ASEAN Journal of
Agriculture and Food Engineering

Journal homepage: https://ejournal.bumipublikasinusantara.id/index.php/ajafe

Mitigation of Spent Crankcase Oil-Contaminated Soil Using Organic and Inorganic Fertilizers: Microbial and Enzymatic Responses with Implications for Sustainable Agriculture and Food Production

M. M. Ebulue[1], B. N. C. Nlemchukwu[2], C. S. Ebulue[3,]*

Federal University of Technology Owerri, Owerri, Imo State, Nigeria
University of Agriculture and Environmental Sciences, Umuagwo, Owerri, Imo State, Nigeria
University of Benin, Benin, Edo State, Nigeria

Correspondence: E-mail: dimpledelfinado@sksu.edu.ph

ABSTRACT

Spent crankcase oil contamination can disturb soil physicochemical conditions, microbial populations, and enzyme activities, thereby reducing soil quality. This study evaluated cow dung (CD), poultry droppings (PD), and NPK fertilizer for mitigating spent crankcase oil-contaminated soil at 1.5, 2.5, and 3.5% contamination levels. Soil pH, total petroleum hydrocarbons (TPH), microbial populations, and catalase, dehydrogenase, acid phosphatase, and alkaline phosphatase activities were determined. Increasing oil contamination increased TPH and reduced soil pH. Fertilizer amendment improved microbial and enzymatic recovery. Poultry droppings showed the strongest overall mitigation response, associated with higher microbial biomass, improved biochemical conditions, and reduced petroleum hydrocarbons. These findings demonstrate the potential of nutrient-rich organic amendments for bioremediation and sustainable agricultural soil management.

ARTICLE INFO

Article History:
Submitted/Received 19 Jun 2026
First Revised 09 Jul 2026
Accepted 15 Sep 2026
First Available online 16 Sep 2026
Publication Date 01 Mar 2026

Keyword:
Agricultural soil;
Bioremediation;
Cow dung;
Poultry droppings;
Soil enzyme activity.

1. INTRODUCTION

Soil quality is fundamental to sustainable agriculture because soil provides nutrients, water, biological habitats, and physicochemical conditions required for plant development and food production. However, soil may be exposed to various anthropogenic contaminants that interfere with these functions. Petroleum hydrocarbons are among the important environmental contaminants because their release can alter soil physicochemical characteristics, microbial communities, nutrient availability, and biological activity. Previous studies have shown that spent engine oil contamination can adversely affect soil conditions and that organic amendments such as cow dung and poultry manure can support the bioremediation of contaminated soil [1, 2]. Xenobiotic contamination can also create metabolic disequilibrium within soil ecosystems, indicating that pollution affects not only chemical characteristics but also biological processes responsible for maintaining soil functionality [2].

Spent crankcase oil is generated during the routine maintenance of vehicles and machinery and contains petroleum hydrocarbons together with compounds accumulated during engine operation. When released directly onto soil, these materials can modify the physical, chemical, and biological characteristics of the soil. Petroleum contamination may initially suppress microbial populations because some hydrocarbon constituents are toxic to microorganisms. However, prolonged exposure can selectively enrich microorganisms capable of utilizing hydrocarbons as carbon and energy sources. These hydrocarbon-degrading microorganisms may subsequently contribute to the natural attenuation of petroleum-contaminated environments. Nevertheless, an increase in hydrocarbon-degrading populations does not necessarily indicate complete ecological recovery because petroleum contamination can continue to disturb soil physicochemical and biological conditions [2, 3].

Petroleum contamination can also influence soil fertility and its potential to support agricultural production. Hydrocarbon accumulation may promote nutrient immobilization, reduce mineral availability, alter cation exchange processes, and impair conditions required for plant growth. Phytotoxic effects have been reported in petroleum-contaminated soils, indicating that hydrocarbon contamination can interfere with plant establishment and nutrient uptake [3]. Similarly, petroleum-contaminated soils may exhibit reduced ecological functionality even when partial hydrocarbon degradation occurs [4]. Therefore, restoration of contaminated soil is relevant not only from an environmental pollution perspective but also to the maintenance of productive soils for sustainable agriculture and food production.

Bioremediation represents an important approach for restoring petroleum-contaminated soils through biological transformation and degradation of contaminants. However, the natural degradation capacity of indigenous microorganisms may become insufficient when relatively high concentrations of petroleum hydrocarbons are introduced into soil [5]. Petroleum hydrocarbons provide an abundant carbon source, but microorganisms responsible for their degradation also require nitrogen, phosphorus, potassium, and other essential nutrients. Nutrient limitation may consequently restrict microbial growth and hydrocarbon degradation. Appropriate nutrient supplementation can stimulate microbial populations and their metabolic activities and thereby enhance biological degradation of petroleum contaminants [6].

Organic and inorganic fertilizers can therefore play important roles in biostimulation-based remediation. Inorganic fertilizers such as NPK directly supply nitrogen, phosphorus, and potassium required for microbial metabolism and biomass development during hydrocarbon degradation [7]. Organic amendments such as cow dung and poultry droppings may provide additional advantages because they supply nutrients, organic matter, and potentially active microbial populations. Organic nutrient supplementation has been reported to enhance microbial utilization of hydrocarbons in crude oil-contaminated soil [8]. The use of biologically derived fertilizer materials is also relevant to agricultural sustainability because nutrient-rich agricultural wastes can be returned to productive systems rather than treated solely as wastes. Biofertilizer approaches have similarly been investigated for improving crop production within agriculture and food engineering applications [9].

The effectiveness of organic amendments depends on their nutrient composition, microbial biomass, amendment dose, and the extent of contamination. Poultry droppings and cow dung can differ substantially in carbon, nitrogen, phosphorus, potassium, organic matter, and microbial populations. These characteristics may influence their capacities to stimulate indigenous microorganisms, modify soil conditions, and promote petroleum hydrocarbon degradation. Comparison with conventional NPK fertilizer is therefore useful for determining whether nutrient-rich animal-derived amendments can support biological recovery under petroleum contamination. Such comparison is particularly relevant to sustainable agriculture because it connects pollution remediation with nutrient recycling and beneficial utilization of agricultural organic residues.

Microbial populations alone, however, are insufficient to describe the biological condition of contaminated soil. Soil enzyme activities provide complementary information because they respond sensitively to changes in microbial activity, soil physicochemical conditions, and contamination. Catalase is associated with the decomposition of hydrogen peroxide and oxidative processes in soil. Changes in catalase activity have been reported following spent engine oil contamination, demonstrating its usefulness for assessing biological disturbance caused by petroleum pollutants [10].

Dehydrogenase activity provides another useful indicator because dehydrogenases are closely associated with respiratory and oxidative metabolism in viable soil microorganisms. Changes in dehydrogenase activity can therefore reflect variations in the overall metabolic activity of soil microbial communities [11]. Studies have demonstrated that dehydrogenase measurements can provide a useful indication of soil microbial metabolic activity and biological responses to environmental disturbance [12]. Thus, monitoring dehydrogenase activity alongside microbial populations can provide additional information about the biological response of contaminated soils.

Phosphatase activities are particularly relevant to agricultural soils because they are closely related to phosphorus cycling and nutrient availability. Acid and alkaline phosphatases contribute to the mineralization of soil organic phosphorus and their activities are influenced by soil properties, microbial processes, nutrient conditions, and pH [13, 14]. Soil acid and alkaline phosphatase activities have also been shown to respond rapidly to changes in soil pH, which is directly relevant to soil fertility and crop production [13]. Consequently, phosphatase activities can provide useful indicators of nutrient-related biochemical changes during contamination and subsequent soil recovery.

Evaluating several physicochemical and biological indicators simultaneously can provide a more comprehensive assessment of remediation performance than relying on a single parameter. Total petroleum hydrocarbons (TPH) indicate the extent of petroleum contamination, while soil pH describes an important environmental condition controlling nutrient availability, microbial activity, and enzyme function. Microbial populations indicate biological responses, whereas catalase, dehydrogenase, acid phosphatase, and alkaline phosphatase activities provide information regarding different biochemical processes occurring within the soil.

