**Original Research Article**

**BIOREMEDIATION OF CRUDE OIL CONTAMINATED SOIL ENHANCED WITH WATERHYACINTH(AQUATIC MACROPHYTE)**

**ABSTRACT**

**Aim:** Crude oil contamination remains a significant environmental challenge, necessitating the development of efficient and sustainable remediation strategies.

**Study Design:** Homogenized powdered Water hyacinth used as an amendment to remediate crude oil contaminated liquid

**Place and Duration of Studies:** Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, P.M.B 1221, Effurun, Delta State**.**

**Methodology:** This study evaluates the potential of water hyacinth in crude oil contaminated soils at concentrations of 5000mg/kg and 10000mg/kg using 15% and 30% powdered water hyacinth respectively. The remediation process was monitored over six weeks by analyzing total petroleum hydrocarbon (TPH) degradation and pH variations.

**Results:** The results revealed a notable reduction in TPH concentrations, with the highest degradation occurring in soils treated with 30% water hyacinth at 10000mg/kg achieving a final concentration below the regulatory threshold of 5000 mg/kg set by the Nigerian Upstream Petroleum Regulatory Commission (NUPRC). Additionally, the pH of the bio stimulated soils increased from acidic to neutral creating a favourable environment for microbial activity and accelerating crude oil breakdown. Statistical analysis demonstrated a significant difference (p‹0.05) between treated and control samples, confirming the effectiveness of eater hyacinth in enhancing microbial remediation.

**Coclusion: The** findings indicate that water hyacinth plays a crucial role in improving biodegradation efficiency through nutrient enrichment and pH buffering. This study underscores the potential of water hyacinth as a cost – effective, eco- friendly and sustainable bioremediation agent for mitigating crude oil contamination in impacted environments, offering valuable insights for environmental efforts.

***Keywords****: Water hyacinth, Total Petroleum Hydrocarbon, Bioremediation,Eco-friendly*

1. **INTRODUCTION**

## Pollution of the environment is one of the major effects of human technological advancement. It results when a change in the environment harmfully affects the quality of human life including effects on animals, micro organisms and plants [40]. Therefore, soil pollution is the appearance in soils of persistent toxic compounds, chemicals, salts, radioactive materials, or disease causing agents, which have adverse effects on plant growth and animal health. The contamination of the environment (mainly terrestrial and aquatic) by crude oil is referred to as crude oil pollution and it is estimated that 80% of crude oil pollution is as a result of spillage [31]. The main losses of petroleum occur during mining, transportation and storage and amount to millions of tons per year. In terms of its negative impact, petroleum, its products and wastes are ranked second after radioactivity. Petroleum spills from mining and processing accidents do significant harm to the ecosystems. In such cases, soil is mainly affected, because it is able to accumulate large amounts of pollutants due to its enormous adsorbing surface area[40]. Petroleum pollution negatively affects soil biogenesis, seriously changes the chemical composition, structure and properties of soil, and reduces soil fertility and arable value. The petroleum spills may turn soils into typical technogenic deserts that are practically absent of biological processes. Petroleum-polluted soils are not suitable for agricultural and recreational uses and are potential sources of contamination of surface and ground waters. Self-restoration of soils may take a long period, of 10 to 30 years or longer, depending on the soil type. Crude oil which is abundantly located in the Niger Delta region of Nigeria is spilled on soil due to pipeline destruction[7]. Previous studies on crude oil pollution in soil had revealed its adverse effects on soil productivity.These studies had been majorly on the effects of pollution on chemical properties of the soil. As a result of crude oil pollution, soil physical properties such as pore spaces might be clogged which reduces soil aeration, infiltration of water into the soil, increased bulk density of the soil which may affect plant growth. Crude oil which is denser than water may reduce and restrict permeability. Some of the effects of crude oil may have adverse effects on soil physical properties include decreased pore spaces. Remediation of petroleum-contaminated land requires a series of measures to clean up and restore soil as a natural environment. The most common of these measures are currently classified as physical, physicochemical, chemical and biological measures. Sometimes, mechanical measures are used depending on the mechanism of action on the soil all in order to achieve sustainable Development Goals (SDGs) framework [42].

