

**REMOVAL OF HEAVY METALS FROM DIESEL-POLLUTED WATER  
USING *KLEBSIELLA SPECIES* ISOLATED FROM DIESEL-POLLUTED SOIL**

**BY**

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## **DEDICATION**

This work is dedicated to the families of Ige, Aina, Adebisi and all those who have helped me in my journey through life.

## **ACKNOWLEDGEMENT**

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## TABLE OF CONTENTS

<b>AUTHORIZATION TO COPY</b>	<b>1</b>
<b>CERTIFICATION</b>	<b>2</b>
<b>DEDICATION</b>	<b>1</b>
<b>ACKNOWLEDGEMENT</b>	<b>2</b>
<b>LIST OF TABLES</b>	<b>8</b>
<b>LIST OF FIGURES</b>	<b>10</b>
<b>ABSTRACT</b>	<b>Error!</b>

Bookmark not defined.

<b>CHAPTER ONE</b>	<b>INTRODUCTION</b>	
.....	<b>Error! Bookmark not defined.</b>	
1.1 General Background	<b>Error! Bookmark not defined.</b>	
1.2 Objectives of the Research Study		2
1.3 Scope of Research Study		2
1.4 Justification		2
<b>CHAPTER TWO</b>	<b>LITERATURE REVIEW</b>	
.....	<b>Error! Bookmark not defined.</b>	
2.1 Origin of Petroleum	<b>Error! Bookmark not defined.</b>	
2.2 Petroleum as a Source of Energy	<b>Error! Bookmark not defined.</b>	
2.2.1 Composition, chemistry and classification of petroleum	<b>Error! Bookmark not defined.</b>	
2.2.2 Fractional distillation of petroleum	<b>Error! Bookmark not defined.</b>	

2.2.3 Diesel oil and its components	<b>Error! Bookmark not defined.</b>
2.3 Oil Spills	<b>Error! Bookmark not defined.</b>
2.3.1 Environmental impact of oil spill	<b>Error! Bookmark not defined.</b>
2.4 Heavy Metals	<b>Error! Bookmark not defined.</b>
2.4.1 Types, occurrence and effects of heavy metals in water on humans	<b>Error!</b>
<b>Bookmark not defined.</b>	
2.5 Bioremediation	<b>Error! Bookmark not defined.</b>
2.5.1 Bioremediation strategies	<b>Error! Bookmark not defined.</b>
2.5.2 Merits and demerits of bioremediation	<b>Error! Bookmark not defined.</b>
2.6 Factors Affecting Bioremediation	<b>Error! Bookmark not defined.</b>
2.6.1 Surface area	<b>Error! Bookmark not defined.</b>
2.6.2 Oxygen	<b>Error! Bookmark not defined.</b>
2.6.3 Temperature	<b>Error! Bookmark not defined.</b>
2.6.4 pH	<b>Error! Bookmark not defined.</b>
2.6.5 Nutrients	<b>Error! Bookmark not defined.</b>
2.6.6 Time	<b>Error! Bookmark not defined.</b>
2.7 Microbial Cultures for Bioremediation	<b>Error! Bookmark not defined.</b>
2.7.1 Klebsiella	<b>Error! Bookmark not defined.</b>
2.7.2 <i>Klebsiella pneumoniae</i>	<b>Error! Bookmark not defined.</b>
2.7.3 <i>Klebsiella oxytoca</i>	<b>Error! Bookmark not defined.</b>
2.8 Pseudomonas	<b>Error! Bookmark not defined.</b>
2.8.1 <i>Pseudomonas fluorescens</i>	<b>Error! Bookmark not defined.</b>

2.8.2 <i>Pseudomonas aeruginosa</i>	<b>Error! Bookmark not defined.</b>
2.8.3 <i>Pseudomonas putida</i>	<b>Error! Bookmark not defined.</b>
2.9 Microbial Growth	<b>Error! Bookmark not defined.</b>
2.9.1 Lag phase	<b>Error! Bookmark not defined.</b>
2.9.2 Exponential (log) phase	<b>Error! Bookmark not defined.</b>
2.9.3. Stationary phase	<b>Error! Bookmark not defined.</b>
2.9.4 Death phase	<b>Error! Bookmark not defined.</b>
2.10 Atomic Absorption Spectrometry	<b>Error! Bookmark not defined.</b>
2.10.1 Terminologies and equations used in atomic absorption spectrometry	<b>Error!</b>
<b>Bookmark not defined.</b>	
<b>CHAPTER THREE MATERIALS AND METHODS</b>	<b>Error!</b>
<b>Bookmark not defined.</b>	
3.1 Experimental Apparatus	<b>Error! Bookmark not defined.</b>
3.2 Materials	<b>Error! Bookmark not defined.</b>
3.2.1 Chemicals/Reagents	<b>Error! Bookmark not defined.</b>
3.2.2 Diesel-polluted-water and diesel-polluted-soil	29
3.2.3 Preparation of 1M HCl and 1M NaOH	<b>Error! Bookmark not defined.</b>
3.2.4 Growth medium for bioremediation experiment	<b>Error! Bookmark not defined.</b>
3.3 Experimental Methods	<b>Error! Bookmark not defined.</b>
3.3.1 Isolation	<b>Error! Bookmark not defined.</b>
3.3.2 Gram staining process	<b>Error! Bookmark not defined.</b>
3.4 Biochemical Characterization of the Bacterial Isolates	<b>Error! Bookmark not defined.</b>



