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Integrated Microbial Bioaugmentation for Remediation of Industrially Contaminated Soil: Impacts on Heavy Metal Reduction, Gas Mitigation, and Geotechnical Sustainability

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Article

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Posted Date: May 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9610169/v1

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Additional Declarations:
No competing interests reported.

Figures are available in the Supplementary Files section.

Title

Integrated Microbial Bioaugmentation for Remediation of Industrially Contaminated Soil: Impacts on Heavy Metal Reduction, Gas Mitigation, and Geotechnical Sustainability

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Abstract

The growing challenge of Industrial contaminated soil along with rising greenhouse gases (GHGs) and toxic air pollutants poses significant threats to environmental and geotechnical sustainability. This research explores the dual role of microbial bioremediation specifically bioaugmentation and biostimulation in addressing these issues. The study focuses on improving soil engineering properties while simultaneously contributing to atmospheric detoxification.

Degraded soil samples are to be collected from industrial zones of Ankleshwar (Gujarat, India) will be treated using selected bacterial cultures (bioaugmentation). A combination of geotechnical and microbial analyses including grain size distribution, Atterberg limits, Standard Proctor test, specific gravity, Heavy metal analysis will be use to assess soil behavior. Advanced microbial characterization techniques such as metagenomic analysis, GC-MS will be applied to identify strains capable of degrading hydrocarbons, PAHs and sequestering gases including NOx, SO_2, VOCs, CO_2, CH_4, and N_2O.

The results demonstrate that integrated microbial interventions enhance both environmental remediation and soil strength offering sustainable and low cost solutions for contaminated sites. This study underscores the transformative potential of soil bacteria in linking climate change mitigation, air pollution control and geotechnical resilience contributing to scalable approaches for industrially impacted regions.

Keywords

Contaminated Soil, Microbial Remediation, Bio augmentation, Greenhouse gas mitigation, Geotechnical sustainability, Soil Bacteria

1. Introduction

Soils are one of the most complex and dynamic components of the Earth system. Soils regulate biogeochemical cycles, filter environmental pollutants, store carbon, maintain hydrological balance, and support infrastructure. Modern challenges such as rapid industrialization, urban expansion, and intensive agriculture have placed unpredicted pressure on soil health. In many industrial clusters, particularly regions like *Ankleshwar* in Gujarat soils have become contaminated with toxic gases, volatile organic compounds (VOCs), polyaromatics, and heavy metals (Prasath, B.B. et. al., 2025). These contaminants not only threaten soil fertility but also deteriorate atmospheric quality by slowly releasing **CO_2, CH_4, N_2O, SO_2, H_2S, and VOCs** trapped within the soil matrix.

Globally, soil ecosystems play an important role as they can act as a source of greenhouse gases (GHGs) or act as biodegradation and other soil health activity. Soil microbes, bacteria, archaea, fungi are key parts to these transformations. They mediate nitrification, denitrification, methanogenesis, methane oxidation, sulphur oxidation, and hydrocarbon degradation processes. Their natural ability to break down toxic chemicals and immobilize pollutants forms the foundation of microbial remediation.

The soil pollution is defined as the contamination of soil by harmful and toxic chemical in enough concentration to harm human health and ecosystem. (Koushal S. et al., 2025) According to Franco et al. (2015), even if this substance does not pose an immediate risk, their presence is classified as pollution if they surpass the typical background levels expected for that specific soil profile.

Industrial activities release a wide spectrum of pollutants into soils, including hydrocarbons, solvents, heavy metal, dyes, pesticides, phenols, inorganic gases, and airborne particulates. These compounds infiltrate the soil through leakage, improper disposal, land deposition, or atmospheric deposition. Over time, contaminants accumulate and interact with soil minerals, organic matter, and microbial communities.

Contaminated soils disrupt microbial activity and increase greenhouse gas emissions, weakening soil strength and altering its natural behavior. These changes also affect permeability, influencing how gases and liquids move through the soil.

Therefore, modern remediation requires integration of microbiology with geotechnics**,** ensuring pollutant degradation without compromising soil mechanical properties. (Beattie et al., 2024, Sarkar et al., 2005).

This study is defined for reduction in soil contamination using soil bacteria and their effects on heavy metal removal, greenhouse gas mitigation and improvement of soil geotechnical properties, with major focus on microbial remediation and sustainable soil enhancement.

2. Materials and Methods

2.1 Study Area and Sample Collection

Soil samples were collected from the Ankleshwar industrial region, Gujarat, which is characterized by high levels of chemical contamination due to industrial discharge. The sampling locations were selected based on visible contamination indicators and proximity to industrial activity zones. Soil samples were collected at shallow depths to capture the most affected layer and stored in airtight containers to preserve their physicochemical characteristics. (Figure 1)

2.2 Gas Chrometography Analysis Of Toxic Gas /Ghg In Air Sample

Air samples containing toxic gases and greenhouse gases (GHGs) were collected from the Amlakhadi, Ankleshwar region using pre-cleaned, airtight Tedlar bags to prevent contamination and loss of volatile components. Samples were transported to the laboratory and analysed promptly to maintain integrity. A known volume of air sample mixed withS acetone was injected into a gas chromatograph (GC) using a gas-tight syringe under optimized operating conditions. (Figure 2) Calibration was performed using external standards (acetone as a reference) following ISO 16000-6 and USEPA TO-15 methods. A calibration curve was developed by plotting peak area against concentration. Gas concentrations in samples were determined using:

Concentration of gas = Peak area of sample/Slope of calibration curve

Compounds were identified based on retention time matching with standards. This method enables reliable quantification of VOCs and GHGs in air samples. (Cerniglia, C.E., et al., 2010)

2.3 Physical and Engineering Properties

Grain size analysis (IS 2720 part 4)

Grain size distribution was determined by sieve analysis in accordance with IS 2720 (Part 4) and IS 1498 (1970). Air-dried soil samples were sieved through a standard set of sieves covering coarse (80 mm to 4.75 mm) and fine fractions (2 mm to 75 μm), selected as appropriate for the soil type. The mass retained on each sieve was recorded to develop the particle size distribution curve. Soil classification parameters, namely the coefficient of uniformity (Cu) and coefficient of curvature (Cc), were calculated using:

- $Cu = D_{60} / D_{10}$
- $Cc = (D_{30})^2 / (D_{10} \times D_{60})$

where D_{10}, D_{30}, and D_{60} represent particle sizes corresponding to 10%, 30%, and 60% finer by weight, respectively. Based on fines content (<75 μm), soils were categorized as coarse-grained (<50% fines) or fine-grained (>50% fines).