Other Remediation processes like, land farming, soil washing, vapour extraction, thermal desorption, composting, incineration and the use of oil booms and solidification have been used for the clean-up of oil contaminated sites; however they are disruptive, labour intensive and relatively expensive processes [13]. Bioremediation which is the use of micro-organisms through the addition of fertilizers or water hyacinth to improve their population or the direct addition of micro-organisms have been studied as means of remediating the harmful effect of crude oil pollution. Bioremediation involves several methods such as bioaugmentation, biostimulation, composting etc has been proven extremely viable and cost-effective[13] .The remediation of polluted sites has become a priority for society because of increase inquality of life standards and the awareness of environmental issues. Over the past few decades there has been avid interest in developing in situstrategies for remediation of environmenta lcontaminants, because of the high economic cost of physicochemical strategies, the biological tools for remediation of these persistent pollutants is the better option [13]. Major focus have been considered on persistent organic chemicals  due to their ubiquitous occurrence, recalcitrance, bioaccumulation potential and carcinogenic activity. The use of water hyacinth as a nutrient source (bio-fertilizer)as an alternative measure which would not only be effective of the polluted site and affordable but in addition encourages local participation in clean up programs because it is environmentally friendly.

1. **MATERIALS AND METHODS**

**2.1 Study Area**

**Uncontaminated Soil** samples were appropriately collected from a farm around college of science FUPRE with GPS coordinate 5.5743oN and 5.8368oE. It was air dried for one month and sieved with 2mm sieve size.

**The water hyacinth** sample were harvested by hand picking during the daytime from Udu River Udu LGA Delta state with GPS coordinates 5.5269oN and 5.7785oE. It was sun dried for a month, after which was taken to the mill and grounded into powder.

**Crude oi**l sample was collected from a flow station in OML 30 Kokori Delta state and was analyzed for Total Petroleum Hydrocarbon (TPH) and water content parameters.

The baseline Analysis for soil and water hyacinth samples were carried out to know the following physiochemical parameters, pH, TPH (Total petroleum Hydrocarbon), TOC(Total Organic Carbon) ,sulphate, Nitrate, phosphate, TotalHydrocarbon utilizing bacteria and fungi, Total heterotrophic bacteria and fungi, metals such as iron, chromium, lead, zinc and nickel, particle sizes, soil texture and water content .

2.2 **Bioremediation Experimental set up**

Glass bottles were used for the bioremediation process in triplicate. 300g of pure garden soil were spiked with two different quantities of crude oil (5000 mg/kg and 10,000 mg/kg) in the natural attenuation setup, which served as the control. Two concentrations of crude oil (5000 mg/kg and 10,000 mg/kg) were employed in different bottles for the biostimulation treatment. Each bottle was biostimulated with 15% (45 g) and 30% (90 g) of dried and powered water hyacinth, respectively. Every three (3) days, 50 milliliters of distilled water were used to maintain moisture. The following parameters were analyzed on weeks 0 through 6 to track the biodegradation of TPH and the remediation of soil polluted by crude oil: pH, TPH, TOC, nutrients (sulfates, phosphate, and nitrate), total heterotrophic bacteria and fungus, and total hydrocarbon-utilizing bacteria and fungi.

**2.3 Method of data analysis**

Data were analyse using SPSS version for pH and TPH resulting to P<0.05

### 3.0 Results and discussion

**Table 1. Baseline composition of crude oil**

|  |  |  |
| --- | --- | --- |
| **TEST RESULTS** | | |
| **PARAMETER** | **UNIT** | RESULTS |
| Total petroleum Hydrocarbon(TPH) | mg/kg | 19541.80 0.3 |
| Water content | % | 10.00 0.3 |

