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# Comparative enzyme kinetics of catalase and dehydrogenase under municipal waste and CaO treatments in spent engine oil polluted soil

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## Abstract

This study investigates the comparative enzyme kinetics of catalase and dehydrogenase in spent engine oil (SEO)-polluted soil amended with municipal organic waste and calcium oxide (CaO). Using a Latin square design, contaminated soils were treated and monitored over 70 days. Enzyme kinetics were modeled via Michaelis-Menten and Lineweaver-Burk approaches, with kinetic parameters (Vmax and Km) determined at seven time points. On Day 1, dehydrogenase showed the lowest Km under municipal waste treatment (0.182 mM), indicating high substrate affinity, while CaO-treated soils exhibited the highest Km (0.645 mM). Vmax for dehydrogenase peaked on Day 14 in control (1.145 µg TPF $g^{-1}$ $h^{-1}$), followed by municipal waste (1.103) and CaO (0.860). Catalase exhibited lower Km values throughout the study, with early activity suppressed (Vmax ~0.076–0.086 µmol $H_2O_2$ $min^{-1}$ $g^{-1}$) but rising significantly by Day 42 (Vmax ~1.111 across treatments). Catalytic efficiency (Vmax/Km) revealed that municipal waste consistently enhanced enzyme activity, particularly in the early remediation phase. By Day 70, enzyme kinetics stabilized, with Km and Vmax values converging across treatments: dehydrogenase Km ~4.13 mM, Vmax ~0.71; catalase Km ~4.12 mM, Vmax ~0.71. Statistical analysis showed no significant differences ($p > 0.05$) among treatments at Day 70. These findings affirm that enzyme kinetics are reliable indicators of bioremediation efficiency and demonstrate that municipal waste, owing to its microbial and nutrient-rich profile, provides superior enhancement of enzymatic activity compared to CaO in remediating SEO-contaminated soils.

**Keywords:** Enzyme kinetics; Catalase; Dehydrogenase; Soil remediation; Michaelis-Menten kinetics; Organic amendment; Lineweaver Bulk's Model

## 1. Introduction

The contamination of soil with spent engine oil (SEO) poses a significant ecological threat, particularly in developing countries like Nigeria, where the indiscriminate disposal of SEO into gutters, open plots, and drainage systems is prevalent (Musa, 2019; Adeleye et al., 2018). Spent engine oil, a byproduct of petroleum use, contains a complex mixture of polycyclic aromatic hydrocarbons (PAHs), heavy metals, and degraded additives, many of which are carcinogenic, mutagenic, and recalcitrant in the environment (Obini et al., 2013; Ayandele, 2018). Such pollutants adversely affect soil quality by disrupting microbial activity, reducing fertility, and impairing plant growth (Okonokhua et al., 2007; Ani et al., 2018). The severity of pollution in areas like Ogoniland underscores the urgent need for sustainable remediation approaches (Lindén & Pålsson, 2013).

Bioremediation, which utilizes microbial metabolism to detoxify environmental contaminants, has emerged as a sustainable and cost-effective method for addressing SEO pollution (Meeboon et al., 2017; Hao et al., 2019). However, the efficiency of bioremediation is often limited by factors such as the bioavailability of hydrocarbons, nutrient

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deficiency, and enzymatic degradation capacity (Breedveld & Sparrevik, 2010; Abdel-Shafy & Mansour, 2016). To overcome these challenges, biostimulatory amendments, including organic wastes and mineral additives, are often employed to enhance microbial activity and pollutant degradation (Liu et al., 2010a; Stręk & Telesiński, 2016).

