THE POTENTIAL OF CASSAVA MILL EFFLUENT IN THE BIOREMEDIATION OF PETROLEUM HYDROCARDON-POLLUTED SOIL

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A RESEARCH THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DORCTOR OF PHILOSOPHY DEGREE IN BIOCHEMISTRY

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CERTIFICATION

This is to certify that this research was carried out and submitted by OBAYUWANA Helen Osemena with matriculation number PG/LSC**0807180** of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City.

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CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of Mrs OBAYUWANA Helen Osemena titled The Potential of Cassava Mill Effluent in the Bioremediation of Petroleum Hydrocardon- Polluted Soil has successfully passed the antiplagiarism test and does not violate any copyright regulations.

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DEDICATION

This thesis is dedicated to El-Roi who has shown me great mercy and saw me through this research journey, all the glory is reserved for Him.

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LIST OF ABBREVIATIONS

AcP	Acid phosphatase
AlP	Alkaline phosphatase
AsA	Ascorbic acid
AAS	Atomic absorption spectrophotometer
Av N.	Available Nitrogen
Av. P.	Available Phosphorus
BTEX	Benzene, Toluene, Ethylbenzene and Xylene
CAT	Catalase
CEC	Cation Exchange Capacity
CME	Cassava mill effluent
CN	Cyanide
C_{org}	Organic carboon
DEH	Dehydrogenases
EA	Exchangeable Acidity
EB	Exchangeable Bases
EC	Electrical conductivity
FDA	Fluorescein diacetate
Fw	Fresh weight
MDA	Malondialdehyde
OM	Organic Matter
PAHs	Polycyclic aromatic hydrocarbons
pNPG	*p*- Nitrophenyl-β-D-glucoside
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TOC	Total organic carbon
TN	Total Nitrogen
TP	Total Phosphorus

TSS	Total Soluble Solids
SLO	Spent Lubricating oil
SOC	Soil Organic Carbon
TDS	Total dissolved solids
THAM	Tris- hydroxyl methyl-amino Methane
THB	Total heterotrophic bacteria
THF	Total heterotrophic fungi
THC	Total hydrocarbon
TPH	Total petroleum hydrocarbon
WHC	Water holding capacity

Abstract

The presence of petroleum-derived compounds in the soil constitutes a significant threat to soil health and agricultural productivity. In the search for the environmentally friendly approach in the bioremediation strategies for restoration of soil health, cassava mill effluent (CME) provides an accessible, available and affordable nutrient-rich organic biowaste of cassava processing for the application of organic amendment and a sustainable biostimulant for restoration of soil health. This study investigated the efficiency of CME as a source of organic nutrients in the biorestoration of hydrocarbon degraded soil for a sustainable bioremediation strategy. This was achieved by polluting clean soil, collected and weighed into containers, with spent lubricating oil (SLO) as the source of petroleum hydrocarbon at 10% (w/w), amended with CME at 2.5% (v/w) at different frequency of daily, weekly, monthly and once for a period of six months but the control soil was not treated. The percentage changes in the quality of the amended soil were monitored at 3 and 6 months periods. Cowpea (*Vigna unguiculata*) were grown on the amended soils at the end of the treatment to evaluate recovery of soil quality and function. Baseline data were collected at the start of the study, and others at 3 and 6 months. For physicochemical changes in the soil, the following parameters were analysed: Soil texture, pH, cation exchange capacity (K^+, Na^+, Ca^{2+}, Mg^{2+}, Al^{3+}), electrical conductivity (EC), total petroleum hydrocarbon (THC), and metals (Fe, Zn, Pb). Nitrogen (N_2) and Phosphorus (P) contents, total organic carbon (TOC) and organic matter (OM) were also analysed. The biological activities assessed include the activities of soil enzymes (urease, phosphatase, dehydrogenase, fluorescein diacetate hydrolase and glucosidase), and microbial load. The grown plants were assessed morphologically and in pigment content, stress adaptability and energy metabolism. The CME and SLO were characterised for their physicochemical and hydrocarbon composition, respectively. All the parameters were analysed using standard laboratory procedures and assay protocols. Data obtained were analysed using Graph Pad version 9.4 and results were expressed as mean ± standard error of mean (SEM) of three replicates. The results showed significant ($p < 0.05$) changes in physicochemical parameters and biological activities in the amended soils which also reflected in the plant's morpho- physiological assessment. This

revealed an effect of CME on soil and plant growth qualities. The percentage effect varied with treatment, with most frequently treated having over 50% recovery while untreated had the least. This was evident in crop yield, SOD, Catalase, MDA, Ascorbic acid and Proline levels. In conclusion, the findings in this study showed that with appropriate management, CME can serve as a sustainable bioremediating agent to restore hydrocarbon- degraded soils, supporting a circular economy, and reduce the need for expensive and harmful physical or chemical bioremediation methods as well as a biofertilizer for nutrient enrichment in soils for crop health and productivity.