Standard proctor test (IS 2720 part 7)

The Standard Proctor test was conducted to determine the optimum moisture content (OMC) and maximum dry density (MDD) of the soil. Air-dried soil passing a 4.75 mm sieve was mixed with varying water contents and compacted in a standard mould in three layers, each receiving 25 blows from a rammer dropped from a specified height. The bulk density was calculated from the mass of compacted soil and mould volume, and the dry density was obtained using:

$$\rho d = \rho / (1 + w)$$

where *w* is the water content. A compaction curve was plotted between dry density and moisture content to determine the OMC and MDD.

Specific gravity (is 2720 part 3).

Specific gravity was determined in accordance with IS 2720 (Part 3) using the density bottle (pycnometer) method for soil passing a 4.75 mm sieve. The clean, dry bottle was weighed (W_1), followed by the addition of oven-dried soil (W_2). The bottle was then filled with distilled water (or kerosene) and weighed (W_3). Finally, the empty bottle filled only with liquid was weighed (W_4). Specific gravity was calculated using these measurements. This method provides accurate results and is suitable for a wide range of soil types.

$$\mathbf{G} = \frac{W2-W1}{(W2-W1)-(W3-W4)}$$

2.4 Physico-Chemical Analysis of Industrial Soil.

Physico-chemical characteristics of contaminated industrial soil were analysed to determine baseline conditions affecting microbial activity and remediation efficiency. (Devatha CP., et al., 2019) (Figure 3)

Methodology for chemical analysis of soil

Ph determination

Soil pH was measured by preparing a suspension of 5 g air dried soil in 25–50 mL distilled water. After settling, pH was determined using indicator strips by comparing colour with a standard chart. (IS 2720 Part 26; ASTM D4972).

Acidity determination

Acidity was determined by titrating a soil suspension (5 g soil in 50 mL distilled water) with 0.05 N NaOH using methyl orange and phenolphthalein indicators. Results were expressed as mg/L as $CaCO_3$:

Calculations were performed as:

Acidity of Methyl orange (mg / L as $CaCO_3$) = $\frac{A\ X\ N\ X\ 1000\ x\ 50}{Sample\ volume}$

Acidity of Phenolphthalein (mg / L as $CaCO_3$) = $\frac{B\ X\ N\ X\ 1000\ x\ 50}{Sample\ volume}$

Total acidity (mg / L as $CaCO_3$) = $\frac{((A+B)\ X\ N\ X\ 1000\ x\ 50))}{Sample\ volume}$

Where:

A: NaOH volume used with methyl orange (ml), B: NaOH volume used with phenolphthalein (ml), N: Normality of NaOH, V = Volume of soil extract / sample (ml)

Alkalinity determination

Alkalinity was measured by titration with standard acid using phenolphthalein and methyl orange indicators. Results were expressed as mg/L as $CaCO_3$:

Calculations:

Alkalinity of Phenolphthalein (mg / L as $CaCO_3$) = $\frac{A\ X\ N\ X\ 50\ X\ 1000}{Sample\ volume}$

Alkalinity (Total) (mg / L as $CaCO_3$) = $\frac{B\ X\ N\ X\ 50\ X\ 1000}{Sample\ VOLUME}$

Where:

A: Volume of acid used up to phenolphthalein endpoint (ml), B: Total volume of acid used (phenolphthalein + methyl orange) (ml), N = Normality of acid (Hcl/ H_2SO_4), V = Volume of soil extract (ml)

Hardness determination (edta method)

Determined by EDTA titration using Eriochrome Black T indicator at pH 10:

Calculation:
Hardness (mg/L as $CaCO_3$) = $\frac{(Volume\ of\ EDTA \times 1000)}{Volume\ of\ sample}$

Chloride estimation

Estimated using Mohr's argentometric method with $AgNO_3$:

Calculation:
Chloride (mg/L) = $\frac{(Volume\ of\ AgNO_3\ (ml) \times Normality \times 1000 \times 35.5)}{Volume\ of\ sample}$

Where:

N: Normality of AgNO3, 35.5: Weight of chloride

Phosphate estimation (stannous chloride method)

Determined by stannous chloride (molybdenum blue) method at 690 nm using a calibration curve. Values were expressed as mg/L as P and converted to PO_4^{3-}. APHA (2017), IS 3025 (Part 31), and ASTM D515.

Final concentration was expressed as mg / L of phosphorus (P), and converted to phosphate (PO_4^{3-}) by multiplying with 3.06.

Phosphate (mg/L as P) = $\frac{Absorbance\ of\ sample}{Calibration\ curve}$

Phosphate (mg / L as PO) = (mg/L as P) x 3.06

Sulphate determination

Measured by turbidimetric method using $BaCl_2$, and concentration was obtained from calibration curve.