**Table 2.Mean physiochemical properties of dried water hyacinth**

|  |  |  |
| --- | --- | --- |
| **PARAMETER** | **UNIT** | RESULTS |
| pH | - | 5.50 0.2 |
| TPH | mg/kg | 2607 0.2 |
| Total Organic Carbon | % | <0.01 0.22 |
| Electrical conductivity | us/cm | 3000 0.12 |
| **NUTRIENT** | | |
| Sulphate | mg/kg | 206.98 0.22 |
| Nitrate | mg/kg | 242.352 0.32 |
| Phosphate | mg/kg | 17.36 0.31 |
| Moisture Content | % | 7.39 0.33 |
| **MICROBIOLOGY** | | |
| Total hydrocarbon utilizing bacteria 103 | cfu/g | 4.58 0.23 |
| Total hydrocarbon utilizing fungi x103 | cfu/g | 4.00 0.24 |
| Total Heterotrophic Bacteria x10-2 | cfu/g | 3.18 0.24 |
| Total heterotrophic fungi x102 | cfu/g | 3.33 0.23 |
| **HEAVY METALS** | | |
| Iron | mg/kg | 6420 0.21 |
| Chromium | mg/kg | 3.30 0.22 |
| Lead | mg/kg | 2.10 0.24 |
| Zinc | mg/kg | 48.50 0.23 |
| Nickel | mg/kg | 6.40 0.25 |

**Table 3.Mean physiochemical properties of uncontaminated soil sample**

|  |  |  |
| --- | --- | --- |
| **PARAMETER** | **UNIT** | RESULTS |
| pH | - | 4.20 0.2 |
| TPH | mg/kg | 71.74 0.2 |
| Total organic carbon (TOC) | % | 0.48 0.22 |
| Electrical conductivity |  | 30.00 0.12 |
| **Nutrient** | | |
| Sulphate | mg/kg | 5.83 0.22 |
| Nitrate | mg/kg | 0.99 0.32 |
| Phosphate | mg/kg | 13.05 0.31 |
| Moisture content | % | 3.59 0.33 |
| **Microbiology** | | |
| Total hydrocarbon utilizing bacteria | cfu/g | 2.17 0.23 |
| Total hydrocarbon utilizing fungi | cfu/g | 2.38 0.24 |
| Total heterotrophic bacteria | cfu/g | 3.03 0.24 |
| Total heterotrophic fungi | cfu/g | 2.08 0.23 |
| **Heavy metal** | | |
| Iron | mg/kg | 8880 0.21 |
| Chromium | mg/kg | 11.02 0.22 |
| Lead | mg/kg | 8.40 0.24 |
| Zinc | mg/kg | 61.20 0.25 |
| Nickel | mg/kg | 6.60 0.25 |

**Table 4 Mean values of soil and water hyacinth texture.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **RESULTS** | | |
|  | SAND | CLAY | SLIT |
| Water hyacinth | 88.60 0.2 | 10.30 0.2 | 1.10 0.2 |
| Soil | 89.80 0.2 | 8.40 0.2 | 1.80 0.2 |

**Table 5.Mean physiochemical properties of Bioremediated soils at WEEK 0**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Unit** | **Control 1 (NA1)** | **Control 2**  **(NA2)** | **BS1A** | **BS1B** | **BS2A** | **BS2B** | **DPR Target limit** | **DPR Intervention Limit** |
| pH |  | 4.10 0.2 | 4.40 0.2 | 6.64 0.2 | 6.60 0.2 | 6.71 0.2 | 6.82 0.2 | NA | NA |
| TPH | mg/kg | 4225 0.2 | 4108 0.2 | 3946 0.2 | 3980 0.2 | 3604 0.2 | 3418 0.2 | 50 | 5000 |
| TOC | mg/kg | 1.46 0.22 | 1.72 0.22 | 2.01 0.22 | 2.08 0.22 | 2.13 0.22 | 2.26 0.22 | NA | NA |
| Sulphate | mg/kg | 12.66 0.22 | 13.02 0.22 | 14.45 0.22 | 15.81 0.22 | 15.89 0.22 | 15.91 0.22 | NA | NA |
| Nitrate | mg/kg | <0.01 0.32 | 0.12 0.32 | 1.038 0.32 | 1.27 0.32 | 1.45 0.32 | 1.49 0.32 | NA | NA |
| Phosphate | mg/kg | 12.44 0.31 | 12.36 0.31 | 12.03 0.31 | 12.05 0.31 | 12.13 0.31 | 12.18 0.31 | NA | NA |
| Total heterotrophic Bacteria x 10-4 | cfu/g | 2.72 0.23 | 2.70 0.23 | 2.14 0.23 | 2.10 0.23 | 2.07 0.23 | 2.04 0.23 | NA | NA |
| Total fungi x 10-4 | cfu/g | 2.00 0.24 | 2.21 0.24 | 1.69 0.24 | 1.52 0.24 | 1.47 0.24 | 1.44 0.24 | NA | NA |
| Total hydrocarbon Utilizing bacteria x 10-2cfu/g | cfu/g | 3.18 0.24 | 3.04 0.24 | 4.80 0.24 | 4.83 0.24 | 4.96 0.24 | 4.99 0.24 | NA | NA |
| Total hydrocarbon Utilizing fungi x 10-2 | cfu/g | 3.00 0.23 | 3.34 0.23 | 4.32 0.23 | 4.49 0.23 | 4.69 0.23 | 4.84 0.23 | NA | NA |