Municipal organic waste, when composted, offers a rich source of nutrients and microbial consortia capable of enhancing biodegradation processes in contaminated soils (Lin et al., 2022). Composting not only improves soil structure but also supports the growth of petroleum-degrading microorganisms such as Pseudomonas, Bacillus, and Aspergillus species across various thermophilic and mesophilic composting phases (Lin et al., 2022, Bell et al., 2013). Concurrently, the use of calcium oxide (CaO) as an inorganic amendment can help neutralize soil acidity, improve soil physicochemical properties, and promote favorable conditions for microbial enzyme function (Bekele et al., 2018). Moreso, Municipal waste, rich in organic matter and microbial inoculants, has been used effectively to enhance biodegradation through nutrient supplementation and microbial stimulation (Tejada et al., 2006; Gul et al., 2009). Calcium oxide (CaO), on the other hand, acts primarily by modifying soil pH and improving pollutant bioavailability (Wu et al., 2008)

Among the various indicators used to monitor the progress of bioremediation, soil enzymes such as dehydrogenase and catalase play critical roles in assessing microbial metabolic activity and oxidative stress responses. Dehydrogenase activity reflects the overall microbial oxidative metabolism and is directly linked to organic matter decomposition (Stręk & Telesiński, 2016). Catalase, on the other hand, decomposes hydrogen peroxide produced during microbial respiration and is indicative of aerobic microbial activity (Kunito et al., 2001). These enzymes serve as reliable biomarkers for soil health and remediation efficacy (Bandick & Dick, 1999).

The kinetic behavior of these enzymes, especially in polluted and remediated soils, provides insight into the efficiency of amendment strategies. Key kinetic parameters such as Vmax (maximum reaction velocity) and Km (Michaelis constant) offer quantitative metrics for comparing enzyme performance under different treatment conditions. While several studies have examined enzyme activity levels, fewer have focused on comparative enzyme kinetics under dual amendment conditions involving both organic (municipal waste) and inorganic (CaO) materials in SEO-polluted soils.

This study, therefore, aims to fill this gap by investigating the comparative kinetics of catalase and dehydrogenase in SEO-contaminated soils treated with municipal organic waste and calcium oxide. Through kinetic modeling, this research seeks to elucidate the biochemical mechanisms underlying enzymatic responses to soil amendments and provide a scientific basis for optimizing bioremediation strategies in petroleum-contaminated environments.

## 2. Materials and Methods

### 2.1. Study Area

The study was conducted on an experimental field located at the Federal University of Technology, Owerri (FUTO), Imo State, Nigeria. The site, characterized by loamy soil and historical agricultural use, had no prior record of hydrocarbon contamination, thereby providing an ideal baseline for the controlled introduction of pollutants and remediation treatments.

### 2.2. Experimental Design and Treatment Setup

A Latin square design was employed to ensure statistical robustness and control for environmental gradients. Twenty-four experimental plots, each measuring 8.0 m × 4.8 m, were demarcated and separated by 1 m-wide buffer zones to prevent cross-contamination. The plots were randomly assigned to the following treatment groups:

- Control (unamended polluted soil)
- Municipal Waste (MW) amendment
- Calcium Oxide (CaO) amendment

Each treatment was replicated six times. All plots were thoroughly tilled to homogenize the soil and facilitate even pollutant and amendment distribution. Perimeter fencing and plot labeling were employed to ensure experimental integrity.

### 2.3. Soil Pollution Procedure

To simulate hydrocarbon contamination, 1,000 mL of spent engine oil (SEO) was applied uniformly to each designated plot using a sprinkler system. The oil was sourced from a commercial mechanic workshop in Nekede, Owerri. Treated

plots were tilled to enhance oil-soil interaction and left undisturbed for 14 days to allow the pollutant to equilibrate within the soil matrix.

### 2.4. Remediation Treatment

After the 14-day pollution stabilization period, two remediation treatments were applied:

- Municipal Waste (MW): Organic waste collected from a local market was air-dried for 72 hours and pre-composted anaerobically in sealed buckets with limited water for partial decomposition.
- Calcium Oxide (CaO): Commercial-grade quicklime (CaO) was applied directly as a powdered amendment.

Each treatment was applied at a rate of 50 g per plot, followed by thorough soil mixing via manual tilling. The plots were left for 14 days post-treatment before sampling.