Despite the established use of nutrient amendments for petroleum-contaminated soils, comparative information on the responses of multiple physicochemical and biological indicators to cow dung, poultry droppings, and inorganic NPK fertilizer remains important. It is particularly useful to determine whether nutrient-rich agricultural organic wastes can support biological and biochemical soil recovery under increasing petroleum contamination. Such information has practical relevance to sustainable agriculture because effective remediation may restore soil conditions required for productive land use while simultaneously providing beneficial applications for animal-derived organic wastes.

Therefore, this study investigated the mitigation potential of cow dung (CD), poultry droppings (PD), and inorganic NPK fertilizer in soil contaminated with spent crankcase oil at different contamination levels. The assessment focused on changes in total petroleum hydrocarbons, soil pH, microbial populations, and the activities of catalase, dehydrogenases, acid phosphatase, and alkaline phosphatase. The study further compared the effectiveness of the three fertilizer amendments in supporting biological and biochemical soil recovery. The novelty of this study lies in the integrated comparison of organic and inorganic nutrient amendments using petroleum hydrocarbon concentration, microbial populations, and multiple enzyme activities under increasing contamination levels. The findings provide insight into the potential use of nutrient-rich organic amendments for restoring contaminated soil conditions relevant to sustainable agriculture and food production.

2. LITERATURE REVIEW

2.1. Petroleum Hydrocarbon Contamination and Soil Biological Responses

Organic amendments have also been investigated for the recovery of spent engine oil-contaminated soil, with cow dung and poultry manure showing potential to stimulate microbial degradation and reduce petroleum contamination [1]. Petroleum hydrocarbon contamination can alter the physicochemical and biological characteristics of soil. Hydrocarbons introduced into soil may modify pH, nutrient availability, aeration, and interactions between soil particles and microorganisms. Previous investigation of soil polluted with spent engine oil demonstrated substantial changes in soil physicochemical properties [2]. Petroleum-derived xenobiotics can also disturb biological equilibrium within the soil ecosystem, making microbial and biochemical responses useful indicators of environmental disturbance [2]. Microbial responses to petroleum contamination are particularly important because microorganisms contribute directly to hydrocarbon transformation and degradation. Immediately following contamination, sensitive microbial populations may decline because of hydrocarbon toxicity and unfavorable soil conditions. With increasing exposure time, however, microorganisms capable of utilizing petroleum hydrocarbons as carbon and energy sources may become enriched. Consequently, microbial population changes during remediation can reflect both the initial biological stress caused by contamination and subsequent adaptation of hydrocarbon-degrading communities [4, 5]. The biological effects of contamination are also closely related to soil enzyme activities. Catalase and dehydrogenase activities have been reported to respond to spent engine oil contamination and can therefore provide useful information regarding microbial oxidative and metabolic processes [10]. Dehydrogenase activity is particularly associated with metabolically active microorganisms and has been widely employed as an indicator of overall microbial activity in soil [11, 12]. Therefore, combining microbial counts with enzyme measurements provides a broader assessment of biological responses to petroleum contamination.

2.2. Nutrient Amendment and Bioremediation

Bioremediation of petroleum-contaminated soil depends on the availability and metabolic capacity of hydrocarbon-degrading microorganisms. Although petroleum provides abundant carbon, microbial degradation can become limited by insufficient nitrogen, phosphorus, potassium, and other nutrients [5-7]. Biostimulation through nutrient addition is therefore used to improve environmental conditions for indigenous microorganisms and accelerate petroleum hydrocarbon degradation. Inorganic NPK fertilizer provides readily available mineral nutrients that can stimulate microbial growth. Organic amendments, in contrast, can simultaneously provide nutrients, organic carbon, and microorganisms. Organic nutrient supplementation has been shown to enhance microbial utilization of hydrocarbons in contaminated soil [8]. These characteristics make animal-derived amendments such as cow dung and poultry droppings potentially useful for bioremediation. The agricultural relevance of these amendments extends beyond pollutant removal. Animal wastes represent nutrient-containing resources that can potentially be reused within agricultural systems. Their beneficial utilization can simultaneously contribute to waste management, nutrient recycling, and soil improvement. Similar concepts involving biofertilizer application have been investigated for agricultural crop production [9]. Therefore, evaluating cow dung and poultry droppings against conventional NPK fertilizer provides information relevant to both contaminated-soil remediation and sustainable agricultural soil management.

2.3. Soil Enzymes as Indicators of Soil Recovery

Soil enzymes are closely associated with microbial metabolism, organic matter transformation, and nutrient cycling. Because enzyme activities respond to environmental conditions, they can serve as sensitive indicators of contamination and subsequent biological recovery. However, different enzymes represent different biochemical processes; consequently, assessment using several enzymes can provide more comprehensive information than measurement of a single enzyme. Catalase participates in the decomposition of hydrogen peroxide generated during biological oxidative processes. Changes in catalase activity can consequently indicate alterations in microbial activity and oxidative conditions. Spent engine oil contamination has been demonstrated to affect both catalase and dehydrogenase activities in soil [10]. Dehydrogenases are intracellular enzymes associated with biological oxidation-reduction reactions and microbial respiration. Their activity is strongly associated with viable microbial cells and has therefore been used as an indicator of overall microbial metabolic activity [11, 12]. Changes in dehydrogenase activity during remediation can provide evidence of biological responses that may not be adequately represented by microbial population measurements alone. Acid and alkaline phosphatases participate in phosphorus cycling by facilitating the release of inorganic phosphate from organic phosphorus compounds. Their activities are strongly influenced by soil conditions, particularly pH [13]. Phosphatase activity is also related to phosphorus availability, microbial processes, and broader soil properties [14]. Monitoring acid and alkaline phosphatases alongside catalase and dehydrogenase can therefore provide an integrated biochemical assessment of contaminated-soil recovery.

3. MATERIALS AND METHODS

3.1. Collection of Soil and Amendments

Soil samples were collected for the preparation of spent crankcase oil-contaminated experimental soils. The organic amendments used were CD and PD, while commercially available NPK fertilizer was used as the inorganic nutrient amendment. The organic materials were characterized before application to determine their physicochemical and biological properties. The pH of the amendments was determined, and their microbial populations were evaluated using standard microbiological procedures. Carbon, nitrogen, phosphorus, and potassium contents were also determined to characterize their nutrient compositions. These measurements were used to compare the nutrient and microbial characteristics of the organic and inorganic amendments before their application to contaminated soil.

3.2. Experimental Contamination and Amendment

Spent crankcase oil was used as the petroleum contaminant. Soil was contaminated ex situ at three contamination levels: 1.5, 2.5, and 3.5%. The contaminated soils were treated separately with cow dung, poultry droppings, and NPK fertilizer. An unamended treatment was maintained as a control for comparison. The treatments were monitored throughout the experimental period to evaluate changes associated with petroleum contamination and fertilizer amendment. Samples were collected at the designated experimental intervals for physicochemical, microbiological, and enzymatic analyses.

3.3. Determination of Soil pH

Soil pH was determined to evaluate changes in soil acidity or alkalinity resulting from spent crankcase oil contamination and fertilizer amendment. Representative soil samples from the different treatments were prepared in an appropriate soil-water suspension, and pH was measured using a calibrated pH meter. The measurements were conducted for the control and amended soils during the experimental period.

3.4. Determination of Total Petroleum Hydrocarbons

TPH were determined to evaluate changes in the concentration of petroleum-derived contaminants in the soil. Hydrocarbons were extracted from representative soil samples using an appropriate organic solvent. The absorbance of the resulting extract was measured spectrophotometrically, and TPH concentration was determined using the corresponding calibration relationship. Changes in TPH during the experimental period were used as an indicator of petroleum hydrocarbon reduction. The performance of cow dung, poultry droppings, and NPK fertilizer was subsequently compared with that of the unamended control.