3.4.1 Triple sugar iron (Tsi) medium	<b>Error! Bookmark not defined.</b>
3.4.2 Sulphide-indole-motility test	<b>Error! Bookmark not defined.</b>
3.4.3 Catalase test	<b>Error! Bookmark not defined.</b>
3.4.4 Citrate utilization test	<b>Error! Bookmark not defined.</b>
3.4.5 Methyl red and voges-proskaur(mrvp) test	<b>Error! Bookmark not defined.</b>
3.4.6 Nitrate reduction	<b>Error! Bookmark not defined.</b>
3.4.7 Oxidation-fermentation test	<b>Error! Bookmark not defined.</b>
3.4.8 Oxidase test	<b>Error! Bookmark not defined.</b>
3.4.9 Sugar fermentation test	<b>Error! Bookmark not defined.</b>
3.5 Heavy Metal Resistance Tests	<b>Error! Bookmark not defined.</b>
3.6 Digestion of Samples for Bioremediation Experiment	<b>Error! Bookmark not defined.</b>
3.7 Bioremediation Experiment	<b>Error! Bookmark not defined.</b>
3.8 Analysis of Variance (Anova)	<b>Error! Bookmark not defined.</b>
3.9 Analytical Methods	<b>Error! Bookmark not defined.</b>
3.9.1 Heavy metal concentration estimation	<b>Error! Bookmark not defined.</b>
3.10 Experimental Design and Optimization	<b>Error! Bookmark not defined.</b>
<b>CHAPTER FOUR RESULTS AND DISCUSSION</b>	<b>Error!</b>
Bookmark not defined.	
4. 1 Isolation and Colonial Characterisation	<b>Error! Bookmark not defined.</b>
4.2 Bioremediation of Heavy Metals in Diesel-polluted-water Using Klebsiella species	<b>Error! Bookmark not defined.</b>

4.2.1 Effect of *klebsiella edwardsii* on bioremediation of heavy metals in diesel-oil-polluted water **Error! Bookmark not defined.**

4.2.2 Effect of *Klebsiella oxytoca* on bioremediation of heavy metals in diesel-oil-polluted water **Error! Bookmark not defined.**

4.2.3 Effect of *klebsiella pneumoniae* on bioremediation of heavy metals in diesel-oil-polluted water **Error! Bookmark not defined.**

4.2.4 Effect of a mixed culture of *Klebsiella edwardsii*, *Klebsiella oxytoca* and *Klebsiella pneumoniae* on Bioremediation of heavy metals in diesel-oil-polluted Water **Error! Bookmark not defined.**