**Table 6. Mean physiochemical properties of Bioremediated soils at WeeK 3**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Unit** | **Control 1**  **(NA1)** | **Control 2**  **(NA2)** | **BS1A** | **BS1B** | **BS2A** | **BS2B** | **DPR target limit** | **DPR intervention limit** |
| pH |  | 4.30 0.2 | 4.70 0.2 | 6.80 0.2 | 6.92 0.2 | 7.04 0.2 | 7.08 0.2 | NA | NA |
| TPH | mg/kg | 3246 0.2 | 3496 0.2 | 2338 0.2 | 2186 0.2 | 2045 0.2 | 2019 0.2 | 50 | 5000 |
| TOC | mg/kg | 1.65 0.22 | 1.84 0.22 | 2.43 0.22 | 2.32 0.22 | 2.63 0.22 | 2.54 0.22 | NA | NA |
| Sulphate | mg/kg | 13.40 0.22 | 13.92 0.22 | 16.90 0.22 | 17.01 0.22 | 17.12 0.22 | 17.18 0.22 | NA | NA |
| Nitrate | mg/kg | 0.28 0.32 | 0.32 0.32 | 1.22 0.32 | 1.30 0.32 | 1.56 0.32 | 1.58 0.32 | NA | NA |
| Phosphate | mg/kg | 12.20 0.31 | 12.18 0.31 | 12.98 0.31 | 12.48 0.31 | 12.56 0.31 | 12.64 0.31 | NA | NA |
| Total heterotrophic Bacteria x 10-4 | cfu/g | 2.58 0.23 | 2.47 0.23 | 2.44 0.23 | 2.32 0.23 | 2.20 0.23 | 2.21 0.23 | NA | NA |
| Total fungi x 10-4 | cfu/g | 2.18 0.24 | 2.20 0.24 | 1.36 0.24 | 1.33 0.24 | 1.27 0.24 | 1.21 0.24 | NA | NA |
| Total hydrocarbon Utilizing bacteria x 10-2cfu/g | cfu/g | 3.46 0.24 | 3.58 0.24 | 4.82 0.24 | 4.98 0.24 | 5.06 0.24 | 5.14 0.24 | NA | NA |
| Total hydrocarbon Utilizing fungi x 10-2 | cfu/g | 3.28 0.23 | 3.45 0.23 | 4.69 0.23 | 4.74 0.23 | 4.82 0.23 | 4.94 0.23 | NA | NA |