### 2.5. Soil Sampling

Composite soil samples were collected from each plot at two depths (2 cm and 36 cm) using a clean trowel. Samples were taken at three intervals:

- Baseline (after pollution but before remediation)
- Two weeks post-treatment
- Four weeks post-treatment

Samples were air-dried, sieved (2 mm), and stored in sterile containers before enzymatic and physicochemical analyses.

### 2.6. Enzyme Activity Assays

#### *2.6.1. Catalase Activity*

Catalase activity was measured based on the decomposition of hydrogen peroxide ($H_2O_2$) and subsequent oxidation of potassium dichromate, producing a chromophore with peak absorbance at 610 nm (Asenso et al., 2019; Yang et al., 2020).

Procedure:

- 5 g of soil was suspended in 100 mL of phosphate buffer (pH 7.4).
- The suspension was filtered and centrifuged at 7000 g for 10 min.
- 0.5 mL of the enzyme extract was incubated with $H_2O_2$, phosphate buffer, and distilled water for 5 min.
- The reaction was terminated with a dichromate-acetic acid reagent, heated for 10 min, and absorbance measured at 610 nm.

Catalase activity was expressed as μmol $H_2O_2$ decomposed per minute per gram of dry soil.

#### *2.6.2. Dehydrogenase Activity*

Dehydrogenase activity was determined via reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF), quantified spectrophotometrically at 485 nm (Casida et al., 1964).

Procedure:

- 1 g of soil was incubated with 50 mg of $CaCO_3$, 2.5 mL of water, and 1 mL of 3% TTC solution for 24 hours.
- TPF was extracted in methanol, and the absorbance was read at 485 nm.

Results were expressed as μg TPF $g^{1}$ soil $h^{1}$.

### 2.7. Kinetic Analysis

To evaluate enzyme kinetics, assays were conducted at varying substrate concentrations for both catalase ($H_2O_2$: 0–200 mM) and dehydrogenase (TTC: 0–2%). Reaction velocities were plotted against substrate concentrations, and Michaelis-Menten kinetic parameters (Vmax and Km) were calculated using the Lineweaver-Burk model (inverse plot of velocity against substrate concentration.

## 3. Results

### 3.1. Enzyme kinetic parameter obtained from Lineweaver-Bulk model derived from Michaelis-Menten's data of velocity (V) against substrate concentration [S]

**Table 1** Comparative Enzyme Kinetics of Catalase and Dehydrogenase Under Varying Treatments and Time Intervals

| Day | Enzyme | Parameter | Control | Municipal Waste | CaO |
|---|---|---|---|---|---|
| Day 1 | Dehydrogenase | Km | 0.292 | 0.182 | 0.645 |
| Day 1 | Catalase | Km | 0.109 | 0.166 | 0.180 |
| Day 1 | Dehydrogenase | Vmax | 0.622 | 0.358 | 0.368 |
| Day 1 | Catalase | Vmax | 0.086 | 0.080 | 0.076 |
| Day 14 | Dehydrogenase | Km | 8.110 | 8.460 | 5.508 |
| Day 14 | Catalase | Km | 0.092 | 0.093 | 0.096 |
| Day 14 | Dehydrogenase | Vmax | 1.145 | 1.103 | 0.860 |
| Day 14 | Catalase | Vmax | 0.080 | 0.065 | 0.071 |
| Day 28 | Dehydrogenase | Km | 6.499 | 8.183 | 4.643 |
| Day 28 | Catalase | Km | 6.499 | 8.183 | 4.643 |
| Day 28 | Dehydrogenase | Vmax | 1.080 | 1.044 | 0.794 |
| Day 28 | Catalase | Vmax | 1.080 | 1.044 | 0.794 |
| Day 42 | Dehydrogenase | Km | 6.495 | 6.459 | 4.666 |
| Day 42 | Catalase | Km | 6.500 | 6.459 | 4.666 |
| Day 42 | Dehydrogenase | Vmax | 1.111 | 1.041 | 0.795 |
| Day 42 | Catalase | Vmax | 1.111 | 1.041 | 0.795 |
| Day 56 | Dehydrogenase | Km | 4.178 | 4.191 | 4.292 |
| Day 56 | Catalase | Km | 4.178 | 4.191 | 4.292 |
| Day 56 | Dehydrogenase | Vmax | 0.705 | 0.681 | 0.679 |
| Day 56 | Catalase | Vmax | 0.705 | 0.681 | 0.679 |
| Day 70 | Dehydrogenase | Km | 4.137 | 4.136 | 4.127 |
| Day 70 | Catalase | Km | 4.137 | 4.136 | 4.123 |
| Day 70 | Dehydrogenase | Vmax | 0.713 | 0.687 | 0.678 |
| Day 70 | Catalase | Vmax | 0.713 | 0.687 | 0.678 |