3.5. Determination of Microbial Population

Microbial populations in the soil and organic amendments were determined using culture-based microbiological techniques. Serial dilutions of representative samples were prepared, inoculated onto suitable culture media, and incubated under appropriate conditions. Colonies were counted after incubation, and microbial populations were expressed as colony-forming units per gram (cfu/g) of sample. Microbial population measurements were conducted during the experimental period to determine the initial effects of petroleum contamination and subsequent microbial recovery following amendment with cow dung, poultry droppings, or NPK fertilizer.

3.6. Determination of Catalase Activity

Catalase activity was determined as an indicator of oxidative biological activity in the soil. The assay was based on the enzymatic decomposition of hydrogen peroxide. Representative soil samples from the different treatments were exposed to the appropriate substrate under controlled assay conditions, and catalase activity was determined from the extent of hydrogen peroxide decomposition. The resulting activities were compared among contamination levels and amendment treatments to evaluate the influence of spent crankcase oil and fertilizer application on soil catalase activity.

3.7. Determination of Dehydrogenase Activity

Dehydrogenase activity was determined as an indicator of microbial respiratory and metabolic activity. Representative soil samples were incubated with an appropriate electron-accepting substrate, and formation of the resulting reaction product was determined spectrophotometrically. Dehydrogenase activities were compared among the control, contaminated, and fertilizer-amended treatments. This measurement was used together with microbial population data to assess changes in biological activity associated with petroleum contamination and subsequent amendment.

3.8. Determination of Acid and Alkaline Phosphatase Activities

Acid and alkaline phosphatase activities were determined to evaluate changes in phosphorus-related biochemical processes. Representative soil samples were incubated with an appropriate phosphate-containing substrate under conditions suitable for acid or alkaline phosphatase activity. The amount of reaction product released during incubation was determined spectrophotometrically. The activities of both enzymes were compared among contamination levels and fertilizer treatments. These measurements provided information regarding the influence of petroleum contamination and nutrient amendment on phosphorus-related biochemical activity in the soil.

3.9. Evaluation of Mitigation Performance

The mitigation performance of the amendments was evaluated by integrating changes in TPH, soil pH, microbial populations, and enzyme activities. Responses obtained for cow dung, poultry droppings, and NPK fertilizer were compared with those of the corresponding unamended contaminated soils. Particular attention was given to whether each amendment reduced petroleum hydrocarbon levels while supporting the recovery of microbial and biochemical activities.

4. RESULTS AND DISCUSSION

4.1. Characteristics of Organic and Inorganic Fertilizer Amendments

The physicochemical and biological characteristics of the fertilizer amendments are presented in **Tables 1 and 2**. PD and CD had nearly neutral pH values of 7.10 ± 0.11 and 7.04 ± 0.10, respectively, whereas NPK fertilizer showed a slightly lower pH of 6.85 ± 0.02. These values indicate that the organic amendments, particularly PD and CD, may help maintain favorable pH conditions during the treatment of spent crankcase oil-contaminated soil.

Table 1. pH of organic and inorganic fertilizer amendments.

PARAMETER	PD	CD	NPK
pH	7.10 ± 0.11	7.04 ± 0.10	6.85 ± 0.02

Note: *Values are expressed as mean ± SD.*

Table 2 shows substantial differences in the chemical composition and microbial biomass of the two organic amendments. PD contained 29.03 ± 0.10% C, 52.00 ± 0.00% organic matter, 2.50 ± 0.04% N, and 1.40 ± 0.01 ppm P, whereas CD contained 23.40 ± 0.10% C, 46.50 ± 0.01% organic matter, 1.56 ± 0.01% N, and 0.10 ± 0.02 ppm P. PD also contained higher concentrations of Na, Mg, and K, whereas CD contained a higher concentration of Ca. These differences indicate that PD provided a comparatively richer nutrient environment.

Table 2. Chemical characteristics and microbial biomass of organic amendments.

PARAMETER	PD	CD
C (%)	29.03 ± 0.10	23.40 ± 0.10
Organic matter (%)	52.00 ± 0.00	46.50 ± 0.01
N (%)	2.50 ± 0.04	1.56 ± 0.01
P (ppm)	1.40 ± 0.01	0.10 ± 0.02
Ca (mEq/100 g)	10.00 ± 0.00	13.20 ± 0.01
Na (mEq/100 g)	44.01 ± 0.01	36.40 ± 0.01
Mg (mEq/100 g)	4.12 ± 0.00	4.04 ± 0.00
K (mEq/100 g)	64.05 ± 0.02	46.42 ± 0.01
Microbial biomass (cfu/g)	1.62×10^9 ± 0.01	7.01×10^8 ± 1.02

Note: *Values are expressed as mean ± SD.*

The microbial biomass was also higher in PD than in CD. The identified microorganisms included *Proteus* sp., *Klebsiella* sp., *Enterobacter* sp., *Pseudomonas aeruginosa*, *Micrococcus* sp., *Bacillus* sp., *Arthrobacter* sp., *Fusarium* sp., *Aspergillus niger*, *Candida* sp., and *Penicillium* sp. The relatively high nutrient content and microbial biomass of PD provide a plausible explanation for its stronger mitigation performance observed in the subsequent treatments. Organic nutrient supplementation has previously been reported to enhance microbial utilization of petroleum hydrocarbons [8].

4.2. Effects of Fertilizer Amendment on Microbial Populations

Changes in microbial populations following amendment with CD, PD, and NPK at contamination levels of 1.5, 2.5, and 3.5% are presented in **Tables 3-5**. The uncontaminated soil contained a microbial population of 6.32×10^8 cfu/g. At 1.5% contamination, PD treatment reached 5.99×10^8 cfu/g on day 28, compared with 4.78×10^8 cfu/g for CD and 4.70×10^8 cfu/g for NPK. At 2.5% contamination, the same general response was observed. On day 28, PD treatment reached 5.39×10^8 cfu/g, whereas CD and NPK treatments reached 4.68×10^8 and 4.47×10^8 cfu/g, respectively. By day 35, microbial populations had declined in all treatments. At the highest contamination level of 3.5%, microbial populations were generally lower than those observed at the lower contamination levels. On day 28, CD, PD, and NPK treatments showed microbial populations of 4.19×10^8, 4.32×10^8, and 3.88×10^8 cfu/g, respectively.

The overall pattern indicates that increasing spent crankcase oil contamination imposed greater stress on soil microbial populations. The initial decline can be associated with hydrocarbon toxicity, whereas subsequent increases may indicate enrichment of microorganisms capable of utilizing petroleum hydrocarbons. The relatively strong response of PD is consistent with its higher initial microbial biomass and nutrient content.

Table 3. Microbial populations at 1.5% contamination following fertilizer amendment (5 mL w/v).

TREATMENT	DAY 14	DAY 28	DAY 35
Soil + oil + CD	$4.60 \times 10^8 \pm 0.01^b$	$4.78 \times 10^8 \pm 0.00^a$	$4.52 \times 10^8 \pm 0.11^c$
Soil + oil + PD	$4.64 \times 10^8 \pm 0.00^e$	$5.99 \times 10^8 \pm 0.10^a$	$4.84 \times 10^8 \pm 0.00^x$
Soil + oil + NPK	$4.66 \times 10^8 \pm 0.01^a$	$4.70 \times 10^8 \pm 0.00^f$	$4.20 \times 10^8 \pm 0.20^v$

Note: Uncontaminated soil (control): $6.32 \times 10^8 \pm 0.02$ cfu/g. Values are expressed as mean ± SD (n = 3). Different superscript letters indicate significant differences ($p < 0.05$).

Table 4. Microbial populations at 2.5% contamination following fertilizer amendment (5 mL w/v).