4.3 Regression Model and Statistical Analysis **Error! Bookmark not defined.**

4.4. Analysis of Copper. **Error! Bookmark not defined.**

4.5 Analysis of Nickel **Error! Bookmark not defined.**

4.5.1 Effect of Time and pH on bioremediation of Nickel **Error! Bookmark not defined.**

4.6 Analysis of Chromium **Error! Bookmark not defined.**

4.6.1 Effect of Time and pH on bioremediation of Chromium **Error! Bookmark not defined.**

4.7 Analysis of Cadmium **Error! Bookmark not defined.**

4.7.1 Effect of Time and pH on bioremediation of Cadmium **Error! Bookmark not defined.**

4.8 Validation of Bioremediation of Heavy Metals. **Error! Bookmark not defined.**

## CHAPTER FIVE CONCLUSION AND RECOMMENDATION

**Error! Bookmark not defined.**

5.1 Conclusions

**Error! Bookmark not defined.**

5.2 Recommendations

**Error! Bookmark not defined.**

## **REFERENCES**

**Error!**

**Bookmark not defined.**

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## LIST OF TABLES

TABLE	TITLE	PAGE
2.1:	Percentage by weight of elements in petroleum	<b>Error! Bookmark not defined.</b>
2.2:	Chain Length, boiling point range and uses of distillation categories of petroleum.	<b>Error! Bookmark not defined.</b>
2.3:	Potential sources of Oil Spills	<b>Error! Bookmark not defined.</b>
2.4:	Current drinking water guidelines of Heavy Metals analysed in this Study	<b>Error! Bookmark not defined.</b>
3.1:	Composition of growth medium	31
3.2:	Coding of experimental factors for bioremediation of heavy metals	39
3.3:	CCRD for two independent factors for bioremediation of heavy metals	<b>Error! Bookmark not defined.</b>
4.1:	Biochemical characterisation of some bacteria obtained from a diesel-oil-polluted soil	<b>Error! Bookmark not defined.</b>
4.2:	Data for bioremediation of heavy metals in diesel-polluted-water using a pure culture of <i>Klebsiella edwardsii</i>	44
4.3:	Data for bioremediation of heavy metals in diesel-polluted-water using a pure culture of <i>Klebsiella oxytoca</i>	48
4.4:	Data for bioremediation of heavy metals in diesel-polluted-water using a pure culture of <i>klebsiella pneumoniae</i>	52
4.5:	Data for bioremediation of heavy metals in diesel-polluted-water using a mixed culture of <i>klebsiella edwardsii</i> , <i>klebsiella oxytoca</i> and <i>klebsiella pneumoiae</i> .	56
4.6:	Results of CCRD for two independent Factors for Bioremediation of copper, nickel, chromium and cadmium using <i>Klebsiella oxytoca</i>	59
4.7:	Design Summary	60
4.8:	Test of Significance for every Regression Coefficient (Copper)	62

4.9: Analysis of Variance (ANOVA) for Regression Equations (Copper)	63
4.10: Regression Coefficient and Significance of Response Surface Cubic Model (Copper)	64
4.11: Experimental Data for Observed Values, Predicted Values and Residual Values (Copper)	65
4.12: Test of Significance for Every Regression Coefficient (Nickel)	69
4.13: Analysis of Variance (ANOVA) for Regression Equation (Nickel)	70
4.14: Regression Coefficients and Significance of Response Surface Cubic Model (Nickel)	71
4.15: Experimental Data for Observed Values, Predicted Values and Residual Values (Nickel)	72
4.16: Test of Significance for Every Regression Coefficient (Chromium)	76
4.17: Analysis of Variance (ANOVA) for Regression Coefficient (Chromium)	77
4.18: Regression Coefficients and Significance of Response Surface Cubic Model (Chromium)	<b>Error! Bookmark not defined.</b>
4.19: Experimental Data for Observed Values, Predicted Values and Residual Values (Chromium)	79
4.20: Test of Significance for Every Regression Coefficient (Cadmium)	84
4.21: Analysis of Variance (ANOVA) for Regression Analysis (Cadmium)	<b>Error! Bookmark not defined.</b>
4.22: Regression Coefficient and Significance of Response Surface Cubic Model (Cadmium)	85
4.14: Experimental Data for Observed Values, Predicted Values and Residual Values (Cadmium)	<b>Error! Bookmark not defined.</b>

## LIST OF FIGURES

FIGURE	TITLE
PAGE	
2.1 Growth Curve for a typical bacterium	<b>Error! Bookmark not defined.</b>
4.1: Graph of Reduction of Copper, Nickel, and Cadmium with time using <i>Klebsiella edwardsii</i> as bioremediating agent	<b>Error! Bookmark not defined.</b>
4.2: Graph of Reduction of Chromium with time using <i>Klebsiella edwardsii</i> as bioremediating agent	
46	
4.3: Graph of Reduction of Copper, Nickel and Cadmium with time using <i>Klebsiella oxytoca</i> as bioremediating agent	<b>Error! Bookmark not defined.</b>
4.4: Graph of Reduction of Chromium with time using a pure culture of <i>Klebsiella oxytoca</i> as bioremediating agent	<b>Error! Bookmark not defined.</b>
4.5: Graph of Reduction of Copper, Nickel and Cadmium with time using a pure culture of <i>klebsiella pneumoniae</i> as bioremediating agent	<b>Error! Bookmark not defined.</b>
4.6: Graph of Reduction of Chromium with time using a pure culture of <i>klebsiella pneumoniae</i> as bioremediating agent	<b>Error! Bookmark not defined.</b>
4.7: Graph of Reduction of Copper, Nickel and Cadmium with time using a mixed culture of <i>Klebsiella edwardsii</i> , <i>Klebsiella oxytoca</i> and <i>Klebsiella pneumoniae</i>	<b>Error! Bookmark not defined.</b>
4.8: Graph of Reduction of Chromium with time using a mixed culture of <i>klebsiella edwardsii</i> , <i>klebsiella oxytoca</i> and <i>klebsiella pneumoniae</i>	<b>Error! Bookmark not defined.</b>
4.9: Parity Plot illustrating the predicted values versus the observed values (Copper)	<b>Error! Bookmark not defined.</b>
4.10: Response surface plot for the interactive effect of Time and pH on bioremediation of Copper	<b>Error! Bookmark not defined.</b>