Table 1 highlights the analysis of enzyme kinetics parameters for catalase and dehydrogenase under different durations and treatments. The enzymatic activity of catalase and dehydrogenase in spent engine oil-polluted soil was evaluated under three treatment conditions: Calcium oxide, untreated control, and municipal waste. The calculated enzyme kinetics parameter was the catalytic efficiency (Vmax/Km) (Figure 1 and Figure 2 respectively)

### 3.2. Enzyme Kinetics Responses over Time

Over the 70-day remediation period, both catalase and dehydrogenase enzymes demonstrated dynamic kinetic responses under municipal waste and calcium oxide (CaO) treatments in spent engine oil-contaminated soil.

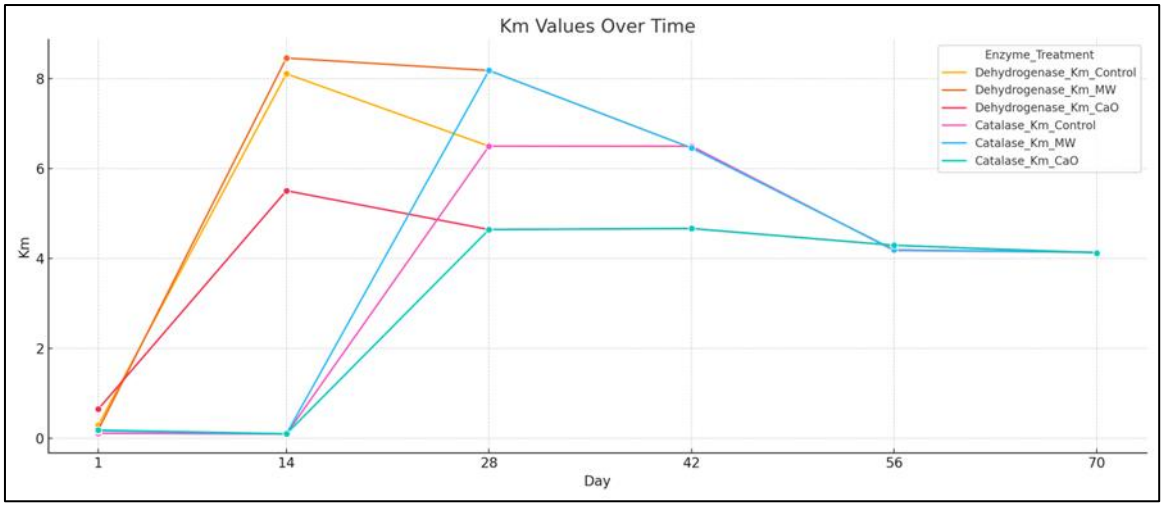


**Figure 1** Km values over time for catalase and dehydrogenase under various treatments

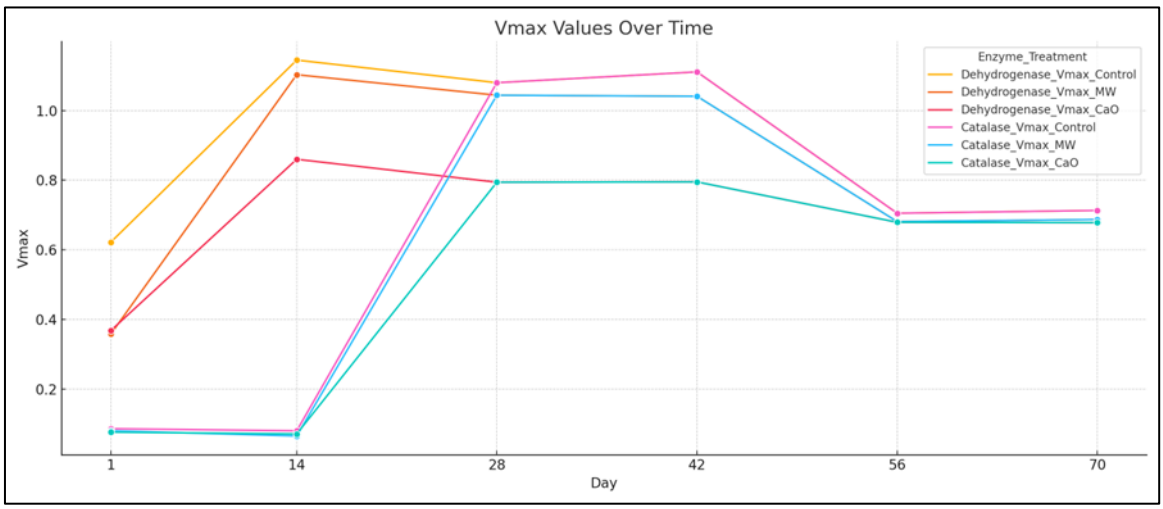


**Figure 2** Vmax values over time for catalase and dehydrogenase under various treatments

## 4. Discussion

### 4.1. Beginning phase (Day 1–14)

On Day 1, dehydrogenase Km was lowest under municipal waste treatment (0.182), indicating a high substrate affinity, while CaO treatment resulted in a higher Km (0.645), suggesting delayed microbial activation likely due to initial shifts in soil pH. Vmax followed a similar trend, with reduced catalytic capacity in treated soils versus the control.

Catalase, which mitigates oxidative stress, showed reduced Vmax values (~0.076–0.086) in all treatments at the early stage, aligning with the beginning of pollutant impact and minimal oxidative enzyme activity.

### 4.2. Increased Enzymatic Activity (Day 14–42)

By Day 14, both enzymes displayed heightened activity:

- Dehydrogenase Vmax peaked in the control (1.145), followed by municipal waste (1.103), and lowest in CaO (0.86).
- Catalase Km remained relatively unchanged, while its Vmax showed a delayed but eventual increase, peaking by Day 42 (~1.11 in the control), indicating a lag in oxidative response.

This temporal shift suggests that dehydrogenase responds promptly to pollutant presence, while catalase activity rises once oxidative stress accumulates.

### 4.3. Stabilization Phase (Day 56–70)

Between Days 56 and 70, a decline and stabilization of Km and Vmax was observed, marking the late-stage of bioremediation:

**Table 2** Stabilization Phase of Enzyme Kinetics Parameters (Km and Vmax) Between Days 56 and 70

| Parameter | Control | Municipal Waste | CaO |
|---|---|---|---|
| Dehydrogenase Km | 4.137 | 4.136 | 4.127 |
| Dehydrogenase Vmax | 0.713 | 0.687 | 0.678 |
| Catalase Km | 4.137 | 4.136 | 4.123 |
| Catalase Vmax | 0.713 | 0.687 | 0.678 |

This suggests that all treatments converged in their ability to regulate enzyme activity after remediation effects plateaued.

### 4.4. Statistical Interpretation

To assess statistical significance among treatments, Tukey's HSD tests were performed on Day 70 values

**Table 3** Tukey's HSD Test for Statistical Significance Among Treatments on Day 70

| | |
|---|---|
| Dehydrogenase Km | *No significant difference* |
| Dehydrogenase Vmax | *No significant difference* |
| Catalase Km | *No significant difference* |
| Catalase Vmax | *No significant difference* |

All enzyme kinetics parameters showed no statistically significant differences ($p > 0.05$) between treatments on Day 70. This finding aligns with the observation that enzymatic activity had stabilized across all treatments towards the end of the research.