TREATMENT	DAY 14	DAY 28	DAY 35
Soil + oil + CD	$4.36 \times 10^8 \pm 0.20^a$	$4.68 \times 10^8 \pm 0.00^b$	$4.21 \times 10^8 \pm 0.00^c$
Soil + oil + PD	$4.50 \times 10^8 \pm 0.10^a$	$5.39 \times 10^8 \pm 0.01^b$	$4.16 \times 10^8 \pm 0.00^c$
Soil + oil + NPK	$4.58 \times 10^8 \pm 0.01^f$	$4.47 \times 10^8 \pm 0.00^d$	$4.04 \times 10^8 \pm 0.02^a$

Note: *Values are expressed as mean ± SD (n = 3). Different superscript letters indicate significant differences ($p < 0.05$).*

Table 5. Microbial populations at 3.5% contamination following fertilizer amendment (5 mL w/v).

TREATMENT	DAY 14	DAY 28	DAY 35
Soil + oil + CD	$4.10 \times 10^8 \pm 0.10^a$	$4.19 \times 10^8 \pm 0.10^b$	$4.17 \times 10^8 \pm 0.01^c$
Soil + oil + PD	$4.06 \times 10^8 \pm 0.01^a$	$4.32 \times 10^8 \pm 0.02^b$	$4.01 \times 10^8 \pm 0.00^c$
Soil + oil + NPK	$3.88 \times 10^8 \pm 0.10^a$	$3.88 \times 10^8 \pm 0.01^d$	$3.88 \times 10^8 \pm 0.00^c$

Note: *Values are expressed as mean ± SD (n = 3). Different superscript letters indicate significant differences ($p < 0.05$).*

4.3. Changes in Total Petroleum Hydrocarbons during Fertilizer Amendment

The effects of CD, PD, and NPK amendment on TPH at 1.5, 2.5, and 3.5% spent crankcase oil contamination are presented in **Figures 1-3**, respectively.

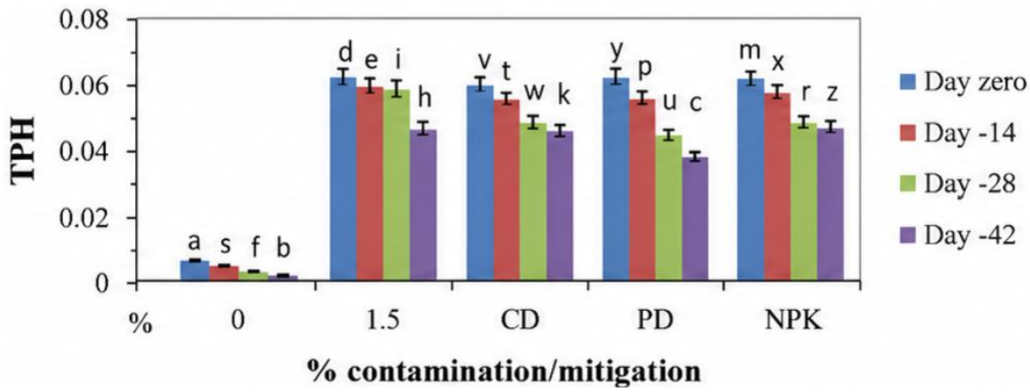

Figure 1. Changes in TPH content of soil contaminated with 1.5% spent crankcase oil following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

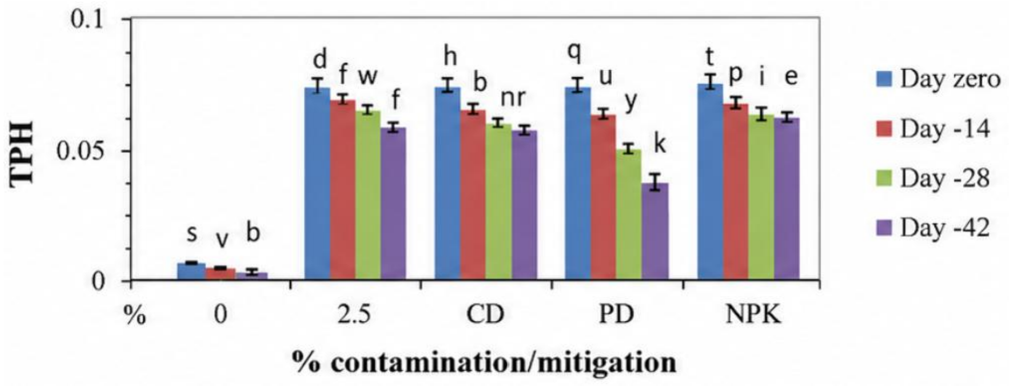

Figure 2. Changes in TPH content of soil contaminated with 2.5% spent crankcase oil following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

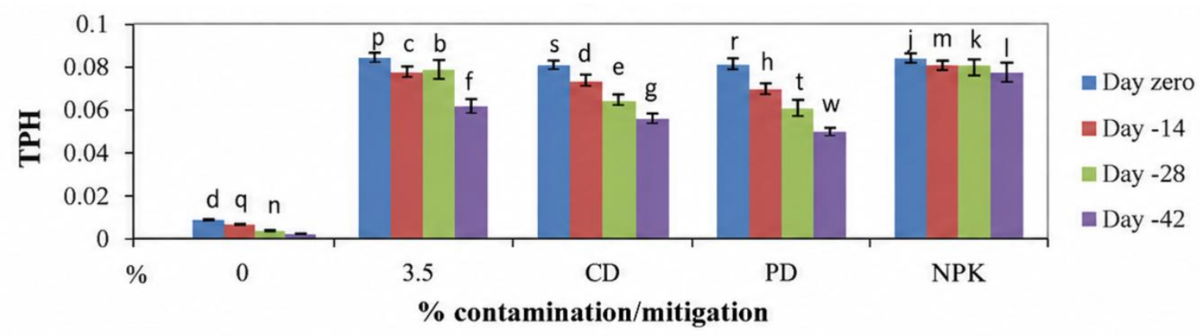

Figure 3. Changes in TPH content of soil contaminated with 3.5% spent crankcase oil following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

TPH generally increased with increasing contamination level and subsequently declined during the treatment period. Among the three amendments, PD produced the strongest TPH reduction. Statistical differences among the lettered bars were reported at $p < 0.05$.

The decrease in TPH following amendment indicates enhanced removal or degradation of petroleum hydrocarbons from the contaminated soil. Nutrient enrichment can support microbial hydrocarbon utilization by supplying nutrients that may become limiting when hydrocarbons provide an abundant carbon source. The stronger response associated with PD may therefore be related to its relatively high nutrient content and microbial biomass. This interpretation agrees with previous observations that organic nutrients can enhance microbial utilization of hydrocarbons [8].

4.4. Changes in Soil pH during Fertilizer Amendment

The effects of spent crankcase oil contamination and fertilizer amendment on soil pH are presented in **Figures 4-6**. Increasing spent crankcase oil contamination reduced soil pH, indicating that petroleum contamination altered the physicochemical environment of the soil. Amendment with CD, PD, and NPK subsequently increased soil pH relative to the contaminated condition, with PD showing the strongest relieving response. Significant differences among lettered bars were reported at $p < 0.05$. The decrease in pH caused by petroleum contamination may influence nutrient availability, microbial metabolism, and enzyme activities. Conversely, the near-neutral pH of PD and CD may help restore more favorable biological conditions during remediation. Soil pH is also an important regulator of phosphatase activity and other biochemical processes [13, 14].

