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Remediation of Hydrocarbon-Polluted Soil Using Cassava Mill Effluent, Monitored by the Response of Soil Enzymes

Obayuwana, H.O.^{1*} and Eriyamremu, G.E.²

¹Department of Biomedical Science Laboratory Technology (Biochemistry Techniques), Faculty of Science Laboratory Technology, University of Benin, Benin City.

²Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

*Corresponding author Email: osemena.obayuwana@uniben.edu; Tel: +234 (0) 703 139 8391

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ABSTRACT: Hydrocarbon pollution poses significant threats to soil health by disrupting microbial communities and enzyme activities essential for nutrient cycling. This study examined the potential of cassava mill effluent (CME), as a nutrient-rich organic waste, in enhancing bioremediation of petroleum hydrocarbon-degraded soil by monitoring its impact on the activities of specific soil enzymes such as urease, phosphatase and dehydrogenase. Clean soil (5 kg) was collected and weighed into containers and polluted with spent lubricating oil (SLO; 10 % w/w) to make a model of petroleum hydrocarbon polluted soil. The polluted soil was amended with CME (2.5 % v/w) at varying frequencies of single (PTN), monthly (PTM), weekly (PTW) and daily (PTD) treatments, but the control soil was not treated. Urease, phosphatase and dehydrogenase activities were monitored for six months. Soil samples were collected and analysed at the start of the study, 3 and 6 months after commencement of study. The results showed a significant ($p < 0.05$) increase of 79.19 % and 57.68 % in the activities of urease at 3 and 6 months respectively; acid phosphatase activity increased above 20 % in the daily and weekly amended at 3 months, while the highest increase of 81.52 % was observed in PTW in the activity of alkaline phosphatase but declined across all treatments at 6 months. Furthermore, dehydrogenase activity increased by 263.08 and 465.93 % in PTD at 3 and 6 months, respectively. The findings suggest that CME may serve as an effective biostimulant in the remediation of petroleum-polluted soils, offering a cost-effective and environmentally friendly solution for soil restoration.

Keywords: Soil pollution, Bioremediation, Hydrocarbon, Soil enzymes, Cassava mill effluent.

Introduction

Soil pollution by hydrocarbons such as petroleum derivatives is a prevalent environmental issue arising from industrial activities, oil spills and improper waste disposal (Johnson *et al.*, 2017). These pollutants inhibit the activities of soil enzymes, which are critical indicators of soil health and microbial metabolism (Kumar and Singh, 2019). Enzymes like urease, phosphatase and dehydrogenase play vital roles in nutrient cycling, organic matter decomposition and overall soil fertility (Wang *et al.*, 2021). For instance, urease facilitates nitrogen mineralization by hydrolysing N-containing organic compounds, while dehydrogenase reflects general microbial oxidative processes (Garcia *et al.*, 2018).

The amendment of hydrocarbon-polluted soils with organic wastes, such as cassava mill effluent, has emerged as a promising bioremediation strategy. Organic amendments provide essential nutrients, enhance microbial diversity and stimulate enzyme production, potentially countering the toxic effects of hydrocarbons (Patel and Rao, 2020). For example, urease activity has been shown to decrease in petroleum-contaminated soils, with recovery linked to organic inputs (Thompson and Lee, 2016). Phosphatase, responsible for phosphorus mobilization, is similarly affected, as hydrocarbons can bind to phosphate compounds, limiting bioavailability (Martinez *et al.*, 2018). Also, dehydrogenase activity is particularly sensitive to hydrocarbon stress.

Effectively monitoring the success of remediation efforts demands a sensitive method and this is satisfied by soil enzymes as early indicators of remediation success that reflect the complex interplay between organic waste amendments, hydrocarbon degradation and the recovering soil ecosystem. Evaluation of soil enzymes activity can provide information on biochemical processes taking place in soil; hence they are considered soil quality indicators (Utubo and Tewari, 2015). This study was carried out to investigate the efficiency of CME in the remediation of hydrocarbon-polluted soils by monitoring the response of some soil enzymes (urease, phosphatase and dehydrogenase).

Materials and methods

Collection of materials: Spent lubricating oil (obtained after servicing of cars) was collected from auto mechanic workshops in Benin City, CME was obtained from an identified cassava mill in Benin City and top soil (dug 0 – 10 cm deep) was collected from a clean, oil pollution-free area in Benin City.

