BIOREMIDATIONANDPHYTOREMDIATIONOFPETROLEUM CONTAMINATED SOIL

Abstract

Remediating soil contaminated by petroleum is a significant environmental problem that calls for long-term and practical solutions. In order to solve this problem, this study investigates the potential benefits of combining phytoremediation with bioremediation procedures. Whereas phytoremediation uses specialized plants to collect and detoxify pollutants, bioremediation makes use of microorganisms' inherent ability to degrade materials. Aim of the search is to assess the viability, effectiveness, and ecological consequences of thesetechniques.Systematicexperimentsrevealedthatpetroleumpollutantsinsoil canbeefficientlyreduced bybothphytoremediationandbioremediation.While phytoremediationdemonstratesthepotentialofspecific plant species to collect and mitigate contaminants, bioremediation benefits from a broad microbial community that breaks down complex hydrocarbons. A few variables that affect the effectiveness of both strategies are temperature, moisture levels, plant selection, and microbial activity. The use of bioremediation and phytoremediation both have benefits and drawbacks, which renders them complimentary methods. Site-specific elements including soil characteristics, pollutant kinds, and weather patterns affect its performance. Thesetechniquesarebeingimproveduponbyresearch,whichalsoincorporatescutting-edgetechnology like genetic engineering and nanoremediation.

Keywords: Contaminatedsoil,Petroleum,Bacteria, Phytoremediation, Bioremedation

1. INTRODUCTION

Phytoremediation, a form of bioremediation uses biological agents like bacteria, enzymes, and plants to removecontaminants,iscoveredintheintroduction. Theterm"remediation"referstotheprocessofcleaning up or getting rid of contaminants using these biological agents. These living things have the ability to mineralize (completely remove) contaminants or bio transform (change them into less hazardous forms) them. This is a procedure that many bacteria and fungus are capable of. Specifically, the utilization of plants to eliminateor repair contaminants is thesubjectof phytoremediation. Certain contaminants can bestored in the shoots or leaves of plants, increasing their bioavailability. Pollutants are either discharged into the atmospherebyvolatilizationorretainedin aninactiveconditionwithinplant cells.Plantportionscanthen be harvested to take out the contaminants that have been trapped. Microorganisms such as fungi, bacteria, and archaea are important in bioremediation.

2.0 MATERIALS

4 PlasticContainer: Theplasticcontainerusedweighs88.7kgitwasusedtoplant theokra and the mixing of the bacteria and the soil together.

Sand(loamysoil): Loamysoil,which consistsprimarilyofsand,silt,andalesserquantityofclay,isideal forplanting.Its mineralmakeupis composedofroughly40–20%sand,silt,and claybyweight.It alsohas the ideal balance, holding moisture while allowing oxygen to reach plant roots and being high in humus (organic matter). Additionally, the soil utilized in this project came from a farm along Umuoma Village, which is located beyond the school's grounds.

Petroleum(1Liter): ThePetroleumproductusedin thisprojectwasgotten afuelstationalong the school road umuoma village.

Okra: Theokraused inthisresearchworkwasobtainedfrommarket(Onitshamainmarket (ose).

Bacteria: Thebacteriausedinthisresearchwasobtainedinthemicrobiologydepartmentlab, Anambra state university, uli.

Glove: Gloveusedwas towearinothertocollectsoilsampleforanalysis.

Foil: Thefoilused was forthecollectionofsoilsample.

Meterrule:Meteruleusedinthis projectwas usedtodeterminetheheightoftheplant.

SETUPFOR PHYTOREMEDIATION

We bought the okra seeds from the major market in Onitsha (ose). These seeds have previously been sun-dried to promoterapid plantgrowth. Thestudy's top-loamy soilwas taken from thevillageof Umuomaalong theuniversityroadatadepthoffivecentimeters,andthepetroleumoil,knownas"Bonnylight,"camefroma commercial station located along the university road in Umuoma, Uli Anambra State, Nigeria. In the laboratory, planting containers weighing a consistent 2.5 kg apiece were set up. The groups underwent three replications, with the labels "treatment CS (control), CSB, CSP, and SP (control)". For three consecutive days, 20 milliliters of water were applied to each treatment and the control planting container days at seven o'clock GMT. In both the treatment and control experiments, three okra seeds were then sown in each planting container. At 7:00 GMT, daily watering continued. It was noted and observed when the seedlings in the treatments and the control experiment emerged The following formula was used to determine the percentage of seedling emergence in each treatment:

100(Totalnumberofseedlings thatsurfaced/Totalnumberofseedssowed)=Emergencyproportion(E %)

Furthermore,thefollowingformulawasusedtofind thegerminationvelocity co-efficient(cov)foreach treatment: The formula for the coefficient of velocity (COV) is as follows: COV = (A1 + A2 +... + A3) / (A1*T1 + A2*T2+...+A5*T5),whereA1,A2,...,andA3standforthenumberofseedlingsatvarioustimes,andT1, T2,..., T5 stand for the corresponding time intervals.