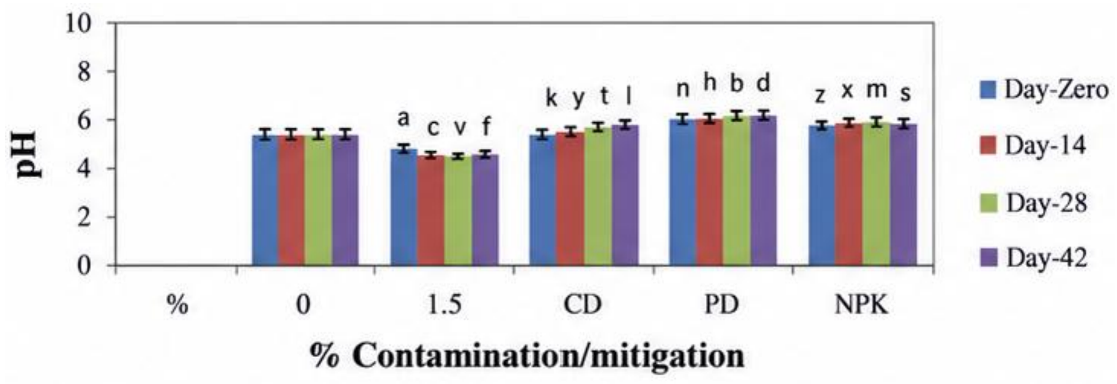

Figure 4. Changes in soil pH at 1.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

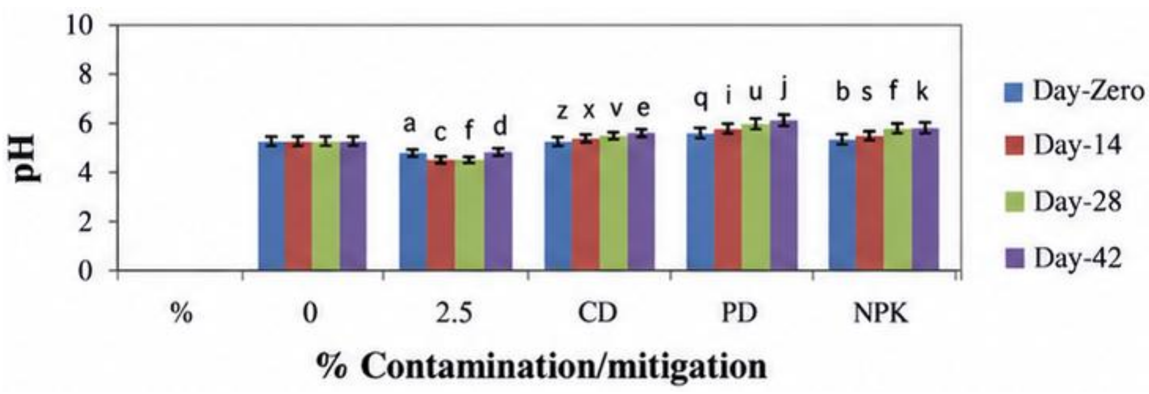

Figure 5. Changes in soil pH at 2.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

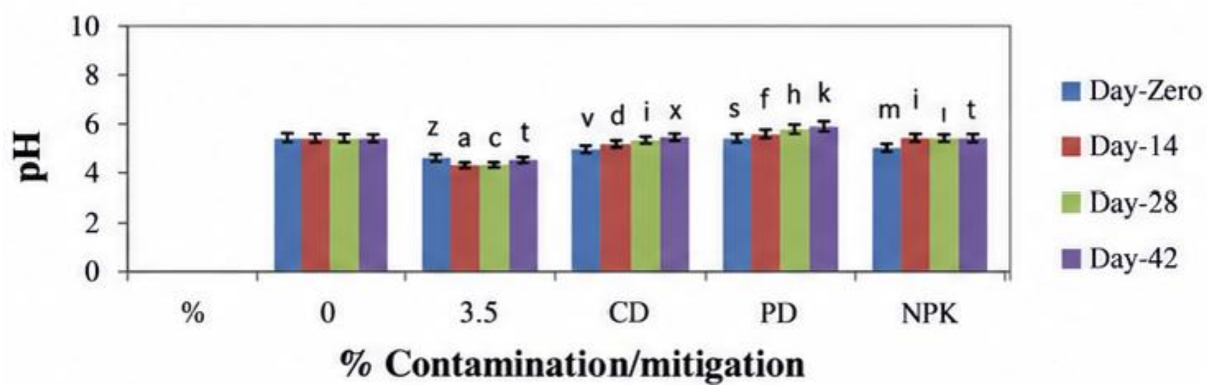

Figure 6. Changes in soil pH at 3.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

4.5. Catalase Activity

Changes in soil catalase activity at 1.5, 2.5, and 3.5% contamination are presented in **Figures 7-9**. Catalase activity was inhibited following spent crankcase oil contamination. Fertilizer amendment relieved this inhibition to different extents, with PD showing the strongest relieving response across the treatments. Significant differences relative to the control were reported at $p < 0.05$. Catalase is associated with the decomposition of hydrogen peroxide and responds to changes in microbial and oxidative conditions. The inhibition observed following contamination indicates disturbance of soil biological processes, whereas recovery after amendment suggests improvement of the soil biochemical environment. Similar responses of soil catalase activity to spent engine oil contamination have been reported previously [10].

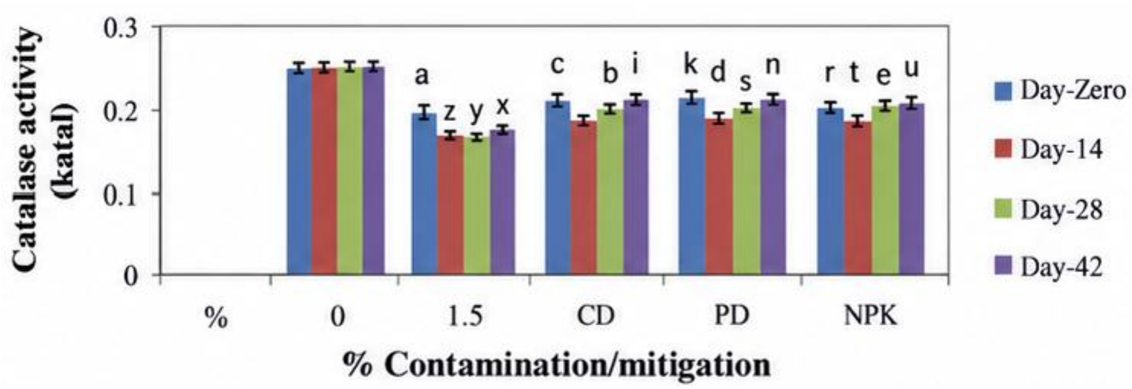

Figure 7. Changes in soil catalase activity at 1.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

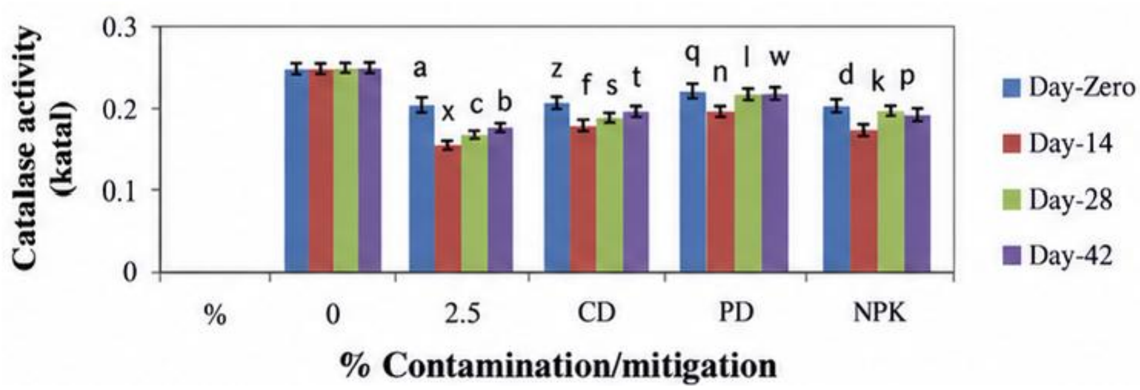

Figure 8. Changes in soil catalase activity at 2.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