Sulphate (mg/L) = $\frac{Absorbance\ of\ sample}{Calibration\ curve}$

Hardness classification (interpretation) based on $caco_3$ equivalent:

Soft: 0–60 mg / L, Medium: 60–120 mg / L, Hard: 120–180 mg / L, Very Hard: >180 mg / L

Determination of total solids

Determined gravimetrically after evaporation and drying at 103–105°C.Following standard procedures adapted from APHA (2017) and IS 3025 (Part 15)**.**

$$\text{Total Solids (mg/L)} = \frac{(X-Y)\ x\ 1000}{Sample\ volume\ (ml)}$$

Where:

X: Total weight (Dried residue + dish (g)), Y: Dish weight (g)

Total dissolved solids (tds)

Measured after filtration and drying at 180°C.

$$\text{Total dissolved solids} = \frac{(X-Y)\ x\ 1000}{Sample\ volume\ (ml)}$$

Where:

X= Final weight of evaporating dish (g), Y= Initial weight of evaporating dish (g)

Total Suspended Solids (Tss)

Calculated from residue retained on filter paper. following standard procedures adapted from APHA (2017) and IS 3025 (Part 16)**.**

$$\text{TSS} = = \frac{(X-Y)\ x\ 1000}{Sample\ volume\ (ml)}$$

Where:

X=Weight of filter+ dried residue (mg), Y= Weight of filter (mg)

The total suspended solids is difference between the total solids (TS) and the total dissolved solids (TDS).

TSS= TS-TDS

Importance:

This analysis was carried out before bioaugmentation to evaluate the existing contamination level and environmental suitability for bacterial survival. These parameters directly influence microbial activity, nutrient availability, and pollutant degradation efficiency. Understanding baseline soil conditions helps in selecting appropriate bacterial cultures, optimizing growth conditions (supported by optical density studies), and ensuring effective remediation of toxic pollutants.

2.5 *D*etermination of Polycyclic Aromatic Hydrocarbon (PAHS) Using Gc-Ms:

Polycyclic aromatic hydrocarbons (PAHs) in soil were determined using gas chromatography mass spectrometry (GC–MS) following USEPA Method 8270D (2007) and ISO 18287 (2006). Soil samples were extracted with organic solvents (e.g., hexane/dichloromethane) and purified prior to analysis. (Figure 4) The extracts were injected into the GC–MS, where compounds were separated and identified based on retention time and characteristic mass spectra (m/z). Quantification was carried out using calibration standards. This method provides sensitive and reliable detection of trace-level PAHs for environmental assessment. (Agamuthu, P. et al., 2013, Lebkowska, M., et al., 2011, Li Q et al., 2020).

2.6 Heavy Metal Analysis of Soil by USEPA Method 3051A.

Heavy metals (Zn, Pb, Ni, Cd, Cr, Hg, and As) were analysed following USEPA Method 3051A using microwave-assisted acid digestion. Air-dried, sieved (<2 mm) soil (~0.5 g) was digested with concentrated HNO_3 in Teflon vessels, then diluted and filtered. Metal concentrations were determined using AAS or ICP-OES with standard calibration and blanks. This method ensures accurate and reliable quantification of metals in soil samples. (Alloway, B.J. (2013), Gadd GM. (2010), Rahman Z, (2020), Kursunoglu, S. (2025), Nies DH. (1999), Ouyang P, et al., (2023))

2.7 Selection of Bacterial Strains for Bioaugmentation

Based on the heavy metal analysis of the contaminated soil sample from Ankleshwar, elevated concentrations of zinc (155.64 mg/kg) and chromium (76.20 mg/kg) were observed, along with moderate levels of nickel (19.20 mg/kg) and lead (18.98 mg/kg). Trace amounts of cadmium, mercury, and arsenic were also detected. These results indicate a multi-metal contaminated environment requiring an efficient and resilient bioremediation strategy. Therefore, bioaugmentation was selected as a suitable remediation approach due to its ability to enhance metal immobilization, transformation, and detoxification through microbial activity. For this purpose, five bacterial strains were selected based on their known heavy metal tolerance, biosorption capacity, and biodegradation potential. (Armanu EG et al., (2025), Verma, N. et al., (2025), Beattie GA, et al., (2025))

Three strains— **1) *Enterobacter Cloacae*, 2) *Pseudomonas oryzihabitans*,** and **3) *Alcaligenes faecalis sub sp*. *Phenolics*** ,were procured from **MTCC, Chandigarh**, as these are well-characterized strains with documented resistance to heavy metals. These bacteria has reported that it is showing good activity such as bioaccumulation, enzymatic reduction, and extracellular polymeric substance (EPS) production, which enhance metal binding and reduce toxicity. (Figure 5) In addition, two strains **4) *Bacillus cereus*** and **5) *Pseudomonas aeruginosa***, were collected from Gujarat University. These strains were selected because of their adaptability to regional climatic conditions and proven efficiency for contaminated soil systems. *Bacillus cereus* is known for its spore-forming ability and high resistance to toxic environments, while *Pseudomonas aeruginosa* exhibits strong metabolic versatility and effective heavy metal biosorption and degradation capabilities. The selected bacterial strains exhibit multiple, complementary mechanisms for heavy metal remediation, including biosorption, intracellular accumulation, enzymatic reduction, biomineralization, and EPS-mediated sequestration. The synergistic action of

these processes results in effective detoxification and stabilization of heavy metals in contaminated soil, supporting the application of microbial bioaugmentation as a sustainable remediation strategy. (Pandey et al., (2019), Vidali M., (2001), Alexander M. (1999), Khan FI et al. (2004))

The bacterial strains used in the present study belong to diverse taxonomic families, reflecting their varied metabolic capabilities and environmental adaptability. *Enterobacter cloacae* is classified under the family Enterobacteriaceae, which comprises facultative anaerobic, Gram-negative bacteria commonly found in soil and water environments. *Pseudomonas oryzihabitans* and *Pseudomonas aeruginosa* both belong to the family ***Pseudomonadaceae***, known for their metabolic versatility and significant role in biodegradation and bioremediation processes. *Alcaligenes faecalis* subsp. *phenolicus* is a member of the family ***Alcaligenaceae***, which includes organisms recognized for their ability to degrade phenolic compounds and survive in contaminated environments. In contrast, *Bacillus cereus* belongs to the family ***Bacillaceae***, a group of Gram-positive, spore-forming bacteria that are highly resilient under adverse environmental conditions. Overall, the presence of these bacterial families highlights their potential application in environmental remediation, particularly in the degradation of pollutants and enhancement of soil quality. (Baweja P, et al. (2020), Somadas et al., (2025), Chia X., et al., (2024), Mitchell JK, et al., (2005), Bodor A, et al., (2020), Kumar, V. et al., (2018), Ventura M, et al., (2007).)