**Table 7.Mean physiochemical properties of Bioremediated soils at Week 6**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Unit** | **Control 1(NA1)** | **Control 2 (NA2)** | **BS1A** | **BS1B** | **BS2A** | **BS2B** | **DPR target limit** | **DPR Intervention Limit** |
| pH |  | 5.06 0.2 | 5.13 0.2 | 7.02 0.2 | 7.21 0.2 | 7.27 0.2 | 7.46 0.2 | NA | NA |
| TPH | mg/kg | 3106 0.2 | 3004 0.2 | 2142 0.2 | 2034 0.2 | 1896 0.2 | 1834 0.2 | 50 | 5000 |
| TOC | mg/kg | 1.83 0.22 | 1.98 0.22 | 2.58 0.22 | 2.67 0.22 | 2.82 0.22 | 2.93 0.22 | NA | NA |
| Sulphate | mg/kg | 14.98 0.22 | 15.06 0.22 | 17.11 0.22 | 17.36 0.22 | 17.53 0.22 | 17.81 0.22 | NA | NA |
| Nitrate | mg/kg | 0.17 0.32 | 0.08 0.32 | 1.34 0.32 | 1.42 0.32 | 2.08 0.32 | 2.24 0.32 | NA | NA |
| Phosphate | mg/kg | 12.10 0.31 | 12.04 0.31 | 13.43 0.31 | 13.56 0.31 | 13.64 0.31 | 13.72 0.31 | NA | NA |
| Total heterotrophic Bacteria x 10-4 | cfu/g | 2.79 0.23 | 2.82 0.23 | 2.36 0.23 | 2.39 0.23 | 2.34 0.23 | 2.38 0.23 | NA | NA |
| Total fungi x 10-4 | cfu/g | 2.41 0.24 | 2.48 0.24 | 1.82 0.24 | 1.78 0.24 | 1.64 0.24 | 1.59 0.24 | NAf | NA |
| Total hydrocarbon Utilizing bacteria x10-2 | cfu/g | 3.86 0.24 | 3.94 0.24 | 5.06 0.24 | 5.22 0.24 | 5.43 0.24 | 5.64 0.24 | NA | NA |
| Total hydrocarbon  Utilizing fungi x10-2 | cfu/g | 3.59 0.23 | 3.65 0.23 | 5.02 0.23 | 5.18 0.23 | 5.39 0.23 | 5.46 0.23 | NA | NA |

**Legends: NA1: natural attenuation 1- control with 5000mg/kg (crude oil)**

**NA2 : natural attenuation 2- control with 10,000mg/kg(crude oil)**

**BS1A: Bio stimulation with 15% powdered water hyacinth with 5000mg/kg crude oil**

**BS1B: Bio stimulation with 15% powdered water hyacinth with 10000mg/kg crude oil**

**BS2A: Bio stimulation with 30% powdered water hyacinth with 5000mg/kg crude oil**

**BS2B: Bio stimulation with 30% powdered water hyacinth with 10000mg/kg crude oil**

**NA: Not Available**

**From the table 8, there was % loss of TPH in all test system including the control. There is a significant difference in % loss of TPH in test system containing 5000ml and 10000ml of crude oil, however % loss of TPH was greater in the test systems containing 30%. Water hyacinth with 5000ml of crude oil.**

**4.0 DISCUSSIONS**

The remediation of crude oil in the various treatment sample was monitored with respect to the varying percentage of water hyacinth, 45g and 90g which is 15% and 30% respectively in crude oil concentration of 5000mg/kg and 10000mg/kg. The results indicated a significant difference in total petroleum hydrocarbon (TPH) and pH in the treatment samples. It was discovered the TPH results fall between 3946mg/kg in 5000mg/kg of crude oil at week 0 to 1834mg/kg in 10000mg/kg of crude oil at week 6 indicating a significant difference at week 0 and week 6 in the treatment samples unlike the control that falls from 4225mg/kg in 5000mg/kg of crude oil at week 0 to 3004mg/kg in 10000mg/kg of crude oil at week 6 indicating no significant difference. The pH on the other hand increased from acidity to alkalinity with the figure of 6.64 to 7.46. The percentage TPH degradation for the 5000mg/kg of crude oil for control 1(NA1) in BS1A and BS2A is 93.3% and 85.3% respectively while the percentage TPH degradation for 10000mg/kg of crude oil for control 2(NA2) for BS1B and BS2B is 96.8% and 83.2% respectively at week 0. The percentage TPH degradation for the 5000mg/kg of crude oil for control 1(NA1) in BS1A and BS2A is 72.0% and 63.0% respectively while the percentage TPH degradation for 10000mg/kg of crude oil for control 2 (NA2) for BS1B and BS2B is 62.5% and 57.7% respectively at week 3. The percentage TPH degradation for the 5000mg/kg of crude oil for control 1 (NA1) in BS1A and BS2A is 68.9% and 61.0% respectively while the percentage TPH degradation for 10000mg/kg of crude oil for control 2 (NA2) for BS1B and BS2B is 67.7% and 61.05% respectively at week 6. Following the TPH percentage calculations, there is reduction with the percentage of TPH from week 0 and week 6. The percentage values for week 0 and week 6 is 80.0% and 59.0% respectively, the results gotten showed that 30% of the water hyacinth in the treatment with 10000mg/kg of crude oil had the highest degradation at week 6 this equally shows significance difference with p-value than 0.05, p<0.05 from the plots. This clearly shows that water hyacinth and time helped with the remediation of the crude oil as seen with the percentage reduction of the TPH from the plot. This is as a result of the microbial activities from the water hyacinth in the treatment samples which helped to reduce the concentration of the TPH compared with the control with no significant difference. The TPH concentration at week 6 is below Nigeria Department of petroleum Resources (DPR) now Nigeria upstream petroleum regulatory commission (NUPRC) target values of 5000 for crude oil indicating that its in compliance with corresponding NUPRC limits.