Where T is the number of days it took in order for seeds to grow and A is the number of seeds that germinated;recordswerekeptforfourweeksfollowingplanting(4WAP)foreach treatment.Usingthemeter rule, the planting height was measured, and the breadth and length of the leaf were also taken, noted, and continuously when a new leaf emerges and reaches a length of 2 cm and a width of 2 cm, it begins to deteriorate and fall off.

SAMPLE COLLECTION

Toguaranteethatwhensampling,asepticconditionsaresatisfiedasstatedbyEziuzorand Okpokwasili

(2009),thatthecompositesoilsamplewasgathered using asterilizedhandtroweland sterileplasticbuckets that was been cleaned with a cotton wool that was soaked at 70% ethanol. Following excavation, the soil used was collected from 4 different locations and then sent to Chukwuemeka Odumegwu Ojukwu University's Microbiology Laboratory for a preliminary physicochemical examination and bioremediation investigation. The fuel was purchased from a for-profit gas station located on University Road in Umuoma, Uli, Anambra State, Nigeria.

SOURCE OFBACTERIAL INOCULUM

Two microbes were isolated from oil contaminated soil of mechanic workshops in Uli but applied topetrolcontaminatedforthedegradation experiment.ThesewerePseudomonassp.3B andSerratiamarcescens9BwerecollectedfromChukwuemekaOdumegwuOjukwuUniversity,UliCampus, Department of Microbiology.

Inordertochoosebacteriaforthecreationofabacterialconsortium, thecapacityforseparatedstrainsto breakdownhydrocarbonswasascertained.Theeffectivenessofisolatedhydrocarbon-degradingstrainswere screened utilizing hydrocarbons as theonly energy sourceon BushnellHaas mediumwith DCPIP indicator. The bacterial strains chosen for the consortium's preparation were those that could break down petroleum hydrocarbons. Prior to the bioremediation study, physicochemical and microbiological studies were conducted. The Sattar group (2022)

BACTERIALCONSORTIUMPREPARATION

Weusedtheprocess describedbySattaretal.(2022)toformthebacterialconsortium.
Initially,wechosenewcolonies(those thatwereonly24hoursold)foreverybacterialstrain thatwasgoing to be part of the consortium. Following that, these colonies were placed into flasks filled with nutritional broth that was sterile.
Cottonplugswereusedtosealtheflasksduringincubationto avoidcontamination.Forsevendays,thewhole flasks used were all incubated at 150 rpm in a shaking incubator. Every 24 hours, we tracked the bacterial strains' development Following incubation, a fresh single bacterial colony (chosen from the isolated strains) was placed in the nutrient broth media, and the mixture was centrifuged for 10 minutes at 5000 rpm. After removal of the liquid supernatant, bacterial pellets were still present. A UVVIS spectrophotometer (Spectrum Lab752N,China)wasusedtoadjusttheopticaldensity(O.D.)ofeachpelletafterithadbeenindividuallyre- suspended in autoclaved distilled water to a value of 1 and Pure cultures of bacterial strains with comparable

volumesandanopticaldensityof1at660nmand106cellsper milliliter(106 cells/mL)were combined to create the final consortium.

BASELINESTUDYANDSOIL CONTAMINATION

The5Lplasticbucketholdingthe2500gofsoilwasfilledwithabout250 mLofgasoline.During thisstage, samples of the contaminated soil were taken for research purposes. A baseline research looks at the current situation to establish the starting point of a project. It also helps with impact monitoring after development and identifies the expected impact level (Ezekoye et al. 2015).

STUDYDESIGN

Thestudywasexperimentallydesignedbyadoptingthemethodof EziuzorandOkpokwasili, (2009) and the details is shown in Table 1 below:

Table1:ExperimentaldesignofBioremedation

BIOREMEDIATIONEXPERIMENT	
Experimental setup	Test experiment
Setup 1(control)	2500gramof soil+ 250mLof petrol + 20mLof water
Setup2	2500gramof soil+250mLof petrol +30mLof bacterialconsortium +20 mLofwater
Setup3	2500gram ofsoil+ 250mLofpetrol+threeseedsofOkraplant+20 mL of water
Step 4 (control)	2500gramof soil+ twoseedof Okraplant +20ml of water

ThesewascarriedoutexsituintheMicrobiologyLaboratory(COOU).Twothousand,five hundredgrams(2500g)ofthesoilwas mixedwith250mLofpetrolandalso waspreparedin3setups using 5 L plastic buckets and were left in the laboratory for 6 days. After contamination, 30 mL\

ofbacterialconsortiumand threeseedsofOkraplantwereaddedto thepetrolpollutedsoiland thecontrolwas notamended either of theadditives and itwas called zero hour as described by Ezekoye etal.(2015).Everytime, anewsterilespatulawasusedtoturnthesamplescarryingnutrientsand control, and 20 milliliters of sterile distilled water was used to wet them every 2 weeks.
Samples weretaken for laboratory analysis at1 week intervals on the1st, 7th, 14th, 21stand 28th days(Romanusetal.,2015).Thebioremediationof petroloilthroughoutthevariousexperimental setups were studied as described below:

PhysicochemicalAnalysis

PhysicalAnalysis

P^HAndConductivity Determination:

ThepH andconductivityweremeasuredusingdigitalmillimeter(DSS–11A,China)by adopting the standard method of AOAC (2012). The study's pH, conductivity, and temperature were measuredin thesamplesthatweretakenonbaseline days1,7,14,21,and28. Foreveryposition, three values were acquired, and the average of the values was utilized.