2.8 Activation of the Lyophilized Culture and Slant Grading

Lyophilized (freeze-dried) microbial cultures were revived by aseptically adding 1 mL of sterile distilled water to the vial to prepare a rehydrated suspension. The suspension was transferred onto a nutrient agar slant using a sterile loop and incubated under optimal conditions for growth. **(Figure 6)**

2.9 Inoculation of Microbial Bacterial Colonies

Selected pure microbial bacterial cultured in liquid nutrient media to promote metabolite production. Six sterile Erlenmeyer flasks were prepared with nutrient broth (100 ml) and inoculated with the selected pure strains (from slant) under aseptic conditions with flame sterilization (Figure 7) Nutrient broth composition: Peptone (0.5 g), beef extract (0.3 g), NaCl (0.5 g) dissolved in 100 mL distilled water. Bacterial strains (*Bacillus cereus*, *Pseudomonas aeruginosa*, *Enterobacter* sp., *Pseudomonas oryzihabitans*, and *Alcaligenes faecalis*) were cultured in nutrient broth at 37 °C for 24 h under shaking conditions. Growth was monitored at 600 nm and standardized using McFarland standards. The prepared inoculum (~100 mL) was added to 1000 g of soil, with moisture maintained throughout the study. (Zhang C, Bennett GN. (2005), Head IM, et al., (2006))

2.10 Compatibility Assessment of Bacterial Strains For Bioaugmentation.

The compatibility of five selected bacterial cultures was evaluated using the agar plate method. Nutrient agar plates were prepared, and each bacterial strain was inoculated on the same plate using streaking (cross-streak method). Following 24 hours of incubation at 37 °C, the plates were assessed for microbial growth, inhibition

zones, or overlapping colonies. The absence of inhibition zones indicated compatibility among bacterial strains, while clear zones suggested antagonistic interactions. The compatibility test is performed to ensure that selected bacterial strains can coexist without inhibiting each other when used as a consortium for bioaugmentation. Compatible bacteria enhance synergistic activity, leading to improved degradation of contaminants and better remediation efficiency. If incompatible strains are used, antagonism may reduce microbial growth and decrease the effectiveness of the treatment process. (DeJong JT. et al., (2023)

2.11 Bioaugmentation of Soil Sample Using Bacterial Culture

Bioaugmentation using five different bacterial strains applied to contaminated soil.

Name of Bacterial Culture,

1) ***13164 –Enterobacter Cloacae,***
2) ***13750 – Pseudomonas oryzihabitans***
3) ***13436 – Alcaligenes Faecalis sub sp. Phenolicus***
4) ***Bacillus Cerus***
5) ***Pseudomonas aeruginosa***

Bioaugmentation was employed to enhance the remediation of contaminated industrial soil by introducing selected heavy metal–resistant bacterial strains. Soil samples were collected, air-dried, sieved, and initially characterized. A measured quantity of bacterial inoculum (consortium) was then uniformly mixed with the soil under controlled moisture conditions, with nutrients added where required. The treated soil was maintained under natural conditions for 15 days with regular mixing to ensure aeration and uniform microbial activity. Moisture was monitored throughout to support bacterial growth. During this period, microbes interacted with contaminants through mechanisms such as biosorption and stabilization, reducing their mobility and toxicity. Post-treatment, soil samples were analyzed to assess contaminant reduction and improvement in soil properties. (Figure 8)

Bacterial Consortium Preparation:

The required volume of bacterial culture was calculated using the formula (1/OD) X Final Volume. Which was used for treatment of 1000 gm soil

Consortia (Five Bacteria Mix)

Individual strain were first grown in broth. The optical density was measured and each culture was adjusted to 0.5 McFarland standard. Following this 1 ml of each adjust culture was inoculated into sterile broth. A bacterial consortium was prepared by aseptically adding 1 ml of each actively growing bacterial broth culture into sterile flask. Incubated at 37 degrees for 24 hr.

2.12 Open Environment Incubation

Incubation of treated soil for 15 days under open environmental conditions with regular moisture maintenance. (Figure 9) The bioaugmentation procedure adopted in this study was based on standard soil microcosm testing

methods (ASTM E1197), soil moisture and sample preparation standards (ASTM D2216; ASTM C702), and microbial standardization techniques using McFarland turbidity standards, along with established bioremediation methodologies reported in literature (Alexander, 1999; Vidali, 2001).

2.13 Microbial Cultures and Bioaugmentation

Five bacterial strains were selected based on their known biodegradation capabilities. These included *Bacillus cereus*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Pseudomonas oryzihabitans*, and *Alcaligenes faecalis*. A microbial consortium was also developed to evaluate synergistic effects. The cultures were grown in nutrient broth under controlled laboratory conditions, and their growth was monitored using optical density measurements at 600 nm (OD_{600}). Soil samples were inoculated with bacterial cultures and incubated for a specified duration. Periodic observations were recorded to assess microbial activity and treatment effectiveness.

3 Results

3.1 Gas Chromatography Analysis

Gas chromatographic analysis revealed prominent peaks at retention times of 5.045 min and 5.313 min, contributing 97% and 99% of the total peak area, respectively. These peaks fall within the typical range of volatile organic compounds (VOCs), indicating significant organic contamination in the soil. The dominance of VOCs suggests the presence of industrial pollutants, which are known to alter soil behavior by reducing inter-particle bonding and shear strength, ultimately affecting bearing capacity and stability. (Table 1) Typical GC retention times: CO_2 (1.5–3.0 min), O_2 (1–2 min), N_2 (0.5–1.5 min), CH_4 (2–5 min), VOCs (5–20 min), H_2S (3–6 min), NH_3 (1–3 min).