[3] in a bioremediation study using water hyacinth as well was able to reduce the TPH concentration from 865.6mg/kg to 335.9mg/kg and increasing the total hydrocarbon utilizing bacteria from 1.7 x 105 to 8.2 x 105cfu/g making water hyacinth effective for remediation. Total organic carbon (TOC) ranged from 2.0mg/kg and 4.93mg/kg which indicate that the carbon energy concentration in the treatment significantly increased with time thereby helping with the remediation of the soil. [3]. pH values ranged from 6.64 and 7.08 in the treatment. This indicates that the water hyacinth helped the treatments from from acidity to alkalinity thereby bringing the contaminated soil to acceptable level. This is as a results of the microbial activities from the water hyacinth which helped with the remediation of the contaminated soil to acceptable level [4]. The total hydrocarbon utilizing bacteria ranged between 4.32 x 10-4cfu/g and 5.46 x 10-4cfu/g. This indicates that the water hyacinth increased the presence of microorganism utilizing hydrocarbon which improved the soil remediation bringing it to significant level [42]

**Fig: 1.: TPH concentrations in bioremediated soils**

Fig 2: pH values of bio remediated soils

4.2 Statistical Interpretation of the TPH

From the above ( fig 1 ) the p - value is less than 0.05 for treatment with 30% water hyacinth, this means there is a significant difference in the 30% treatment between the 5000mg/kg and 10000mg/kg crude oil contaminated samples. There is however no significant difference with 15% water hyacinth treatment between the 5000mg/kg and 10000mg/kg crude oil contaminated soil samples. There is however more TPH loss in 15% treatment when compared with the control.

**5.1 Conclusion**

This work was intended to determine the effects of water hyacinth application on soil contaminated with crude oil at the concentration of 5000mg/kg and 10000mg/kg at 15% and 30% of powdered water hyacinth, respectively.Remediation with these varying percentage of water hyacinth for a period of six weeks showed that remediation occurred most with 10000mg/kg of crude oil stimulated with 30% of water hyacinth played a major role in the remediation of the crude oil contaminated soil with respect to the decrease in the TPH concentration.

Also, the TPH had P-value less than 0.05 indicating significant difference in the effects of water hyacinth on the treatment samples with time. This is as a result of the enhancement of microbial activities from the water hyacinth which was added to the bioremediation microcosm.

ThewaterhyacinthalsohadabufferingeffectonthetestmicrococsmbyincreasingthepHofthebiostimulatedsoilstoapHrangefavourableforbiodegradation,whencomparedwiththecontroltestsoils.Therefore,theuseofwaterhyacinthiseffectiveintheremediationofcrudeoilcontaminatedsoil

## REFERENCES

1Abdel-Shafy, H., and Mansour, M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*. *25*. 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011.

2 Achten, C., and Hofmann, T. (2009). Native polycyclic aromatic hydrocarbons (PAH) in coals-A hardly recognized source of environmental contamination. *The Science of the total environment. 407*(8), 2461-73. https://doi.org/10.1016/j.scitotenv.2008.12.008.

3 Agu, G. E., (2023) Bioremediation of crude oil contaminated soil using water hyacinth. *World journal of advanced research 18*(03):880-888. https://doi.org/10.30574/wjarr.2023.18.3.1124.