Examining Chemicals

NitrateDetermination: Using aspectrophotometer (Astell, UV - Vis Grating, 752 W), thenitratelevel was measured at 470 nm using the Brucine method as described by UNEP (2004) and also one milliliter of a clean test tube was put into soil filtrate, and also another test tube was filled with one milliliter of distilled water as a blank solution. Both test tubes were first filled with a half milliliter of Brucine reagent. Next, two milliliters of concentrated sulfuric acid were added, and the mixture was agitated to ensure homogenization. Theresultantsolutionwasmeasuredat470nmusing a 752NUV-VISspectrophotometerafterbeing allowed to cool to ambient temperature until it turned yellow.

DeterminationOfPhosphate:

Using a colorimetric approach, the phosphate content was calculated in accordance with UNEP criteria from 2004. At 660 nm, spectrophotometric measurements were made, and the results were compared to water standards that had been generated in the same way. A solution of one-tenth of 2.5% glacial acetic acid was made and used to extract the phosphate. In a 100 mL conical flask, it was combined with 2 grams of the sampleandswirledfor10minutes.A50mLsample extractwasthenobtainedand autoclavedfor30 minutes

at121^{o}CusingK2S2O8andH2SO4.Fivemilliliters ofammoniummolybdbatewereaddedto themixture duringautoclaving inordertocreateheteropolymolybdophosphoricacid.At30oC,stannouschloridewas used to further decrease this in an aqueous sulfuric acid media causing the creation of a molybdenum blue complex at 30^{o}C, A prepared water standard was used to assess the blue color's intensity using spectrophotometry. This method's detection limits is 0.01mg/L.

TOTALORGANICCARBON DETERMINATION:

The total organic carbon (TOC) was determined using the colorimetric method developed by Nelson and Sommers in 1975. A neat Pyrex conical flask was filled with one gram of the sample, five milliliters of potassium chromate solution, and seven and a half milliliters of strong sulfuric acid. After that, the mixture was heated for 15 minutes on an electromagnetic heater to cause reflux. After allowing the sample to reach roomtemperature,distilledwaterwasusedtodiluteittoafinalvolumeof100mL. Next,usingferroin asan indicator, after then, thesamplesolution was titrated with 0.2 M ferrous ammoniumsulfatein 20 milliliters. Potassium chloride, an oxidant, and sulfuric acid were combined in a blank solution, which was titrated against the sample A value log was kept. The computation adhered to a particular process for determining TOC.

%TOC=Sampletitre×0.003×100-Blanktitre Sample
mass

Theformulausedto calculatetotalorganicmatter (TOM) was % TOM = %TOCX1.724.

DeterminationOfTotalPetroleumHydrocarbon(Tph)Content:

The total petroleum hydrocarbons (TPH) was measured using the spectrophotometric technique that was created by Akpoveti et al. in 2011. N-Hexane was used as the extractive solvent in this examination, which wascarriedout atawavelengthof640nm.First,amechanicalshakerwasusedto agitateonegramof the soil sample for ten minutes after it had been dissolved in ten milliliters of hexane. The mixture was then filtered through Whatman's filter paper, and the filtrate was gently diluted by mixing extract a single milliliter from the with fifty milliliters of hexane. Using n-hexane as the blank reference, the absorbance of this diluted solution was measured at 460 nm using a UV-Vis spectrophotometer (model 752N). The total amounts of petroleum hydrocarbons were Throughout a period of 56 days, at intervals of two weeks.

QualityControl:

Inordertoensuretheaccuracyand consistencyof theprocess,weproducedproceduralblanksandstandard solutions the outcomes. During the TPH determination process, we performed replicate studies to get an average result that will be used to evaluate accuracy.

4.0 RESULT ANDDISCUSSION

RESULT OFPHYTOREMEDIATION

Theexperiment'sfindingsdemonstrateacleardifferencebetweenplantsgrowninsoilcontaminatedwith crude oil and those cultivated in soil that is not. Unlike seedlings planted in untreated soil (control), Abelmoschus esculentus treated with oil began to germinate after 6 days.

Table2:SeedlingEmergence(E%)And Co-EfficientOfVelocity(Cov)OfAbelmoschus Esculentus.

Treatment (Concentration ml)	E%	COV
1	100	
2	100	
3	66	
4	33	

Table 2 shows how crude oil affects Abelmoschus esculentus seedlings' emergence rate (e %) andthe velocityco-efficient(COV).Incleansoil,emergenceratesof100%wererecorded.Ata5%probabilitylevel, notable distinctions were seen between Abelschus esculentus plants in crude oil-contaminated and uncontaminated soil. The seedling emergence rate (e %) and COV declined as the oil content rose. Abelmoschusesculentuswasalsofoundtohaveyellowingleaves,falling leaves,stuntedgrowth,withsome plants dying four weeks after planting in a soil which was treated with a crude oil while others survived.