3.2 Geotechnical Properties of Soil

Grain Size Distribution:
The soil exhibited a coefficient of uniformity (Cu) of 8.9 and coefficient of curvature (Cc) of 0.49, indicating moderately graded sandy soil. However, contamination may have modified the soil fabric by occupying pore spaces, thereby influencing permeability and drainage characteristics. (Figure 10, Table 2)

Compaction Characteristics:
The untreated soil showed an optimum moisture content (OMC) of 14.52% and a maximum dry density (MDD) of 1.877 g/cm³. The presence of contaminants likely reduced compaction efficiency due to interference with particle arrangement and lubrication effects, limiting the achievable density. (Figure 11)

Specific Gravity:
A reduction in specific gravity from 2.428 (untreated soil) to a range of 2.192–2.382 after treatment was

observed. This change indicates a decrease in dense contaminant content and reflects alterations in soil composition due to microbial activity.

Liquid Limit

The liquid limit of the soil was **38.32%**, indicating **medium plasticity**. Contaminants, particularly organics, tend to increase plasticity by modifying clay-water interactions.

3.3 Physico-Chemical Characteristics

The soil exhibited neutral pH, absence of acidity, moderate alkalinity, and low hardness. A notably high sulphate concentration (~1183 mg/L) was observed, confirming industrial influence. Elevated total solids content further supports the presence of accumulated contaminants in the soil matrix. (Table 3)

3.4 Heavy Metal and PAH Analysis

Initial analysis indicated the presence of significant concentrations of heavy metals such as zinc, chromium, nickel, and lead, while PAHs were below detectable limits. Heavy metals are known to interfere with microbial processes and soil biochemical cycles, contributing to environmental degradation and toxicity. (Table 4)

3.5 Microbial Growth and Compatibility

Optical density measurements confirmed successful microbial growth across all bacterial strains. Compatibility testing showed no inhibitory interactions among the selected strains, indicating their suitability for consortium-based bioaugmentation. The consortium demonstrated enhanced growth, suggesting synergistic microbial interactions. (Figure 12)

OD_{600} and cell counts: *Bacillus cereus* (1.450, 1.96×10^9 cells/mL, 69 mL), *Pseudomonas aeruginosa* (1.720, 2.32×10^9, 59 mL), *Enterobacter cloacae* (2.038, 2.75×10^9, 50 mL), *Pseudomonas oryzihabitans* (1.874, 2.53×10^9, 54 mL), *Alcaligenes faecalis* (1.538, 2.08×10^9, 65 mL), consortia (1.910, 2.58×10^9, 53 mL for 1000 g soil). (McFarland, J. (1907).

3.6 Heavy Metal Reduction after Bioaugmentation

A substantial decrease in heavy metal concentrations was observed following microbial treatment. The highest removal efficiency was achieved by the bacterial consortium, particularly for lead (~83%) and nickel (~63%). Individual strains also contributed to metal reduction through mechanisms such as biosorption, bioaccumulation, and enzymatic transformation. Variations in zinc concentration in some treatments may be attributed to microbial-induced mobilization of bound metals.(Table 5, 6)

3.7 Environmental and Geotechnical Implications

Heavy metal contamination disrupts microbial activity and influences greenhouse gas emissions, including methane (CH_4) and nitrous oxide (N_2O). The reduction of contaminants through bioaugmentation helps restore microbial balance and minimizes such emissions. Additionally, microbial activity improves soil structure by enhancing particle interaction and reducing heterogeneity, leading to better engineering performance. (Ivanov V, Chu J. (2008))

4 Discussion and Analysis:

The reduction in heavy metal concentration after microbial remediation treatment indicates effective biodegradation and immobilization of contaminants by soil bacteria. Pollutant concentration decreases. The experimental results demonstrate a significant variation in heavy metal concentrations after treatment with different bacterial strains and their consortia, indicating the involvement of multiple microbial remediation mechanisms. A notable reduction in chromium (Cr) concentration from 76.20 to lower values across treatments suggests effective bioreduction and biosorption, particularly by *Pseudomonas oryzihabitans* (77.55) and *Enterobacter cloacae* (68.25), which are known for enzymatic conversion of toxic Cr(VI) to less toxic Cr(III) (Gadd, 2010). Similarly, *Pseudomonas aeruginosa* and *Alcaligenes faecalis* also contributed to chromium removal through redox transformation and extracellular binding. In the case of zinc (Zn), an increase in concentration in some treatments (e.g., *Enterobacter cloacae* and *Pseudomonas oryzihabitans*) indicates possible mobilization or solubilization of bound zinc from soil matrices due to microbial activity, rather than direct removal. This behavior is consistent with microbial production of organic acids and chelating agents that enhance metal bioavailability (Wang & Chen, 2009). Nickel (Ni) and lead (Pb) showed a consistent decrease across all bacterial treatments, reflecting efficient biosorption and intracellular accumulation mechanisms. *Bacillus cereus*, due to its thick peptidoglycan cell wall, exhibited strong adsorption capacity, while *Enterobacter cloacae* and *Pseudomonas aeruginosa* demonstrated active uptake and sequestration of these metals. The lowest concentrations observed in consortia for Ni (7.15) and Pb (3.20) indicate a synergistic effect, where combined microbial activity enhances overall removal efficiency.(Chiemchaisri, C. et al., (2008), Zhu, B. et al., (2024), Wang, Y et al., (2022), DeJong. et al., (2010))

Overall, the results confirm that the selected bacterial strains utilize a combination of biosorption, bioaccumulation, enzymatic reduction, and extracellular polymeric substance (EPS) mediated binding to reduce heavy metal toxicity. These findings support the applicability of microbial bioaugmentation as an effective and sustainable approach for remediation of heavy metal–contaminated soils (Nies, 1999; Gadd, 2010). (Table 7)

Percentage reduction of heavy metal after bioaugmentation

Percentage reduction (%) = $\frac{\boldsymbol{Initial\ Value - Final\ Value}}{\boldsymbol{Initial\ Value}}\ \boldsymbol{x\ 100}$

Consortia show highest overall reduction, especially: Pb ≈ **83%** , Ni ≈ **63%**
Enterobacter & Bacillus: Less effective, even increase in Zn → not suitable alone from above data

Microbial consortia exhibited the highest overall removal efficiency, particularly for pb and Ni, indicating synergistic microbial activity. The percentage reduction analysis indicates that microbial consortia exhibited the highest efficiency in heavy metal removal compared to individual bacterial strains, particularly for lead and nickel. Some strains showed negative reduction suggesting metal mobilization instead of stabilization

Impact of heavy metal contamination on greenhouse gas and toxic gas emission

from soil.