4.11: Parity plot of predicted value versus actual values (Nickel)**Error! Bookmark not defined.**

4.12: Response surface plot for the interactive effect of Time and pH on bioremediation of Nickel **Error! Bookmark not defined.**

4.13: Parity Plot showing the predicted values against actual values (Chromium) **Error! Bookmark not defined.**

4.14.: Response surface plot for the interactive effect of Time and pH on concentration of Chromium **Error! Bookmark not defined.**

4.15: Parity Plot of Predicted Values against Actual Values (Cadmium)**Error! Bookmark not defined.**

4.16: Response surface plot for the interactive effect of Time and pH on bioremediation of Cadmium **Error! Bookmark not defined.**

## ABSTRACT

This project investigated the microorganisms indigenous to a diesel-oil-polluted soil sample, their potential use in bioremediation of heavy metals in diesel-oil-polluted water using ex situ technique and factors affecting bioremediation. This was with a view of reducing the hazards that arise due to heavy metal pollution.

A diesel-oil-polluted soil sample was collected from a mechanic workshop. A working sample was prepared in a conical flask by suspending 0.5 g of the diesel-oil-polluted soil sample in 50 ml of sterile distilled water (dilution of  $10^{-2}$  w/v) in a 100 ml conical flask in which some glass chips were inserted. The flask was carefully agitated to obtain a uniform suspension. Dilutions of  $10^{-4}$  and  $10^{-6}$  were carried out in test tubes A and B. One milliliter of each dilution in tubes A and B was transferred into properly labelled petri dishes respectively. Culturing was done by making use of 20 ml molten sterile Eosin Methylene Blue agar and left to stand for the medium to set. They were incubated at 35 °C for 48 h. Colonial characteristics of the mixed culture obtained was observed. The differential colonies were isolated by sub-culturing into nutrient agar slants and labelled accordingly for use. The 18 h cultures were gram stained to study the morphological characteristics of the cultures and to ensure their purification. The ability of the isolates to grow on a heavy metal polluted medium was investigated. A digestion process was carried out and the sample was analyzed for Copper, Nickel, Chromium and Cadmium using atomic absorption spectrometry. Two hundred and fifty millilitres of bushnell-haas medium was distributed into each conical flask in which 10 ml of diesel-polluted water was added. Sterilization by autoclaving was done at 121 °C for 15 min. The medium was inoculated in a sterile environment with pure and mixed cultures of the *Klebsiella species* and labelled appropriately. They were incubated in a New Brunswick Gyrotory shaker for 16 days. Samples were withdrawn at four days interval for analysis. Design-Expert 8.0.3.1 was used for the design, modelling and optimization using time and pH as the two variables.

The isolated organisms isolated and used for the bioremediation studies were *Klebsiella edwardsii*, *Klebsiella oxytoca*, *Klebsiella pneumoniae* and *Pseudomonas cepacia*. When *Klebsiella edwardsii* was used as bioremediating agent, concentration of Cu, Ni, Cr and Cd reduced by 87.28, 81.55, 98.30 and 92.59% respectively. *Klebsiella oxytoca* removed 85.55, 80.58, 98.56 and 96.29% of Cu, Ni, Cr and Cd respectively from the original sample. When the polluted water was treated with *Klebsiella pneumoniae*, percentage reduction in concentration of Cu, Ni, Cr and Cd were 89.59, 83.49, 98.49 and 88.88% respectively. In the mixed culture of *Klebsiella edwardsii*, *Klebsiella oxytoca* and *Klebsiella pneumoniae*, percentage reduction in values were 82.65, 83.49, 98.27 and 92.59% for Cu, Ni, Cr and Cd respectively. In the optimization studies, optimum pH and time for the bioremediation of the heavy metals of interest were 7.46 and 16 days respectively.

This study revealed that *Klebsiella species* is an efficient bioremediating agent for Cu, Ni, Cr and Cd. It also showed that pH and time have a significant effect on bioremediation of heavy metals.