Table3:ResultOfSeedlings HeightsOn ContaminatedAndNon-Contaminated Soil

Treatment	Seedlingheights	Seedlingheights
Weeks	Contaminated soil+okra	Not-contaminated soil

0	0	0
1	2 cm	4.73cm
2	2.5cm	8.54
3	3.5cm	16.5
4	4.5 cm	32.3

TABLE3

The consequences of varying quantities of crude oil on the growth of Abelmoschus esculentus seedlings are shown in **Table 3.** Plants which grew in uncontaminated soil showed notable differences from those which was grown in a soil with comparable levels of crude oil. Regardless of the oil concentration, statistical analysisshowedthattheplantsinuncontaminatedsoilfared andwasdoingwellmorethan thoseoncein oil- polluted soil. But the effectivenessof the plants in oil-polluted

BASE LINE FEATURES OF DIESEL IMPACTED SOIL: The baseline physicochemical and microbiologicalproperties ofthediesel-impactedsoilwas summarizedinTable1.Accordingtotheresults, the following parameters were observed: pH 7.11, conductivity 0.57 mS/cm, temperature 16.50 °C, nitrate 12.09µg/kg,phosphate3.90mg/kg, totalorganiccarbon6.72%,totalorganicmatter740.44mg/kg,and total residual oil content 740.44 mg/kg.

Table4:Baselinephysicochemicalpropertiesofpetrolimpactedsoil

Parameter	Value
Ph	7.11
Conductivity(mS/cm)	0.57
Nitrate(NO_3)(µg/kg)	16.50
Phosphate(PO_4) (mg/ kg)	12.09
Total organiccarbon(% TOC)	3.90
Totalorganicmatter(%TOM)	6.72
Totalresidualoilcontent(mg/kg)	740.44