Heavy Metals - GHGs emission

Heavy metal contamination significantly influences greenhouse gas emissions by altering soil microbial processes and biochemical cycles (Wang et al., 2021).

Methan (CH_4) emission

Studies have reported that heavy metal pollution can increase methane emissions due to shifts in microbial community structure (Wang et al., 2021).

Nitrous oxide (N_2O)

Heavy metals affect denitrification processes in soil, leading to variations in nitrous oxide emissions (Hammelehle et al., 2008).

Reduction of Heavy metal concentration in industrial soil using selected bacterial strain

The presence of heavy metals disrupts soil microbial activity, resulting in incomplete degradation of pollutants and increased gaseous emissions (Holtan-Hartwig et al., 2002).

1) ***Pseudomonas aeruginosa* –**

In this present study metal absorption of Cr, Ni and Pb ions by *Pseudomonas aeruginosa* was observed on the soil sample of ankleshwar industrial area. The atomic spectrometry results confirm the reduction in these ions in the soil. The result indicates that *pseudomonas aeruginosa* was most effective in lead 79.19% decreases. whereas bioremediation of zinc is less. Although *pseudomonas aeruginosa* was degrading metal at selected concentration. These results are in concurrence with the results of similar studies carried out by Awasthi et al, 2015 that proved Cr, Ni, Zn, Pb to be the heavy metal showing maximum affinity towards bioremediation by *P. aeruginosa*. (Li, X. et al., (2023))

2) ***Bacillus Cereus* –**

In this present study metal absorption of Cr, Ni and Pb ions by *Bacillus Cerus* was observed on the soil sample of ankleshwar industrial area. The atomic spectrometry results confirm the reduction in these ions in the soil. The result indicates that *Bacillus Cerus* was most effective in lead 66.91%. whereas the observed increases in zinc after treatment is attributed to microbial induced mobilization on metals from the soil matrix. Although *Bacillus Cerus* was degrading metal at selected concentration. These results are in concurrence with the results

of similar studies carried out by Anamika das et al, 2018 that proved the heavy metal showing maximum affinity towards bioremediation by *Bacillus Cereus.*

3) ***Enterobacter cloacae –***

In this present study metal absorption of Cr, Ni and Pb ions by *Enterobacter Cloacae* was observed on the soil sample of ankleshwar industrial area. The atomic spectrometry results confirm the reduction in these ions in the soil. The result indicates that *Enterobacter Cloacae* was most effective in lead 64.38%. whereas bioremediation of zinc is very less. Although *Enterobacter cloacae* was degrading metal at selected concentration. These results are in concurrence with the results of similar studies carried out by Anamika Das et al, 2018 that proved Pb to be the heavy metal showing maximum affinity towards bioremediation by *Enterobacter cloacae*.

4) ***Pseudomonas Oryzihabitans –***

In this present study metal absorption of Zn, Ni and Pb ions by bacteria strain was observed on the soil sample of ankleshwar industrial area. The atomic spectrometry results confirm the reduction in these ions in the soil. The result indicates that *pseudomonas Oryzihabitans* was most effective in lead 70.91%. whereas bioremediation of chromium is less. Although *pseudomonas Oryzihabitans* was degrading metal at selected concentration. These results are in concurrence with the results of similar studies carried out by *T. Płociniczak* et al, 2013 that proved Zn to be the heavy metal showing maximum affinity towards bioremediation by *P. Oryzihabitans*.

5) ***Alcaligenes Faecalis sub sp. Phenolicus –***

In this present study metal absorption of Cr, Ni, Zn and Pb ions by bacteria strains was observed on the soil sample of ankleshwar industrial area. The atomic spectrometry results confirm the reduction in these ions in the soil. The result indicates that *Alcaligenes faecalis sub sp. phenolics* was most effective in lead 75.61%. Although *Alcaligenes faecalis sub sp. phenolics* was degrading metal at selected concentration. These results are in concurrence with the results of similar studies carried out by Ndeddy Aka et al, 2016 that proved Cr, Ni to be the heavy metal showing maximum affinity towards bioremediation by *P. aeruginosa*.

6) ***Consortia –***

In this present study metal absorption of Cr, Ni, Zn and Pb ions by bacteria strain was observed on the soil sample of ankleshwar industrial area. The atomic spectrometry results confirm the reduction in these ions in the soil. The result indicates that *pseudomonas aeruginosa, Bacillus Cereus, Enterobacter Cloacae, pseudomonas Oryzihabitans, Alcaligenes faecalis sub sp. phenolics* was most effective in lead 83.14%. whereas bioremediation of Cr, Zn, Pb and Ni is effective in consortia observed after 15 days. All the selected metal decrees in effective manner. No reduction in percentage of heavy metal negatively or increases in original metal concentration. Although, The strain bacterial consortia was metal degrading at selected concentration. Similar conclusion observed in the metal degradation using consortia. These results are in concurrence with the results of similar studies carried out by Shaza Y. A. Qattan, 2025 that proved the heavy metal showing maximum affinity towards bioremediation by *P. aeruginosa*.

The metal uptakes results are indicated in the table 5.10 and 5.11. From observation after 15 days the bacteria showed exponential growth.