## **CHAPTER ONE**

### **INTRODUCTION**

#### **1.1 Background to the Study**

Nigeria is one of the major producers of crude oil in the world and contamination of the environment through its exploitation, refining, transportation and storage has steadily increased. Crude or refined oil and related products enter into land, land waters, estuaries or open ocean through oil well blowouts, tanker accidents, accidental rupture of oil pipelines and spills of used petroleum products. In the Niger Delta Area alone, there have been over 550 reported cases of crude oil spillage since 1976, releasing about 2.8 million barrels of crude oil into the environment (Korie-Siakpere, 1998; Odiye, 1999). All these activities lead to environmental pollution which poses a great threat to terrestrial and aquatic life and causes disruption in the ecosystem. The impact of pollution cannot be underestimated since some hydrocarbon components are carcinogenic in nature (Shukla and Singh, 2012).

It is a common practice in Nigerian cities and towns to allocate large portions of land of about 5 hectares or more, to groups of small scale auto-mechanic businesses and these are designated as villages where they locate their workshops and repair yards to offer their services to the public. Activities conducted in these shops involve working with oils, greases, petrol, diesel, battery electrolyte which may be accidentally spilled unto bare soil (Adelekan and Abegunde, 2011). Diesel oil, which is one of the major products of crude oil, constitutes a source of pollution in our environment (Nwaogu *et al*, 2008). Diesel pollution can occur through leakage from storage tanks, refueling of vehicles, wrecks of oil tankers and improper disposal by diesel tankers and by mechanics (Venosa *et al*, 1996).

Diesel oil pollution also occurs through careless and indiscriminate disposal of used diesel oil in fuel stations and depots. This has led to reduced plant growth on agricultural land. Suggested reasons for the reduced plant growth in diesel oil contaminated soils range from direct toxic effect on plants (Baker, 1982) and reduced germination (Udo and Fayemi, 1975) to unsatisfactory soil condition due to insufficient aeration of the soil because of the displacement of air from the space between the soil particles by diesel oil. Due to these activities, heavy metals in diesel oil can be deposited in the soil and enter the human food web thereby constituting risk to the ecosystem as they tend to bioaccumulate and can be transferred from one food chain to another. Consequently they are discovered in various food chains where the results are usually detrimental to plants, animals and humans alike (Abdu and Muazu, 2007). Diesel oil pollution can also lead to an increase in heavy metals concentration in water which could have harmful effects on humans and the environment. The contaminants are able to infiltrate deep into the layer of underground waters and pollute the groundwater as well as the surface water. As a result, the World Health Organisation has set some specified limits for heavy metals concentration in water (Ileckukwu and Okonkwo, 2012).

Biological treatment is an innovative technology available for heavy metal polluted wastewater. Since microorganisms have developed survival strategies in heavy metal polluted habitats, their different microbial detoxifying mechanisms such as bioaccumulation, biotransformation, biomineralization or biosorption can be applied either ex situ or in situ to design economical bioremediation processes (Malik, 2004 ; Lin C, 2005). Cases of diesel oils spills are common in the environment. Therefore this work studied the extent and rate to which *Klebsiella edwardsii*, *Klebsiella oxytoca* and *Klebsiella pneumoniae* isolated from a diesel-oil polluted soil can be used to remove Copper, Nickel, Chromium and Cadmium metals from diesel-oil-polluted water.

## **1.2 Objectives of the Research Study**

The objectives of this research work are:

- (i) to isolate, characterize and identify the microorganisms present in a diesel-oil-polluted soil.
- (ii) to use the microorganisms isolated from (i) to reduce the heavy metal concentration in diesel- oil-polluted water.
- (iii) to check the effects of pH and time on the rate of bioremediation of the heavy metals of interest.

## **1.3 Scope of Research Study**

The overall objective of this research study was to use *Klebsiella edwardsii*, *Klebsiella oxytoca* and *Klebsiella pneumoniae* to bioremediate heavy metals in water that was polluted with diesel fuel. The optimization was carried out using Design-Expert 8.0.3.1. software to generate the experimental runs using Central Composite Rotatable Design by taking into consideration two factors: pH and time.

## **1.4 Justification**

Petroleum is a leading contaminant due to its production, transport, use and disposal (Jagadevan and Mukherji. 2004). According to Baker and Herson (1994), the national priority list of sites polluted by petroleum is about 1,200 with potential sites numbering about 32,000. Bioremediation has proven to be an improved alternative technology to destroy or render harmless various contaminants. This study will provide information on the best conditions for the bioremediation of heavy metals using the *Klebsiella spp.* It will also show that microorganisms in polluted environments can be used for environmentally friendly purposes.