BIOREMEDIATIONPROFILE

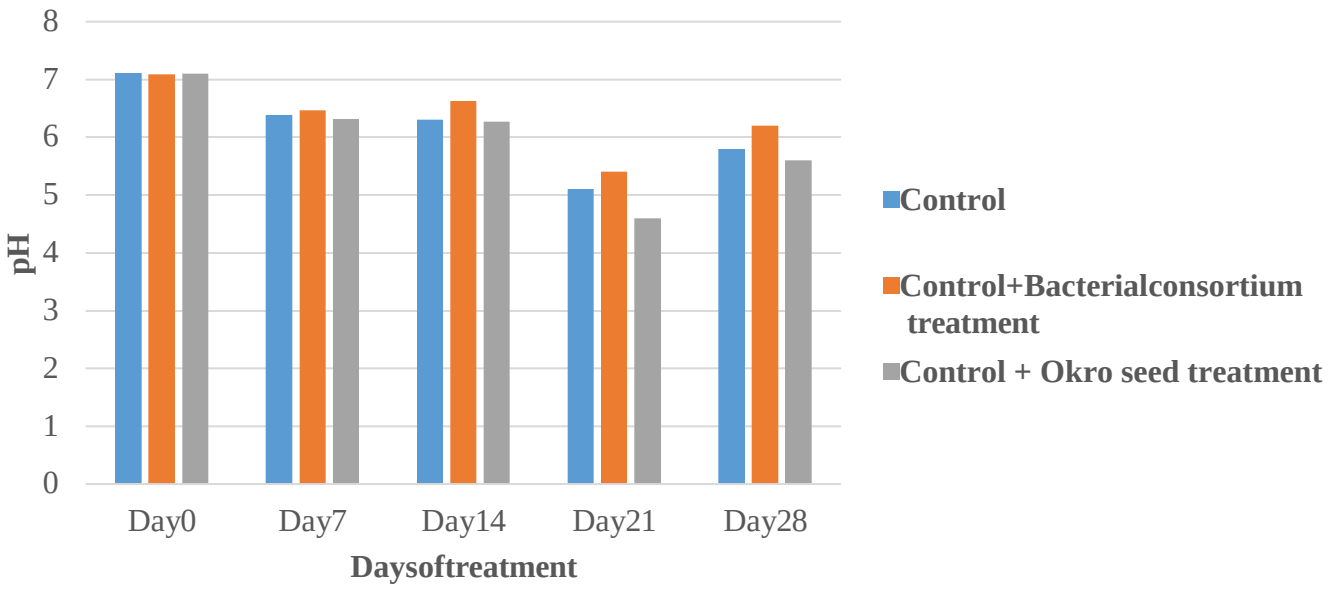

chart1:pHvariationsduringbioremediationtreatmentofpetrolcontaminatedsoil

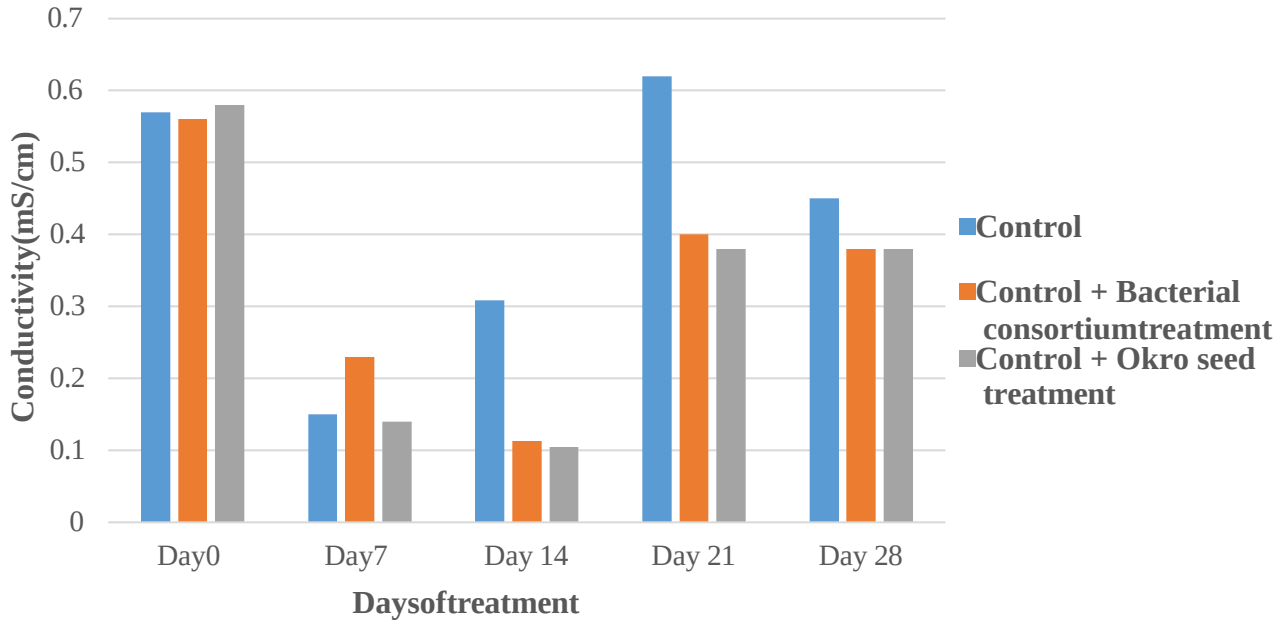

chart2:Conductivityvariationsduringbioremediationtreatmentofpetrolcontaminatedsoil