4 Aislabie J, Saul, D.J., (2016) Bioremediation of hydrocarbon contaminated polar soils. *Extremophiles, 10:171-179doi: 10.1007/50092-2005-0498-4.*

5 Al Disi, Z., Jaoua, S., Al-Thani, D., Almeer, S., and Zouari, N. (2017). Considering the Specific Impact of

Harsh Conditions and Oil Weathering on Diversity, Adaptation, and Activity of Hydrocarbon-Degrading Bacteria in Strategies of Bioremediation of Harsh Oily-Polluted Soils. *BioMed Research International. 2017*. 11. https://doi.org/10.1155/2017/8649350.

6 Ali, H., Khan, E., and Anwar Sajad, M. (2013). Phytoremediation of heavy metals—Concepts and applications. *Chemosphere 91*(7) 869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075.

7 Apulu, O.G., Potravny, I.M., Sukhorukova,I.V. (2021). Methods of justification and selection of technologies for remediation of oil contaminated land. Ecol. Ind. Russ. 25:38-43.

8 Allamandola, Louis. (2011) cosmic distribution chemical complexity (NASA Archived from the original). pp 39-42.

9 Anil, H.s., Lao S.N., Srividya D., (2019). Advances in cloning, structural and bioremediation aspects of nitrilehydratases. “molecular Biology Reports.46(4):4661-4673.

10 Ansari Mi,and Malik, A., (2007). Biosorption of nilkel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial waste water. Bioresources technology 98 916):314a-53.

11 Anzenbacher, P. and Anzebacherova E., (2011) Cytochrome P450 and Metabolism of Xenobiotics cell mol life sci 58:737-747.CPYIA: Widor roles in cancer progression and prevention. BMC Cancer 9(1):187.

12 Araka, Perez.P, Okparanma, Reuben .N and Josaiah. M.., (2019) Diagnostic screening of organic contaminant level in solidified/ stabilized pre treated oil-based drill cuttings. 5(10) : e02644. doi : 10.1016/j.heliyon. 2019. E02644.

13 Azubuike, C.C, Chikere, C.B, and Okpokwasili GC., (2016) – Biorcmediation techniques –classification based on site of application: principle, advantages, limitations and prospects. World journal of microbiology and biotechnology. 32(11):180.

14 Baird, W.M., Hooven, L.A, and Mahadevan, B., (2015) “Carcinogenic polycyclic aromatic hydrocarbon DNA adducts and mechanisms of action”.Environmental and molecular Mutagenesis 45(2-3):106-114.

15 Baker, R..S, Moorc.A.T., (2013). Optimizing the effectiveness of insitu bioventing: at sites suited to its use polluting 32(7):44-47.

16 Banitz.T,Frank. and K, Wilkly., (2016) spatial metrics as indicators of biodegradation benefits from bacterial dispersal networks. Ecolind 60:34-63. Doi:10.10161j.ecolind.2016.06.021.

17 Bargar, R, Lioydjr, L, and Dr, Williams KH., (2018) Bioremediation of Uranium contaminated groundwater: a system approach to subsurface biogeochemistry current opinion in biotechnology 24(3):480-97Doi:10.10161jlopbio.2012.10.008.

18 Baric. M, Pierro, L, and Pietrangeli.B., (2014).Polyhydroxyalicanoatc (PHB) a slow releaseelection donor advanced in situ biocmediation of chlorinated solvent contaminated aquifers. New Biotechnology 31:377-382.doi:1010161j.nbt.2013.10.008.

19 Barr.D., (2017). biological methods for assessment and remediation of contaminated land. Case studies construction industry research and information association, London.

20 Barry, Carolyn., (2019). “Slick Death: Oil spll treatment kills coral” Science News.172(5): 67.Doi:10.1002/scin. 2019.5591720502.

21 Batters .s. (2014). “Space molecules point to organic origins New scientist. Retrieved2009-12-11..

22 Bautista, H, Rahman, K.M.M., (2016). Effects on Wildlife and Habitats “International Research Journal 1 (43): 93:96.Doi:10.1845411RJ.2016.43.143.

23 Bautista, H, Rahmam, K.M.M., (2016).Effects of crude oil pollution in the tropical rainforest biodiversity of Ecuadorian Amazon region. (journal of biodiversity and environmental sciences8(2):249-254.