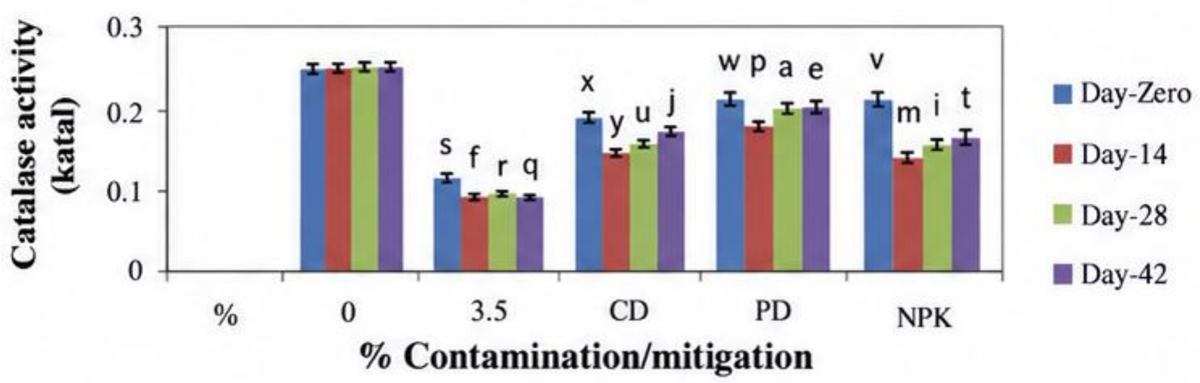

Figure 9. Changes in soil catalase activity at 3.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

4.6. Dehydrogenase Activity

The effects of spent crankcase oil contamination and fertilizer amendment on dehydrogenase activity are shown in **Figures 10-12**. In contrast to catalase, dehydrogenase activity was stimulated following spent crankcase oil contamination. Following fertilizer amendment, dehydrogenase activity changed toward the corresponding treated conditions, with PD producing the strongest relieving response. Significant differences among the lettered bars were reported at $p < 0.05$. Dehydrogenase activity reflects microbial respiratory and oxidative metabolism. Its stimulation following petroleum contamination may indicate increased metabolic activity among microorganisms capable of utilizing hydrocarbon substrates. The subsequent changes after amendment demonstrate that dehydrogenase activity responded dynamically to both contamination and remediation conditions. This observation is consistent with the use of dehydrogenase as an indicator of soil microbial metabolic activity [10-12].

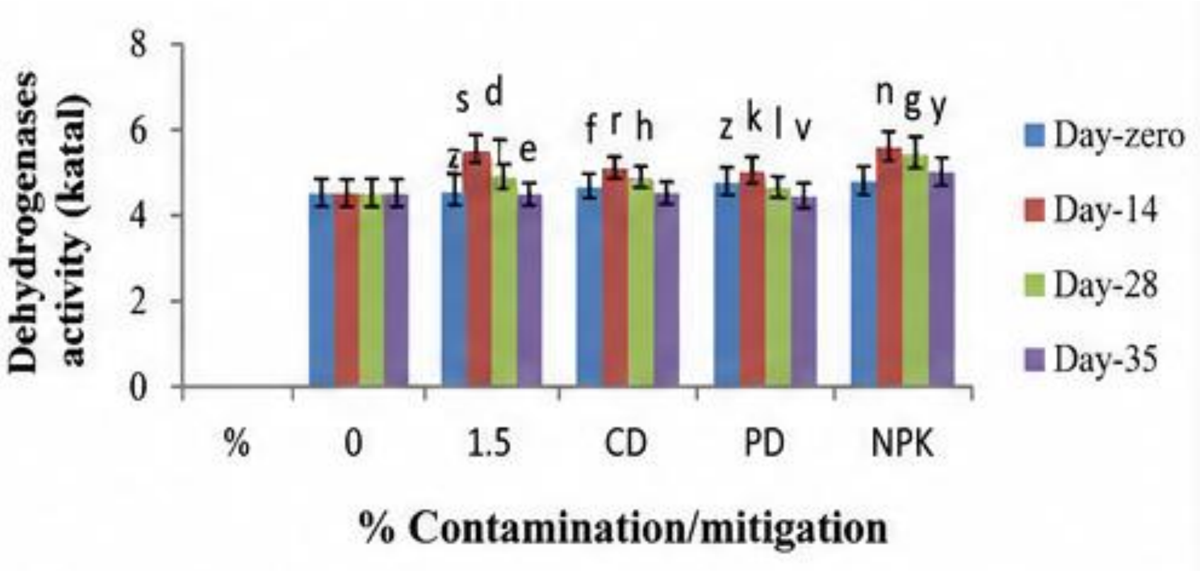

Figure 10. Changes in soil dehydrogenase activity at 1.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

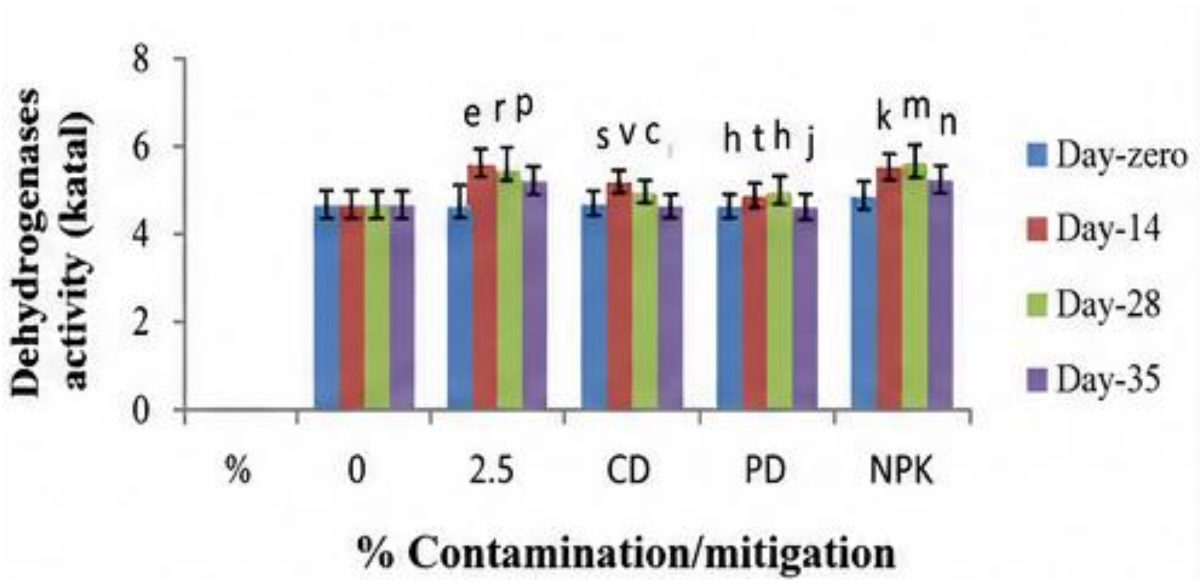

Figure 11. Changes in soil dehydrogenase activity at 2.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