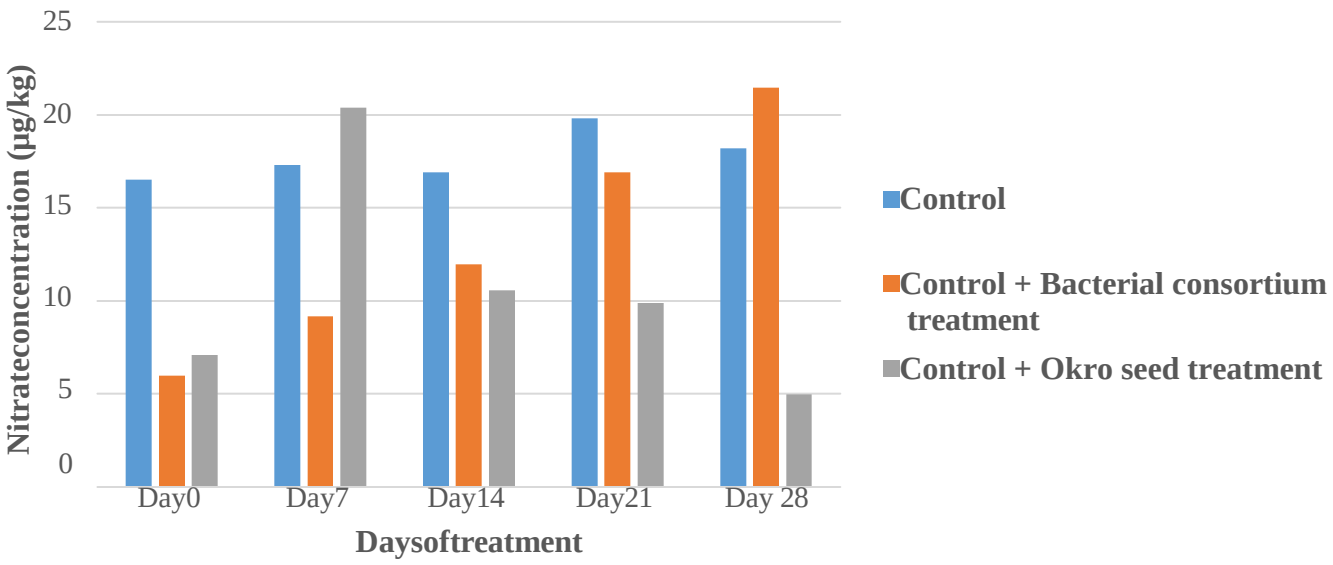

chart3:Nitratevariationsduringbioremediationtreatmentofpetrolcontaminatedsoil

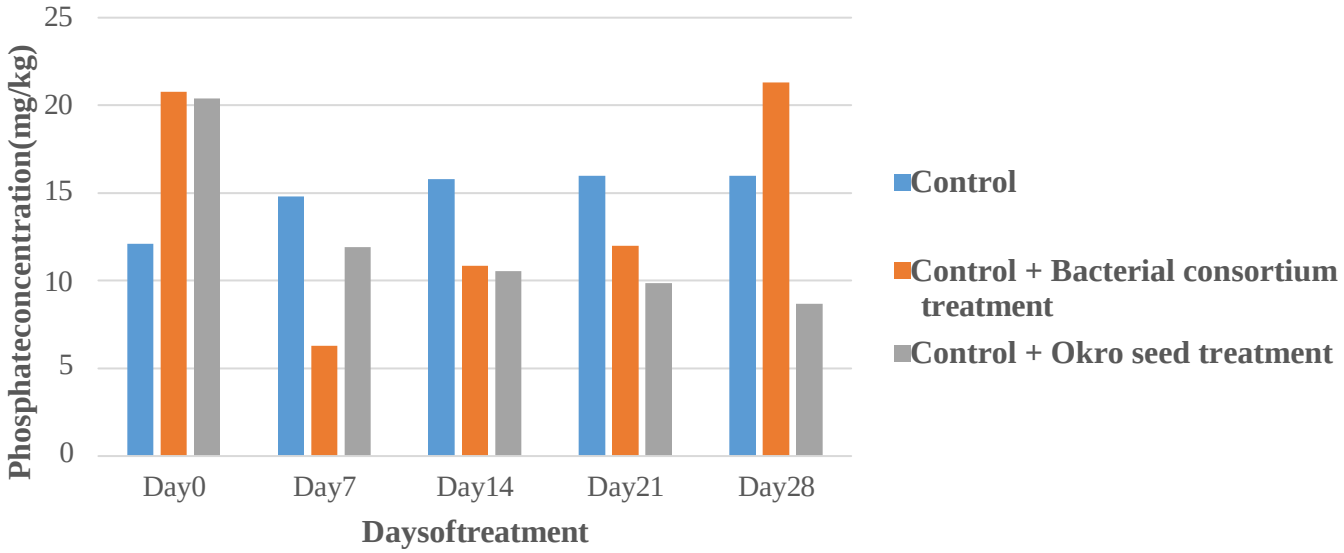

Chart4:Phosphatevariationsduringbioremediationtreatmentofpetrolcontaminatedsoil

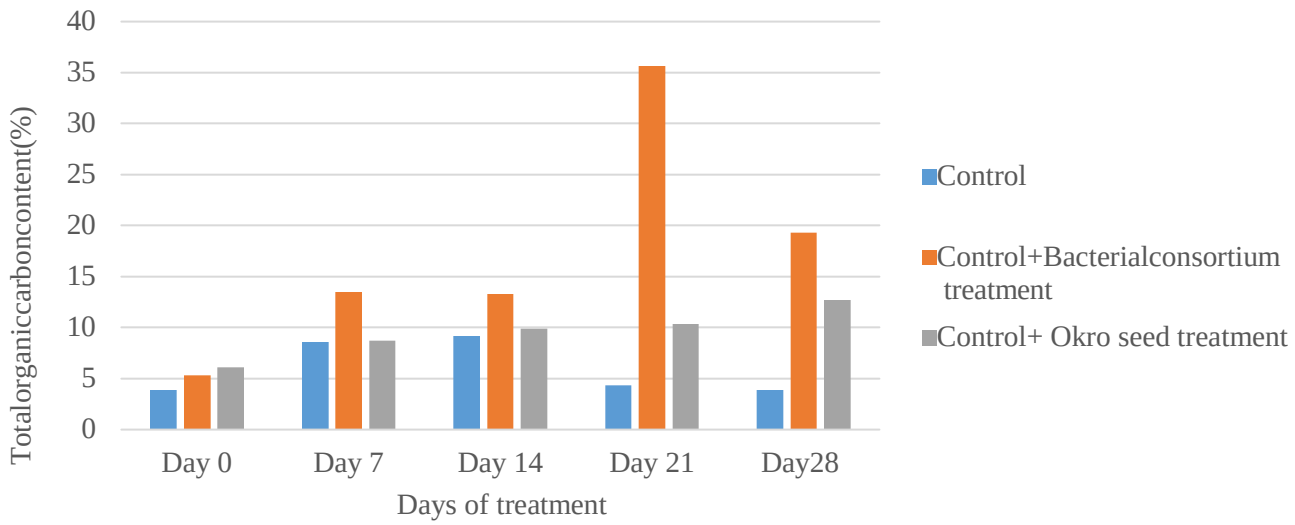

Chart5:Totalorganiccarbonvariationsduringbioremediationtreatmentofpetrol contaminated soil