Environmental link

Soil contaminated with heavy metals acts as a source of greenhouse gases due to its impact on microbial dynamics and soil biochemical processes. Heavy metal contamination significantly affects greenhouse gas emissions from soil by altering microbial activity and biochemical processes. The presence of metals such as lead, cadmium, and zinc disrupts microbial communities, leading to increased methane emissions and variations in carbon dioxide flux (Wang et al., 2021). Additionally, heavy metals influence denitrification pathways, resulting in altered nitrous oxide emissions (Hammelehle et al., 2008). These findings reveal the vital role of heavy metals in regulating gaseous emissions from contaminated soils. Heavy metal contaminated soil acts as source of greenhouse gases due to their influence on microbial mediated carbon and nitrogen transformation. (Chen H. et al., (2024), Basheer, S. et al., (2024), Serrano-Silva, N., et al., (2014), Cox A., et al., (2020)).

Chromium (Cr) – Highest Risk Parameter

Strongest microbial enzyme inhibitor, disrupts nitrogen cycle severely, causes incomplete denitrification and leads to accumulation of Nitrous Oxide (N_2O)

Zinc (Zn) – Second Highest Risk

Present at the highest concentration, suppresses aerobic microbes and promotes anaerobic macrozones

Nickel (Ni) – Moderate Risk

Inhibits methane-oxidizing bacteria, allows methane accumulation and Supports sulphate - reducing bacteria

Lead (Pb) – Lowest GHG Risk, but Toxic

Low mobility, Strong soil binding and Less influence on carbon/nitrogen cycles. Lead is toxic to health, but least influential for climate-relevant GHG generation.

5 Conclusion

The findings of this study confirm that microbial bioaugmentation is an effective strategy for the remediation of heavy metal–contaminated soils. A significant reduction in metal concentrations was observed after treatment, with lead and nickel showing the highest removal efficiencies, particularly in the microbial consortium. Chromium reduction was also evident, primarily through bio reduction processes, while slight variations in zinc indicate possible microbial mobilization rather than direct removal. The comparative performance of individual bacterial

strains and the consortium highlights the importance of synergistic interactions. The consortium consistently demonstrated superior removal efficiency, suggesting that combined microbial activity enhances mechanisms such as biosorption, bioaccumulation, and enzymatic transformation. From a geotechnical perspective, contamination adversely influenced soil properties, including compaction behavior and particle interaction. The untreated soil exhibited an optimum moisture content of 14.52% and maximum dry density of 1.877 g/cm³, along with a specific gravity of 2.428. Following treatment, the reduction in specific gravity (2.192–2.382) indicates changes in soil composition due to microbial activity and partial removal or transformation of contaminants. These changes reflect an improvement in soil structure and stability. Physico-chemical analysis confirmed the presence of industrial contamination, particularly high sulphate levels and detectable heavy metals, whereas PAHs were below quantification limits. Grain size analysis indicated a well-graded sandy soil, which is susceptible to contamination-induced changes in pore structure and permeability. Microbial growth analysis demonstrated active proliferation and compatibility among the selected strains, with the consortium showing enhanced performance. In addition to reducing contaminant levels, microbial activity plays a role in restoring soil biochemical processes, which may help in reducing greenhouse gas emissions associated with contaminated soils.

Overall, the study demonstrates that bioaugmentation not only mitigates environmental contamination but also improves soil engineering properties. The combined environmental and geotechnical benefits highlight its potential as a sustainable and efficient approach for the restoration and management of contaminated industrial soils

Acknowledgement

The author acknowledges the importance of microbial biotechnology literature and previous research that contributed to the development of this review.

Conflict of interest

The author declares no conflicts of interest

CRedit author statement

Priyanka Oza: Contributed to conceptualization, literature collection, data interpretation and manuscript drafting.

Prof. Dr. Manish V. Shah: Contributed to critical review, technical inputs, and editing the draft of manuscript.

Prof. Shalini R. Singh: Contributed to conceptualization, literature collection, data interpretation and manuscript drafting.

Dr. Rakesh Panchal: Contributed to critical review, technical inputs, and editing the draft of manuscript.

Dr Jaimin Pandya: Contributed to conceptualization, literature collection, data interpretation and manuscript drafting.

All authors reviewed and approved the final version of the manuscript.

Funding declaration: No funding is received.

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Parameter	Report 1	Report 2
Major peak RT	5.045 min	5.313 min
Major peak area	4,933,375	14,500,949
% contribution	97 %	99 %
Minor peaks	10-38 min	10-35 min
Baseline behaviour	Gradual rise at 30-35min	Gradual rise at 30-35min

Table 1: Report data

Property	Value
D60	1.60
D30	0.375
D10	0.18
Cu	8.9
Cc	0.49

Table 2: Sieve analysis values

SR. NO.	PARAMETER	RESULT	UNIT	TEST METHOD
1	**pH**	**7**	**-**	**GU/SOP/MIC/505/2**
2	**Acidity**	**Absent**	**-**	**GU/SOP/MIC/505/2**
3	**Phenolphthalein Alkalinity**	**20 as caco3**	**mg/l**	**GU/SOP/MIC/505/2**
4	**Hardness**	**15 as caco3**	**mg/l**	**GU/SOP/MIC/505/2**
5	**Chloride**	**28.4**	**mg/l**	**GU/SOP/MIC/505/2**
6	**Sulphate**	**1183.745**	**mg/l**	**GU/SOP/MIC/505/2**
7	**Phosphate**	**27.214**	**ug**	**GU/SOP/MIC/505/2**
8	**TDS**	**0.57**	**g**	**GU/SOP/MIC/505/2**
9	**TSS**	**81.415**	**g**	**GU/SOP/MIC/505/2**
10	**Total Solids**	**81.985**	**g**	**GU/SOP/MIC/505/2**

Table 3: Chemical characteristic of soil

Sr. No.	Parameter	Result	Unit	Test Method
1	Zinc	155.64	mg/Kg	USEPA Method 3051A
2	Lead	18.98	mg/Kg	USEPA Method 3051A
3	Nickel	19.20	mg/Kg	USEPA Method 3051A
4]	Cadmium	0.69	mg/Kg	USEPA Method 3051A
5	Chromium	76.20	mg/Kg	USEPA Method 3051A
6	Mercury	0.09	mg/Kg	USEPA Method 3051A
7	Arsenic	2.08	mg/Kg	USEPA Method 3051A
8	Naphthalene	BQL	mg/Kg	GGMPL/SOP/Soil & Sediment/60
9	Fluoranthene	BQL	mg/Kg	GGMPL/SOP/Soil & Sediment/60
10	Phenanthrene	BQL	mg/Kg	GGMPL/SOP/Soil & Sediment/60
11	Pyrene	BQL	mg/Kg	GGMPL/SOP/Soil & Sediment/60
* BQL = Below Quantification Limit. (QL = 0.025)				

