizanioides* are able to promote the growth of decomposing microorganisms, accelerating the biodegradation process (Sinha et al., 2021; Oyedeki et al., 2022).

However, although BS2 and CS2 showed a high number of colonies, their TPH degradation rate was lower than that of the eggshell less treatment (BS1 and CS1). This indicates that population increase is not always linear with increased degradation. One possible cause is an imbalance in the C/N/P ratio due to the addition of eggshells that can interfere with the efficiency of bacterial metabolism (Zhang et al., 2023). In addition, excessive pH increases can inhibit the activity of certain enzymatic degradation that are sensitive to alkaline conditions (Putra et al., 2021).

3.1 Plant Growth Rate

The height of kale and *Vetiveria zanioides* on the 0th, 7th, and 15th days is presented in the graph **Figure 4**. The growth rate of Kale plants ranges from 0.8 – 1.5 cm/day and *Vetiveria zanioides* ranges from 0.1 – 0.2 cm/day according to **Figure 5**.

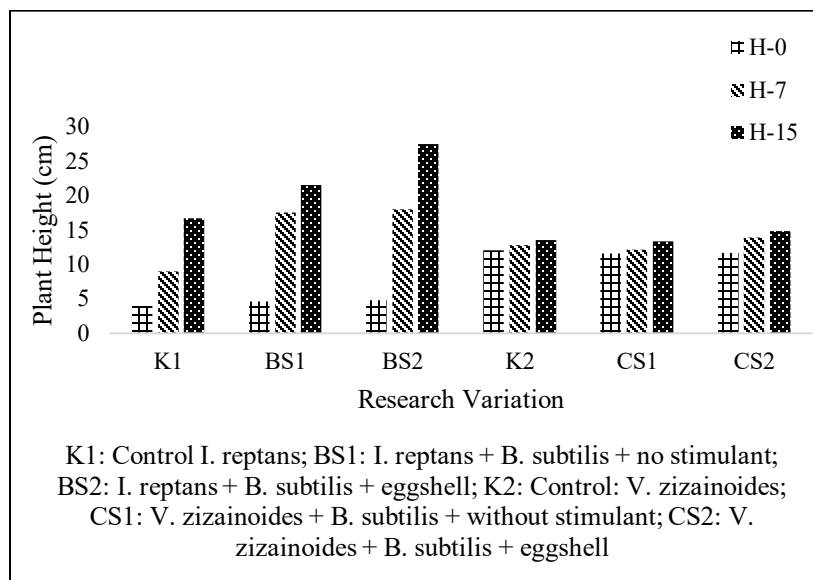


Figure 4. Plant Height *Ipomea reptans* Poir and *Vetiveria zizanioides*

Based on **Figure 4**, shows that the plant height data on days 0, 7, and 15. The BS2 variety (*Ipomoea reptans* + *B. subtilis* + eggshell) recorded the highest plant height on day 15 (about 27 cm), followed by BS1 (no stimulant) and K1 (control). In contrast, varieties that use *Vetiveria*

zizanioides (K2, CS1, and CS2) show much slower growth, with the average plant height reaching only about 13–15 cm on day 15.

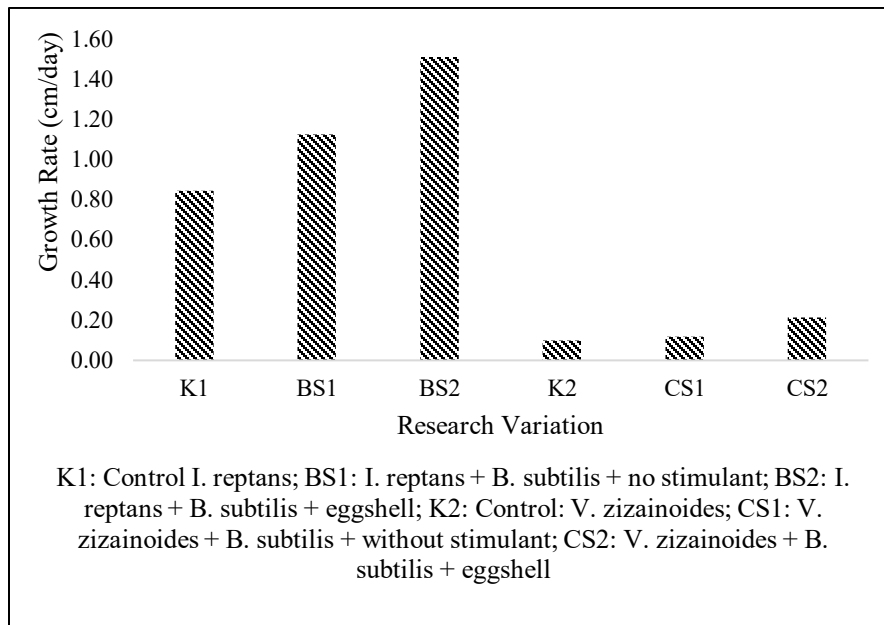


Figure 5. Plant Growth Rate *Ipomea reptans* Poir and *Vetiveria zizanioides*

Based on the growth rate graph in **Figure 5**. BS2 has the highest growth rate, around 1.5 cm/day. This indicates a better physiological response of the plant to the improved soil environment. In contrast, the plant growth rate in CS1 and CS2 is only around 0.2–0.3 cm/day, indicating slower adaptation or less than optimal soil conditions.