24 Bell, Bethan, Caccioholo, Mario., (2017). “Black tide: when the British bombed an oil spill retrieved 2020-01-09.

25 Bertolini, Massimo, Bevilacqua, Maurizio., (2018). Oil pipeline spill cause analysis. A classification free approach, “Journal of quality in maintenance engineering 12(2). 186-198.Doi:10.1108113552510610667192.

26 Besaltatpour. A, and Hajabbasi. M., (2014). Land farming process effects on biochemical properties of petroleum contaminated soils. Soil sediment contaminant J 20.234-248.doi:10.1080115320 383.2011.J46447.

27 Bhattacharya. M, and Guchhait. S., (2015). Waste lubricating oil removal in a batch reactor mixed bacterial consortium a kinestic study. Bioprocess Bio-system Eng 38:2095-2106. doi: 10.10071500449-015-1449-9.

28 Bojar.D., (2018).“Building a circular economy with synthetic biology” phys.org.

29 Boopathy R., (2010). Factors limiting bioremediation technologies”. Bioresource technology 74:63-7Doi:101016150960-8524(99)00144-3.

30 Bostrom, C.E, Gerde, and P. Itanberg, A., (2016). “cancer risk assessment indication and guidelines for polycyclic aromatic hydrocarbons in the ambient air environmental health perspectives. 110 (suppl.3).451-488.

31 Bulanova, A.V, Gretskova,I.V., Muratova, O.V.(2005) Sorption properties of sorbents used for cleaning up soils from oil pollution. Vestn. Samara Univ. Nat. Sci.Ser. 3:150-158.

32 Cambria,M.T,Minniti.Z.Librando, V. Cambria. A., (2018) Degradat in of polycyclic aromatichydrocarbons by (Rigidopruslignosus and its laccase in the presence of redox meditators. ApplBiochem Biotechnology) 149:1-8.

33 Campbell, Roberton,Clifford Krauss .,(2010). “Gulf Spill is the largest of its kind, scientistsay “the new your times”.

34 Canak, S .Berezjjev, Borojevic.K, Asotic.J, Ketin. S., (2019) “Bioremediation and greenchemistry” Fresenius Environmental Bulletin 28 (4):3056-306x.

35 Canas.A.Alcalde, M. Plou. F, Martinez, M.J,Martinez.A.T.Camarero., (2017) Transformation of poly cyclic aromatic hydrocarbons by laccase is stronglyenhanced by phenolic compounds present in soil. (Environ Sci.Technol 41:2964-2971).

36 Capotorti. G. Cesti,Plombardi.A.Guglie, Metti. G., (2015) formation of sulfate conjugatesmetabolites in the degradation of phenanthrene, anthracene,pyreneandbenzopyrene by the ascomyeaspergillusterreuspolycyclaromat comp 2 25:197-213.

37. Carey, Bjorn.,(2015) “life building blocks abundant in spac e” space.com. retrieved2014-03-03.

38 Castillo, Maximiliano, Metta-Magana, Alejandro J.Fortier, Skye.,(2016), “Isolation ofgravimetricallyqunntifiable alkali metal arenides using 18 crown 6”m New journal of chemistry 40(3): 1923-1926.doi:10.1039/C5NJ02841H.

39 Duran, Nelson; Esposito, Elisa., (2020) potential applications of oxidative enzymes and phenoloxidase-like compounds in waste water and soil treatment: A review applied catalysis B: Environmental. 1(2):305-318. doi:10.1016/s0926-3373(00)00168-5.

40. Elinskiy, V.I., Akmedov, R.M., Ivanova, Y.A., (2020) The problem of environmental pollution in oil production: Topical issue. Vestn. Moscow Univ. Minist. Intrn. Aff. Russ. 38-43.

41 Ikwa, L.O., Mmecha, M.I., Umoh, J.D.,(2015) Investigation of soil ecosystem Variables affected by oil spill in Odagwa Etche Rivers State, Nigeria. Journal of Biopesticides and Agriculture. 1:45-60.

42. Udeh N.U and Nwaogazie I.L,. (2013) Bioremediation of crude oil contaminated soil using water hyacinth. Advancedin Applied science research 4(2):362-369.

Extremophiles, 10:171-179 doi:10.1007/50092-2005-0498-4.