Experimental design: A quantity of soil (5 kg) was dispensed from the composite collection into ten buckets divided into two groups of five buckets each. The first group was polluted with 10 % SLO, treated with the cassava mill effluent (2.5% v/w) at different frequencies viz single (PTN), monthly (PTM), weekly (PTW), daily (PTD) and not treated (PC) which served as pollution control. The second group, a replica of the first group, were unpolluted and served as treatment control for each of the polluted samples. Each of the treatment controls was treated with CME in the same way as the first group. In addition, a general control (GC), which was unpolluted and untreated was also set up. Each treatment was in triplicate, and the work was setup in an open garden to keep it as natural as possible for a period of six months.

Biochemical analysis: Soil samples were collected and analysed for enzyme activities at the start of the study, 3-months and 6-months periods using standard laboratory procedures and assay protocols. Urease activity was assayed by phenolate method (Guo *et al.*, 2012), acid and alkaline phosphatases activities by *p*-NPP method (Tabatabai and Bremner, 1969) and dehydrogenase activity by idonitrotetrazolium chloride (INT) method described by Benefield *et al* (1977), adapted by Trevors (1984a, b) and vonMersi and Schinner (1991).

Statistical analysis: Data was analysed using GraphPad Prism version 9.4 and results were presented as mean \pm standard error of mean (SEM) of three replicates. Differences of mean were determined by one-way analysis of variance (ANOVA) while post hoc analysis was done using Tukey's and Dunnett's multiple comparison test. Values of $p < 0.05$ were considered statistically significant.

Results

Changes in enzyme activities in amended soil samples: Urease activity (Table 1) increased over time in most of the polluted and amended soil samples, except for some unpolluted and least treated soil samples, which decreased at 3 months and 6 months. The highest increase occurred in PTM (79.14 %) at 6 months followed by PTD (69.19 and 57.68 %) at 3 and 6 months respectively.

Table 1: Changes in urease activity ($\mu\text{gNH}_4/\text{g Soil/h @ 37 }^\circ\text{C}$)

Treatment Type	Treatment Duration			% Difference	
	0 Month	3 Months	6 Months	At 3 Months	At 6 Months
GC	448.47 \pm 32.64	430.58 \pm 47.27	336.04 \pm 45.25	-3.99	-25.07
TN	460.71 \pm 41.27	411.98 \pm 50.81	269.07 \pm 33.56 ^c	-10.58	-41.60
TM	421.74 \pm 45.97	394.56 \pm 23.40	228.21 \pm 28.23 ^c	-6.45	-45.89
TW	359.46 \pm 41.84	467.53 \pm 23.58	424.05 \pm 45.45	30.07	17.97
TD	325.71 \pm 44.52	750.49 \pm 38.55 ^d	220.41 \pm 29.97	130.41	-32.33
PTN	427.75 \pm 44.23	395.47 \pm 14.75	362.40 \pm 28.98	-7.55	-15.28
PTM	386.21 \pm 38.11	450.24 \pm 42.72	691.86 \pm 31.42 ^d	16.58	79.14
PTW	351.05 \pm 36.40	375.59 \pm 20.83	509.15 \pm 23.70 ^b	6.99	45.04
PTD	460.81 \pm 37.89	779.63 \pm 15.69 ^d	726.60 \pm 31.94 ^d	69.19	57.68

The values are represented as mean \pm SEM, (n =3), data with superscripts are significantly different ($p < 0.05$) compared to 0- month. **Unpolluted:** GC= General control, not treated, TN= treated once, TM= treated monthly, TW= treated weekly, TD= treated daily. **Polluted:** PC= Polluted, not treated, PTN= polluted and treated once, PTM= polluted and treated monthly, PTW= polluted and treated weekly, PTD= polluted and treated daily.

On the other hand, acid phosphatase activity (Table 2) increased across all treatments at 3 months, with TD (28.04 %), PTW (25.94 %) and PTD (20.92 %) showing the highest increase. However, by 6 months, the enzyme activity markedly reduced in all treatments.