The relationship between plant growth and TPH's remediation ability is strongly seen in the third graph. BS2 showed the highest rate of TPH degradation (68% on day 15), suggesting that the combination of *Ipomoea reptans* plants, *B. subtilis* bacteria, and eggshells had a very effective synergy in breaking down hydrocarbon fractions in the soil. The role of *B. subtilis* in the biodegradation process of complex organic compounds has been confirmed in many studies, as these bacteria produce enzymes such as dehydrogenase and peroxidase that accelerate the degradation of toxic compounds (Wu et al., 2020).

Kale (*I. reptans*) is an annual, fast-growing, and has a relatively short life cycle, usually around 30–45 days until harvest (Sutaryo et al., 2020). This plant is known to be adaptive to flooded conditions and has a shallow but fast-spreading fibrous root system. These characteristics allow kale to utilize nutrients quickly and stimulate the growth of rhizosphere microbes in a short period of time. Kale also has the physiological ability to tolerate heavy metal contaminants and hydrocarbons through phytoextraction and phytodegradation processes (Widiastuti et al., 2021).

In contrast, vetiver (*V. zizanioides*) is a perennial grass plant with a long lifespan, able to live up to many years. Vetiver has a deep and very dense fibrous root system, capable of reaching depths of more than 3 meters, which makes it very effective in strengthening soil structures and

controlling erosion (Truong et al., 2017). This root system also increases the contact zones of the microbes involved in remediation. However, because vetiver has a slower growth rate, its effects on soil quality improvement and degradation of pollutants such as hydrocarbons tend to last longer than kale (Kisku et al., 2019).

In addition, the addition of eggshells as a biostimulant improves soil pH and increases cation exchange capacity (KTK), which ultimately improves microbial conditions and nutrient availability for plants (Ahmed et al., 2022). The calcium carbonate content in the eggshell helps neutralize soil conditions that are too acidic due to kerosene contamination, supporting root growth and nutrient absorption.

CONCLUSION

Based on the comprehensive analysis of the research findings, it can be conclusively determined that the integrated application of phytoremediation with bioaugmentation presents a highly effective strategy for the remediation of kerosene-contaminated soil. The study successfully demonstrates that *Vetiveria zizanioides*, when augmented with *Bacillus subtilis*, achieved the highest Total Petroleum Hydrocarbon (TPH) removal efficiency of 70% (632 mg/kg/day), significantly outperforming other treatments. This superior performance is attributed to the synergistic relationship between vetiver's extensive root system—which enhances soil aeration and provides a large surface area for microbial colonization—and the hydrocarbon-degrading enzymatic capabilities of *B. subtilis*. The finding that *Ipomea reptans Poir* also exhibited substantial remediation potential (68% TPH removal with bacterial augmentation) further underscores the viability of plant-based solutions. However, the paradoxical outcome concerning the eggshell biostimulant—increasing bacterial population (Log CFU/ml) while reducing TPH degradation efficiency—highlights a critical nuance: enhancing microbial abundance does not automatically translate to improved pollutant breakdown, likely due to pH-induced shifts in microbial community structure or metabolic pathways.

For future research, several avenues are recommended to build upon these findings. First, a detailed microbial community analysis (e.g., using 16S rRNA sequencing) should be conducted to elucidate the specific shifts in rhizosphere microbiome structure and function caused by the addition of eggshells, identifying which bacterial taxa are favored or inhibited and their respective roles in hydrocarbon degradation. Second, investigating the optimal dosage and particle size of eggshell powder, as well as exploring other organic amendments with buffering capacities, could help fine-tune the biostimulation process to avoid alkaline stress while still providing beneficial minerals. Third, long-term mesocosm or field studies are essential to validate these laboratory results under realistic environmental conditions, assessing the sustainability of remediation and the potential for residual toxicity. Finally, future studies should explore combining these botanical-bacterial systems with other remediation techniques, such as electrokinetics or nano-enhanced remediation, to develop multi-pronged strategies for addressing higher levels of contamination or mixed pollutants.

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