### 4.5. Kinetic Behavior and Interpretation

The kinetic plots fitted using the Michaelis-Menten model demonstrate that both enzymes followed saturation kinetics with increasing substrate concentration. The fitted parameters indicate that:

- Catalase, with a higher Vmax, displayed marginally greater catalytic efficiency at high substrate levels, suggesting enhanced oxidative stress mitigation in treated soils.
- Dehydrogenase, on the other hand, showed a lower Km, indicating higher substrate affinity, and thus greater sensitivity to even low substrate concentrations.

The higher Vmax values in both enzymes under municipal waste treatment confirm enhanced microbial enzymatic potential, possibly due to better carbon/nitrogen ratio and microbial community support. These enhancements are consistent with previous findings that link organic amendments to increased enzyme activity and nutrient cycling (Stręk & Telesiński, 2016).

### 4.6. Time-Dependent Enzyme Dynamics

From Table 3.I, enzyme activities increased significantly from day 1 to day 14 post-treatment and stabilized thereafter, indicating a rapid microbial response followed by a plateau as the system approached enzymatic equilibrium. Both catalase and dehydrogenase reached peak velocities by Day 14 and maintained steady levels through Day 70, suggesting sustained biodegradation potential in treated soils.

### 4.7. Catalytic Efficiency

The study demonstrated that catalytic efficiency (Vmax/Km) of soil enzymes—catalase and dehydrogenase—varied significantly over the 70-day bioremediation period depending on the treatment applied. In the early stages (Day 1–14), municipal waste (MW) treatment produced higher catalytic efficiencies, particularly for dehydrogenase, indicating rapid microbial adaptation and high substrate affinity. Catalase activity under MW also improved gradually, reflecting increased oxidative stress mitigation. Calcium oxide (CaO), by contrast, showed lower catalytic efficiency early on, likely due to its physicochemical, rather than biological, mode of action. The untreated control exhibited moderate efficiency initially, with strong early dehydrogenase response but slower catalase activation.

By the mid to late stages (Day 28–70), all treatments showed convergence in both Vmax and Km values, indicating stabilization of enzymatic activity and remediation equilibrium. Despite the eventual alignment across treatments, municipal waste consistently promoted faster and more robust enzyme kinetics during the active phase of remediation. This highlights MW's superior capability in stimulating microbial activity and accelerating pollutant degradation, making it a more effective bioremediation amendment than CaO. The trend in catalytic efficiency confirms that enzyme kinetics, particularly Vmax/Km, are sensitive and reliable indicators of bioremediation progress and amendment efficacy.

## 5. Conclusion

This study demonstrated the effectiveness of municipal organic waste and calcium oxide (CaO) as soil amendments in enhancing enzymatic activity and promoting bioremediation of spent engine oil (SEO)-contaminated soil. The results revealed significantly elevated activities of catalase and dehydrogenase enzymes in amended soils, with municipal waste treatment producing the highest reaction velocities and enzymatic responses.

Kinetic modeling showed that both enzymes followed Michaelis-Menten kinetics, with municipal waste-treated soils exhibiting superior maximum reaction rates (Vmax) and lower Michaelis constants (Km), indicating enhanced catalytic efficiency and substrate affinity. Dehydrogenase demonstrated a lower Km value compared to catalase, suggesting a higher substrate affinity and more immediate microbial response to pollution stress.

The early increase in enzymatic activity—stabilizing after 14 days—suggests rapid microbial adaptation and sustained biodegradation potential. In contrast, CaO provided moderate improvement, likely through its pH-buffering properties, but was less effective than organic waste in stimulating microbial metabolism. These findings highlight the utility of enzyme kinetics as reliable biomarkers in evaluating bioremediation efficiency and confirm that municipal waste, due to its organic richness and microbial stimulation capacity, is a superior amendment for restoring polluted soils.

## Compliance with ethical standards

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*Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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