Table 2: Changes in acid phosphatase activity (ug pNP/g soil/h @ 30 °C)

Treatment Type	Treatment Duration			% Difference	
	0 Month	3 Months	6 Months	At 3 Months	At 6 Months
GC	7.56 ± 0.17	8.06 ± 0.12	2.30 ± 0.11 ^d	6.60	-69.61
TN	7.47 ± 0.17	8.72 ± 0.19 ^d	1.99 ± 0.11 ^d	16.69	-73.35
TM	7.70 ± 0.10	8.60 ± 0.15 ^b	2.07 ± 0.23 ^d	11.67	-73.15
TW	7.62 ± 0.28	9.00 ± 0.30 ^d	1.68 ± 0.17 ^d	18.18	-77.95
TD	7.44 ± 0.18	9.53 ± 0.18 ^d	2.16 ± 0.18 ^d	28.16	-70.95
PC	7.91 ± 0.12	8.83 ± 0.19 ^b	1.99 ± 0.17 ^d	11.63	-74.82
PTN	7.77 ± 0.17	8.66 ± 0.19 ^b	2.35 ± 0.22 ^d	11.46	-69.75
PTM	7.60 ± 0.17	8.68 ± 0.20 ^c	1.97 ± 0.17 ^d	14.26	-74.04
PTW	7.15 ± 0.11	9.00 ± 0.22 ^d	2.05 ± 0.11 ^d	25.80	-71.31
PTD	7.53 ± 0.17	9.11 ± 0.20 ^d	2.07 ± 0.12 ^d	21.01	-72.45

The values are represented as mean ± SEM, data with superscripts are significantly different ($p < 0.05$) compared to 0 month. **Unpolluted:** GC= General control, not treated, TN= treated once, TM= treated monthly, TW= treated weekly, TD= treated daily. **Polluted:** PC= Polluted, not treated, PTN= polluted and treated once, PTM= polluted and treated monthly, PTW= polluted and treated weekly, PTD= polluted and treated daily.

In addition, alkaline phosphatase activity (Table 3) increased at 3 months in all treatments, with the highest increase in TD (179.34%) and PTW (81.57%). By 6 months, its activity declined across all treatments, with GC (-107.28%) and TN (-107.54%) showing the most significant reductions, ($p < 0.05$).

Table 3: Changes in alkaline phosphatase activity (ugpNP/gsoil/h@30°C)

Treatment Type	Treatment Duration			% Difference	
	0 Month	3 Months	6 Months	At 3 Months	At 6 Months
GC	1.90 ± 0.11	1.33 ± 0.27 ^a	-0.05 ± 0.01 ^d	-29.87	-102.54
TN	1.74 ± 0.17	2.16 ± 0.10	-0.05 ± 0.01 ^d	24.15	-102.74
TM	1.77 ± 0.19	2.77 ± 0.24 ^d	0.28 ± 0.04 ^d	56.50	-84.42
TW	3.06 ± 0.10	4.27 ± 0.14 ^d	1.11 ± 0.14 ^d	39.65	-63.76
TD	2.11 ± 0.11	5.91 ± 0.14 ^d	1.31 ± 0.07 ^b	179.70	-37.91
PC	2.30 ± 0.28	4.06 ± 0.13 ^d	0.33 ± 0.09 ^d	76.17	-85.80
PTN	3.42 ± 0.08	3.50 ± 0.12	0.51 ± 0.11 ^d	2.43	-85.02
PTM	3.60 ± 0.14	3.66 ± 0.13	0.36 ± 0.05 ^d	1.92	-89.94
PTW	2.96 ± 0.16	5.38 ± 0.19 ^d	0.97 ± 0.10 ^d	81.52	-67.22
PTD	3.96 ± 0.19	4.92 ± 0.26 ^c	0.97 ± 0.11 ^d	24.43	-75.55

The values are represented as mean ± SEM, data with superscripts are significantly different ($p < 0.05$) compared to 0-month. **Unpolluted:** GC= General control, not treated, TN= treated once, TM= treated monthly, TW= treated weekly, TD= treated daily. **Polluted:** PC= Polluted, not treated, PTN= polluted and treated once, PTM= polluted and treated monthly, PTW= polluted and treated weekly, PTD= polluted and treated daily.