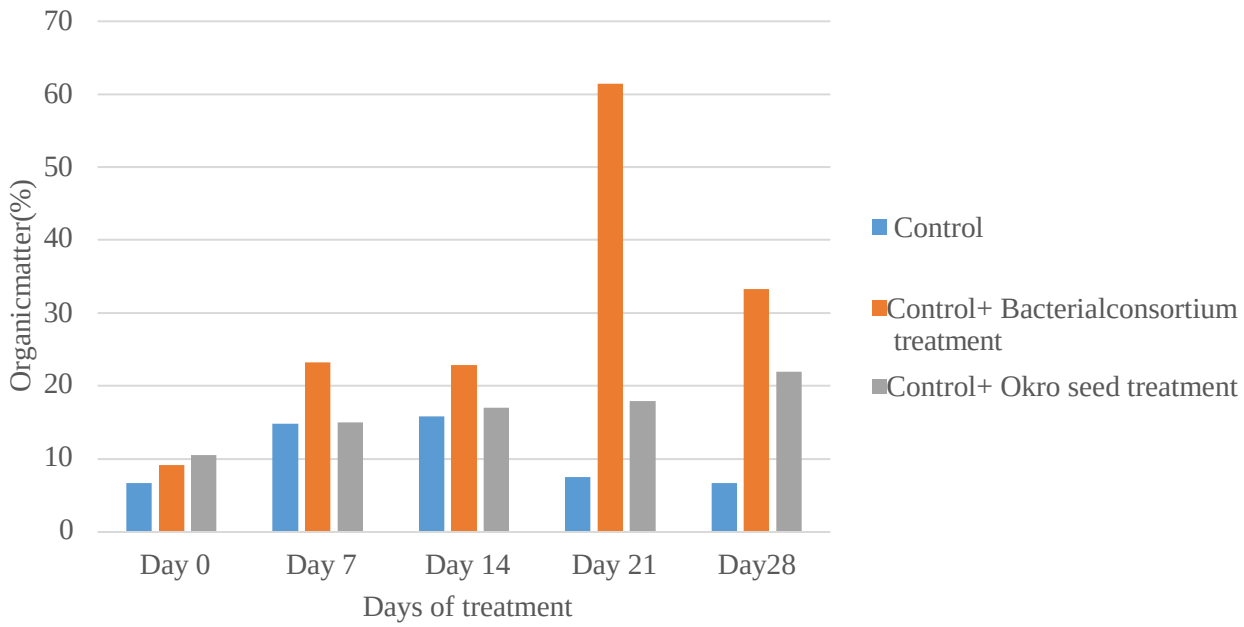

Chart6:Organicmattervariationsduringbioremediationtreatmentofpetrol contaminated soil

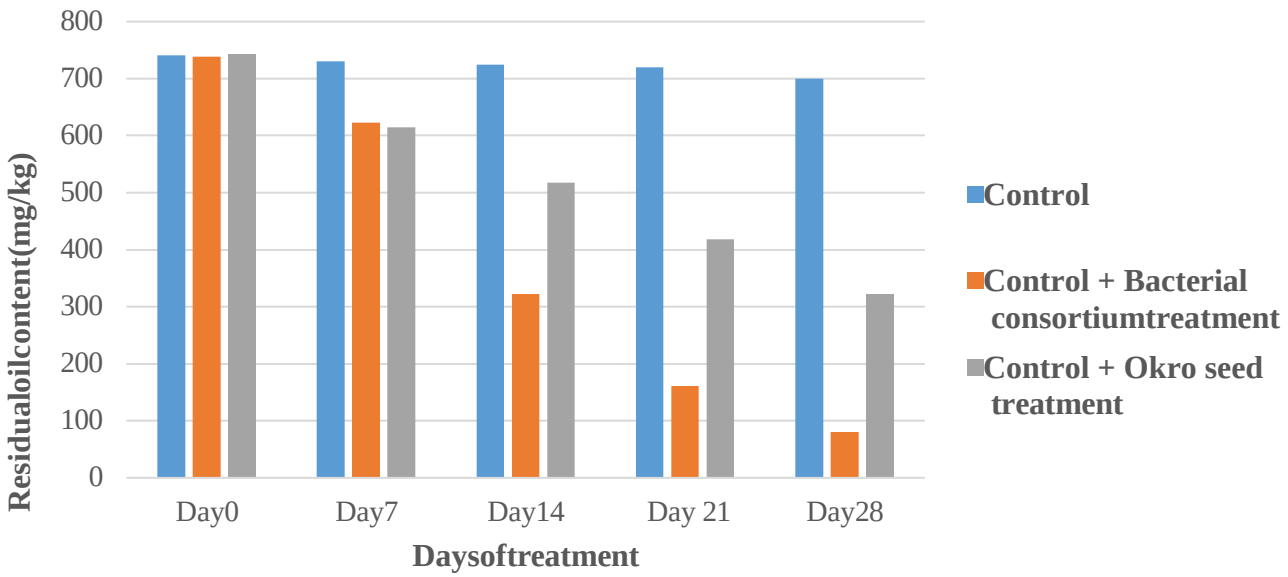

Chart7:Residualoilcontentvariationsduringbioremediationtreatmentofpetrol contaminated soil