Table 4: Heavy Metal and PAH value

	Original	*Pseudomonas aeruginosa*	*Bacillus Cerus*	*Enterobacter Cloacae*	*Pseudomonas Oryzihabitans*	*Alcaligenes Faecalis sub sp. Phenolicus*	*Consortia*
Chromium (Cr)	*76.20*	48.13	67.15	68.25	77.55	60.17	49.97
Zinc (Zn)	*155.64*	131.2	162.59	175.16	119.96	157.86	124.14

Nickel (Ni)	***19.20***	**8.43**	**11.75**	**11.52**	**8.27**	**8.19**	**7.15**
Lead (Pb)	***18.98***	**3.95**	**6.28**	**6.76**	**5.52**	**4.63**	**3.20**

Table 5: Heavy metal analysis (After treatment) value

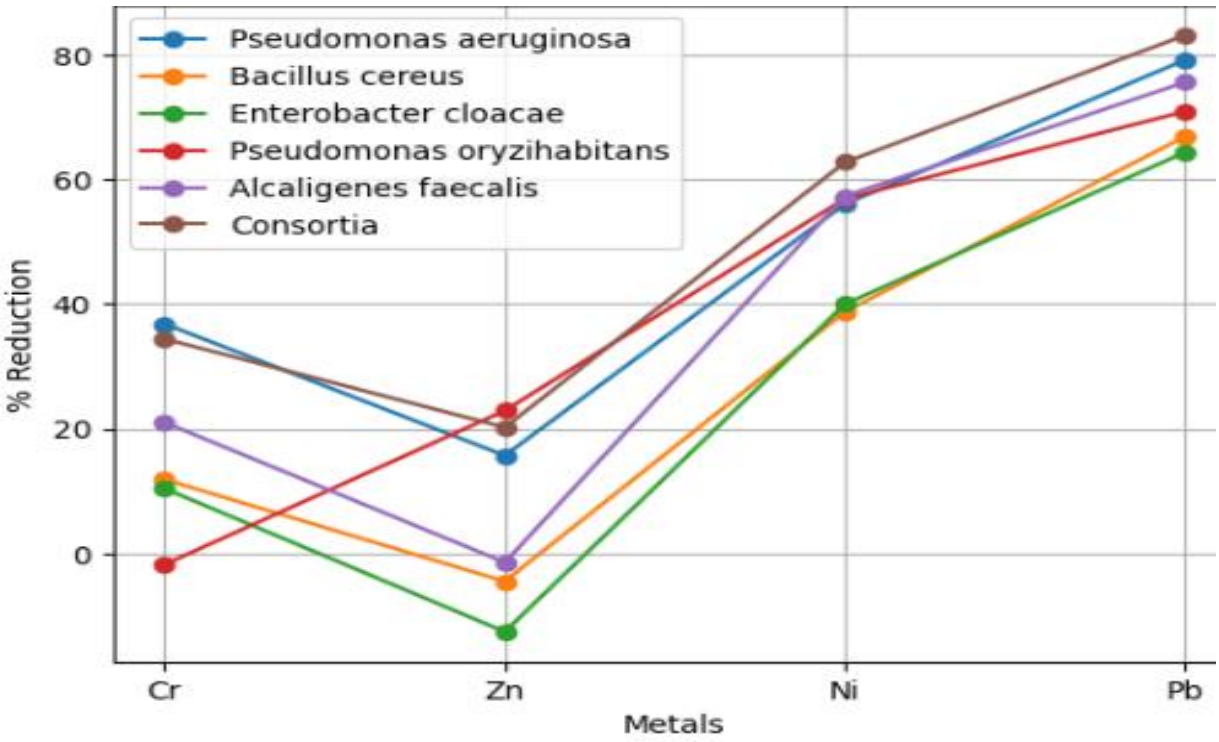


Table 6: Heavy metal analysis (After treatment

	Pseudomonas aeruginosa (%)	*Bacillus Cereus (%)*	*Enterobacter Cloacae (%)*	*Pseudomonas Oryzihabitans (%)*	*Alcaligenes Faecalis sub sp. Phenolicus (%)*	*Consortia (%)*
Chromium (Cr)	**36.84**	**11.88**	**10.43**	**-1.77**	**21.04**	**34.42**
Zinc (Zn)	**15.71**	**-4.47**	**-12.55**	**22.94**	**-1.43**	**20.24**

Nickel (Ni)	**56.07**	**38.80**	**40**	**56.93**	**57.34**	**62.76**
Lead (Pb)	**79.19**	**66.91**	**64.38**	**70.91**	**75.61**	**83.14**

Table 7 Percentage reduction of heavy metals after bioaugmentation

Fig1: Soil and air sample of Amla khadi, Ankleswar

Fig2: GC Analysis of Ankleswar air sample

Fig3: Physiochemical testing

Fig4: GC-MS

Fig5: Bacterial strain (Freeze dried) – MTCC, Chandigrah

1) ***Enterobacter Cloacae – MTCC 13164*****,**

2) ***Pseudomonas Oryzihabitans*** **– MTCC 13750**

3) ***Alcaligenes faecalis sub sp. Phenolics – MTCC 13436***

Fig6: Slant Grading

Fig7: Nutrient broth of bacillus Cerus

Fig8: Bioaugmentation of soil sample

Fig9: Open Environmental Incubation

Fig10: Sieve analysis

Fig11: Compaction Curve of untreated soil

Figure 12: Bacterial compatibility assessment

Supplementary Files

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