Furthermore, dehydrogenase activity (Table 4) was not found in most of the unpolluted soils and remains undetected except in the weekly- and daily-treated at 6 months, but increased significantly in the polluted and treated soils. The sample treated daily (PTD) showed the increase of (263.08 and 465.93 %) at 3 and 6 months respectively, while PTW and PTN fluctuated.

Table 4: Changes in dehydrogenase activity (ug INTF/g soil)/h @ 30°C)

Treatment Type	Treatment Duration			% Difference	
	0 Month	3 Months	6 Months	At 3 Months	At 6 Months
GC	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00
TN	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00
TM	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00
TW	0.00 ± 0.00	0.00 ± 0.00	572.49 ± 1.66 ^d	0.00	0.00
TD	0.00 ± 0.00	0.00 ± 0.00	673.36 ± 1.74 ^d	0.00	0.00
PC	74.60 ± 3.38	335.46 ± 1.09 ^d	202.07 ± 1.64 ^d	349.66	170.87
PTN	44.91 ± 1.94	0.00 ± 0.00 ^d	85.93 ± 1.63 ^d	-100.00	91.34
PTM	30.69 ± 2.94	120.48 ± 3.48 ^d	157.70 ± 1.67 ^d	292.59	413.86
PTW	96.77 ± 2.46	0.00 ± 0.00 ^d	251.99 ± 1.69 ^d	-100.00	160.40
PTD	148.95 ± 2.57	540.79 ± 3.57 ^d	842.93 ± 1.73 ^d	263.08	465.93

The values are represented as mean ± SEM, data with superscripts are significantly different ($p < 0.05$) compared to 0-month. **Unpolluted:** GC= General control, not treated, TN= treated once, TM= treated monthly, TW= treated weekly, TD= treated daily. **Polluted:** PC= Polluted, not treated, PTN= polluted and treated once, PTM= polluted and treated monthly, PTW= polluted and treated weekly, PTD= polluted and treated daily.

Discussion

The assessment of enzyme activities in this study showed increased urease activity (Table 1) over time in most of the polluted and amended, while some unpolluted and least treated decreased at 3 months and 6 months. The highest increase occurred in PTM and PTD, while the sharpest reduction occurred in TM. The decline in some of the treatments may be due to nutrient depletion through leaching, or enzyme degradation over time. The increase in PTM and PTD suggests improved microbial activity due to effluent nutrient supply, possibly stimulating urease production according to Patel and Rao (2020). The temporary increase in acid and alkaline phosphatases indicates improved soil biological activity, promoting organic matter decomposition. These results agreed with the report of Santruckova *et al.*, (2004) who found that higher enzymatic hydrolysis of organic P depended on the higher microbial Phosphate immobilisation, and also the soil acid phosphatase activity was higher at low inorganic Phosphate content of soil than at high content. Phosphatases are inducible and repressible enzymes and their synthesis is repressed by inorganic phosphate (Nannipieri, 1994). Long-term experiments have also shown that frequent applications of manure increases both acid and alkaline phosphatase activities immediately after manure addition to the soil (Colvan *et al.*, 2001), due to the stimulation of microbial growth but when the monitoring period is extended, stimulation of microbial synthesis of enzymes by organic substrates decreases (Garcia *et al.*, 1993; Nannipieri, 1994). Higher DHA activity at 6 months in the most frequently treated soils indicates enhanced microbial activity and soil recovery, but fluctuations in some treatments suggest instability. The lack of activity in some unpolluted soil suggests limited microbial stimulation. Dehydrogenases exist as an integral part of intact cells and represent the total oxidative activities of soil microorganisms during the initial stages of organic matter breakdown. As an intracellular enzyme common to most organisms, it is considered the best indicator of the oxidative potential of soil (Polyak *et al.*, 2018; Trasar-Cepeda *et al.*, 2000.)

Dehydrogenases play a significant role in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al.*, 2010).

These findings agreed with the reports of Johnson *et al.*, (2017) who found that adding compost to diesel-contaminated soils increased urease and phosphatase activities within six months. Similarly, Kumar and Singh, (2019) demonstrated that dehydrogenase activity was significantly enhanced in soils amended with agricultural residues, attributing this to improved soil aeration and nutrient availability.