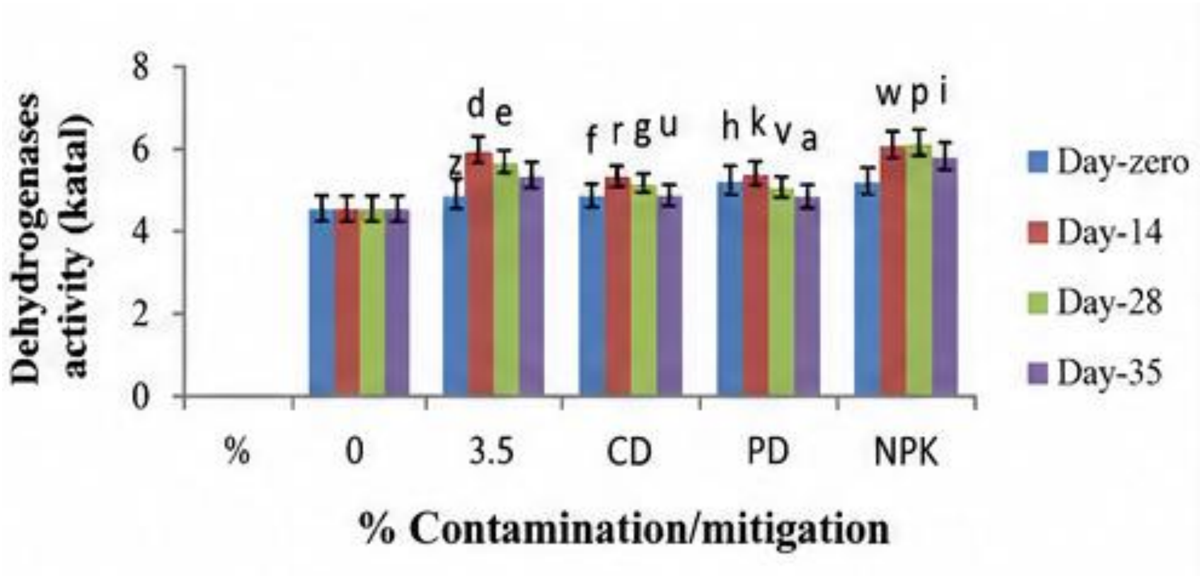

Figure 12. Changes in soil dehydrogenase activity at 3.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

4.7. Acid Phosphatase Activity

Acid phosphatase activity at the three contamination levels is presented in **Figures 13-15**. Acid phosphatase activity was inhibited following spent crankcase oil contamination and subsequently recovered during fertilizer amendment. Among the amendments evaluated, PD showed the strongest mitigating response. Significant differences among treatments were reported at $p < 0.05$. Acid phosphatase contributes to phosphorus cycling through the mineralization of organic phosphorus compounds. Its inhibition indicates that spent crankcase oil affected biochemical processes associated with phosphorus mobilization. Recovery following amendment suggests that fertilizer addition improved conditions supporting phosphatase activity. Since phosphatase activity is strongly influenced by soil pH, nutrient availability, microbial processes, and other soil properties [13, 14], this enzyme provides a useful additional indicator of biochemical recovery.

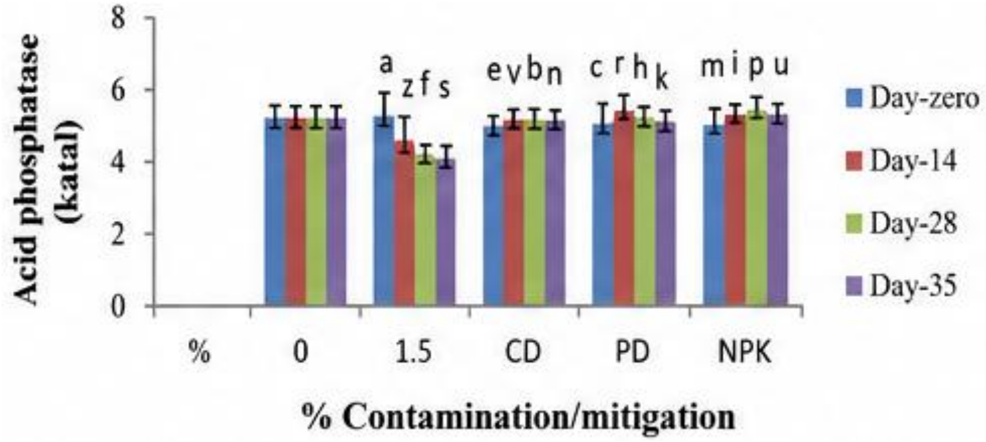

Figure 13. Changes in soil acid phosphatase activity at 1.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

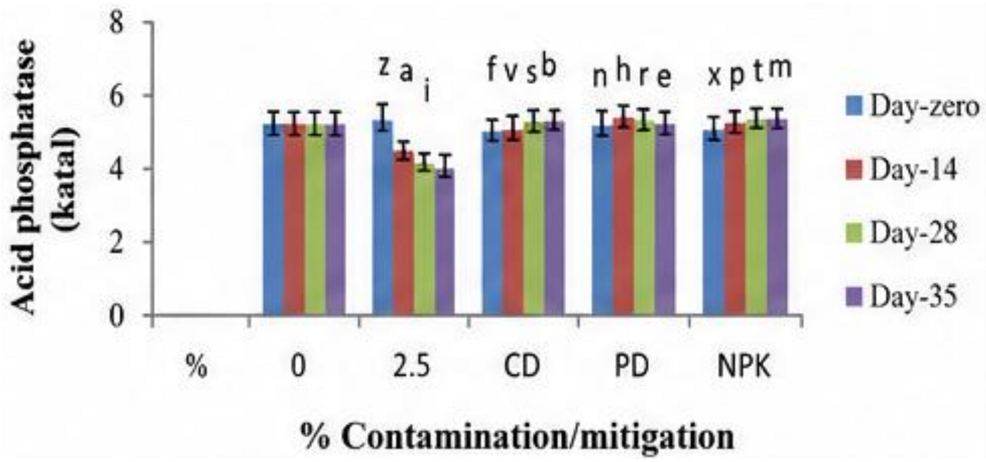

Figure 14. Changes in soil acid phosphatase activity at 2.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

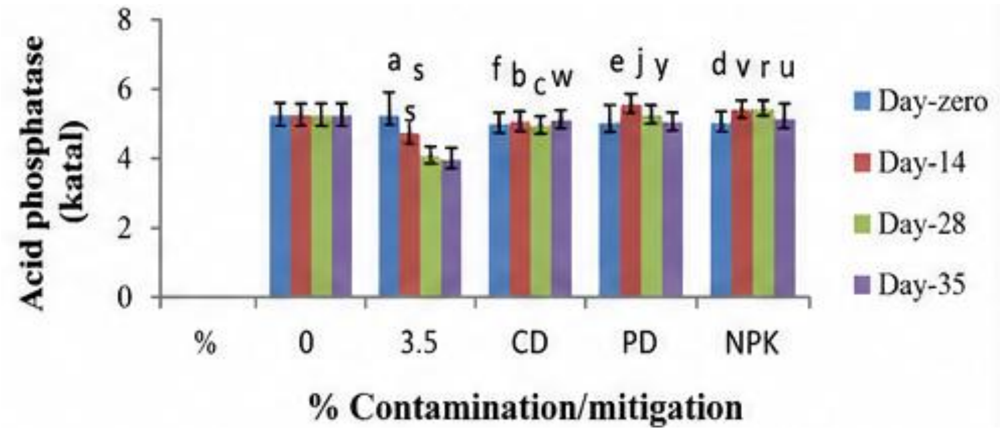

Figure 15. Changes in soil acid phosphatase activity at 3.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

4.8. Alkaline Phosphatase Activity

Alkaline phosphatase activities at 1.5, 2.5, and 3.5% contamination are presented in **Figures 16-18**. Unlike acid phosphatase, alkaline phosphatase activity was stimulated following spent crankcase oil contamination. Following fertilizer amendment, the activity declined, with PD showing the strongest relieving response. Different letters indicate statistically significant differences at $p < 0.05$. The contrasting responses of acid and alkaline phosphatases indicate that petroleum contamination did not uniformly suppress soil enzyme activities. Whereas acid phosphatase was inhibited, alkaline phosphatase was stimulated. These contrasting responses may result from differences in enzyme sensitivity to pH, microbial activity, nutrient availability, and other physicochemical changes caused by petroleum contamination [13, 14]. Therefore, simultaneous evaluation of several enzymes provides a more complete assessment of biological soil recovery than measurement of a single enzyme.