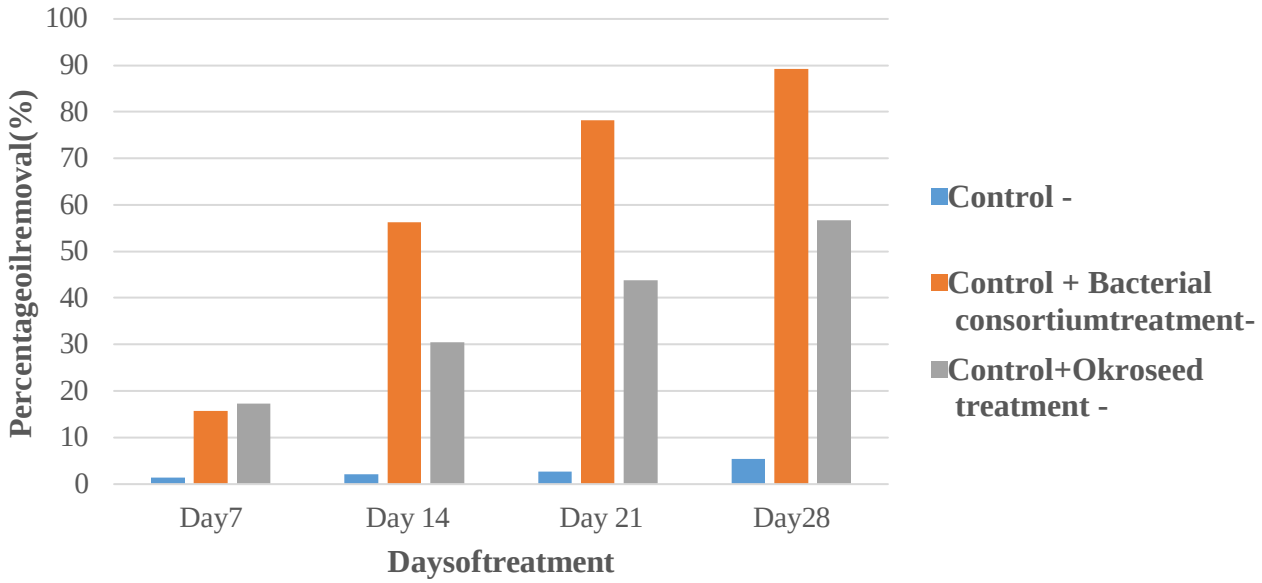

Chart8:Percentageoilremovalduringbioremediationtreatmentofpetrol contaminated soil

V CONCLUSIONS

In conclusion, the study experiment on bioremediation and phytoremediation of petroleum Contaminated soil has shed light on the effectiveness and potential of these environmentally Friendly remediation methods. Both bioremediation and phytoremediation have demonstrated Theircapabilitiesinreducingpetroleumcontaminantsinsoil,contributingtotherestorationof Ecological balance. The results gotten from this project is considering various Elements,includingsoilconditions,plantselection,andmicrobialactivity,whenimplementing these Methods. Bioremediation, which harnesses the natural degradation capabilities of Microorganisms,offersapromisingapproachforbreakingdowncomplexhydrocarbonsintoless Harmful compounds. Phytoremediation, utilizing specialized plants to extract and detoxify Contaminants, showcases its ability to mitigate petroleum contamination while promoting plant Growth and ecosystem recovery.

Through systematic experimentation, it has become evident that the success of both methods is influenced by factors like temperature, moisture levels, and initial contamination levels. The optimization of these conditions plays a pivotal role in achieving efficient and sustainable remediation outcomes. This study not only adds to the body of knowledge surrounding bioremediation and phytoremediation but also provides valuable insights for environmental practitionersandpolicymakers.Thepracticalrecommendationsgeneratedfromthisresearchcan guide the selection and application of remediation strategies in real-world scenarios, ensuring effective pollution management while minimizing negative impacts on soil health and the ecosystem.

VII RECOMMENDATION

Recommendations derived from this study emphasize the importance of site-specific Assessments, optimization of environmental conditions, plant-microbe interactions, and long Term monitoring. Integrating microbial inoculation into the rhizosphere of plants enhances the Synergistic effects of bioremediation and phytoremediation. Native plant species, diverse Plantingstrategies,andabalancednutrientmanagementplan contributeto theoverallsuccessof The combined methods. Public awareness and community engagement are crucial aspects of Promoting sustainable petroleum-contaminated soil remediation. The findings from this study

Provide valuable insights for environmental practitioners, policymakers, and stakeholders Involved in pollution management. By adopting a holistic approach that combines Bioremediationandphytoremediation,contaminatedsitescanberestoredwhileminimizing Ecological impact, contributing to a cleaner and healthier environment.

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