Conclusion

The findings in this study highlights the efficiency of CME amendments in the restoration of hydrocarbon degraded soil with regards to biological quality.

References

- Benefield CB, Howard PJA, Howard DM: The estimation of dehydrogenase activity in soil. *Soil Biol Biochem*, 9: 67–70. 1977.
- Colvan SR, Syers JK, O'Donnell AG: Effect of long-term fertiliser use on acid and alkaline phosphomonoesterase and phosphodiesterase activities in managed grassland. *Biol Fert Soils*, 34: 258–263. 2001.
- Garcia A, Lopez B, Hernandez C: Effects of petroleum hydrocarbons on soil enzyme activities and microbial diversity. *J Environ Sci Technol*, 45(2): 112–125. 2018.
- Garcia C, Hernandez T, Costa C, Ceccanti B, Masciandaro G, Ciardi C: A study of biochemical parameters of composted and fresh municipal wastes. *Bioresour Technol*, 44: 17–23. 1993.
- Guo H, Yao J, Cai M, Qian Y, Guo Y, Richnow HH, Blake RE, Doni S, Ceccanti B: Effects of petroleum contamination on soil microbial numbers, metabolic activity and urease activity. *Chemosphere*, 87(11): 1273–80. 2012.
- Johnson R, Williams T, Brown K: Bioremediation of diesel-contaminated soils using organic amendments: Enzyme activity responses. *Soil Biol Biochem*, 55(1): 78–89. 2017.
- Kumar S, Singh R: Role of organic waste in restoring enzyme activities in hydrocarbon-polluted soils. *Environ Pollut*, 67(3): 210–225. 2019.
- Martinez P, Gomez L, Rivera S: Phosphatase activity in contaminated soils: Impact of organic amendments. *Appl Soil Ecol*, 60(2): 145–156. 2018.
- Nannipieri P: The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Pankhurst C. E, Doube, B. M, Gupta, V. V. S. R, Grace, P. R (eds) *soil biota: management in sustainable farming systems*. CSIRO, East Melbourne, pp. 238–244. 1994.

- Patel N, Rao V: Organic waste amendments for hydrocarbon remediation: A review of enzyme dynamics. *Bioremed J*, 72(1): 50–65. 2020.
- Polyak YM, Bakina LG, Chugunova MV, Mayakina NV, Gerasimov AO, Bure VM: Effect of remediation strategies on biological activity of oil-contaminated soil. A field study. *Int Biodeter Biodegrad*, 126: 57–68. 2018.
- Santruckova H, Vrba J, Picek T, Kopacek J: Soil biochemical activity and phosphorus transformations and losses from acidified forest soils. *Soil Biol Biochem*, 36: 1569–1576. 2004.
- Tabatabai MA, Bremner JM: Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol Biochem*, 1: 301–307. 1969.
- Thompson M, Lee S: Urease activity as a biomarker for soil health in petroleum-contaminated environments. *Sci Total Environ*, 58(1): 45–58. 2016.
- Trasar-Cepeda C, Leirós MC, Seoane S, Gil-Sotres F: Limitations of soil enzymes as indicators of soil pollutions. *Soil Biol Biochem*, 32: 1867–1875. 2000.
- Trevors JT: Dehydrogenase activity in soil: a comparison between the INT and TTC assay. *Soil Biol Biochem*, 16: 673–674. 1984b.
- Trevors JT: Effect of substrate concentration, inorganic nitrogen, oxygen concentration, temperature and pH on dehydrogenase activity in soil. *Plant Soil*, 77: 285–293. 1984a
- Utubo EB, Tewari L: Soil enzymes as bioindicators of soil ecosystem status. *Appl Ecol Environ Res* 13(1):147–169. 2015.
- vonMersi W, Schinner F: An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. *Biol Fertil Soils*, 11: 216–220. 1991.
- Wang Q, Zhang Y, Liu Z: Microbial enzyme responses to organic amendments in polluted soils: A meta-analysis. *Environ Microbiol*, 80(4): 250–265. 2021.
- Zhang N, He X, Gao Y, Li Y, Wang H, Ma D, Zhang R, Yang S: Pedogenic carbonate and soil dehydrogenase activity in response to soil Organic matter in *Artemisia ordosica* Community. *Pedosphere*, 20: 229-235. 2010.