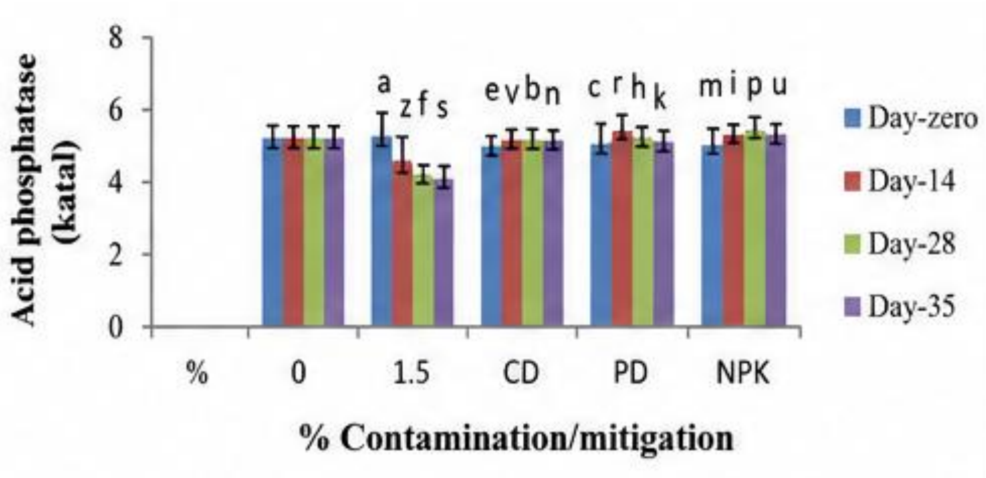

Figure 16. Changes in soil alkaline phosphatase activity at 1.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

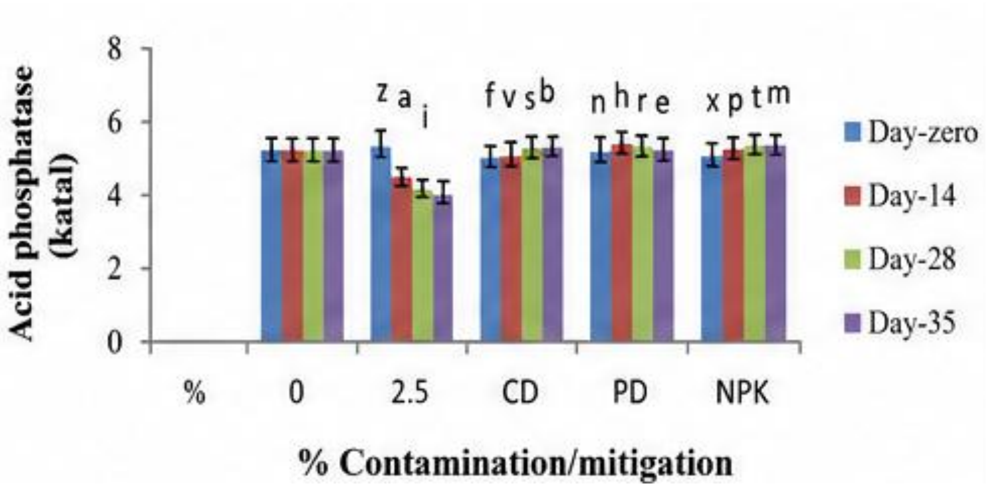

Figure 17. Changes in soil alkaline phosphatase activity at 2.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

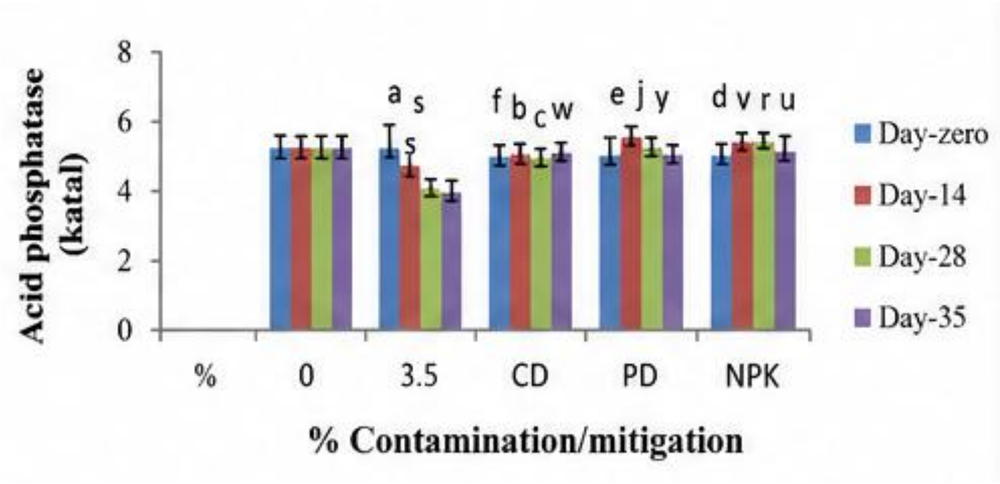

Figure 18. Changes in soil alkaline phosphatase activity at 3.5% spent crankcase oil contamination following amendment with CD, PD, and NPK fertilizer (5 mL w/v).

4.9. Integrated Mitigation Performance and Agricultural Implications

When the results are considered collectively, a consistent relationship emerges among amendment characteristics, microbial responses, petroleum hydrocarbon reduction, pH recovery, and soil enzyme activities. PD exhibited the highest microbial biomass and relatively high concentrations of several nutrients (**Tables 1 and 2**). Correspondingly, PD generally supported higher microbial populations during treatment (**Tables 3-5**) and produced the strongest TPH mitigation across the contamination levels (**Figures 1-3**).

Improvement in soil pH following amendment (**Figures 4-6**) was accompanied by recovery of catalase (**Figures 7-9**) and acid phosphatase (**Figures 13-15**), both of which had been inhibited by contamination. In contrast, dehydrogenase (**Figures 10-12**) and alkaline phosphatase (**Figures 16-18**) were stimulated following contamination and subsequently changed after fertilizer amendment. These responses demonstrate that spent crankcase oil affected different biochemical pathways in different ways rather than uniformly suppressing soil biological activity.

The overall performance of PD supports the concept that organic amendments can contribute simultaneously to nutrient supply and biological stimulation during petroleum hydrocarbon remediation. From an agricultural perspective, the use of nutrient-rich animal-derived organic wastes can potentially connect waste utilization, nutrient recycling, contaminated-soil recovery, and sustainable soil management. This interpretation is also consistent with the broader agricultural use of biofertilizer materials [9]. However, the present study did not measure crop growth, yield, food quality, or food safety. Therefore, the agricultural implication should be interpreted specifically as restoration of soil conditions potentially relevant to future agricultural use, rather than as direct evidence of improved crop productivity or food production.

The findings should also be interpreted within the conditions of the present ex situ experiment. The relative performance of CD, PD, and NPK may differ with soil type, petroleum composition, amendment dosage, environmental conditions, and field management practices. Further field-scale studies are therefore necessary to optimize amendment dosage and determine whether the improvements observed in soil biochemical properties can support subsequent agricultural production.

5. CONCLUSION

Spent crankcase oil contamination altered soil physicochemical and biological characteristics, as demonstrated by increased TPH, reduced soil pH, changes in microbial populations, and contrasting enzyme responses. Catalase and acid phosphatase activities were inhibited, whereas dehydrogenase and alkaline phosphatase activities were stimulated. Amendment with cow dung, poultry droppings, and NPK fertilizer promoted soil recovery to different extents. Poultry droppings showed the strongest overall mitigation response, corresponding with their relatively high nutrient content and microbial biomass and reflected in stronger TPH reduction, pH recovery, microbial responses, and biochemical recovery. These findings demonstrate the potential of nutrient-rich organic amendments for bioremediation and sustainable soil management. However, because crop performance was not evaluated, future field studies should examine amendment dosage, soil recovery, crop growth, and the suitability of remediated soils for agricultural production.

6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

7. REFERENCES

[1] Hanson-Akpan, R. I., Obiekezie, S. O., Makut, M. D., Ekeleme, K. I., & Muhammed, I. A. (2023). A comparative study on the bioremediation of spent engine oil contaminated soil using cow dung, poultry manure and saw dust. *International Journal of Research and Innovation in Applied Science*, *8*(9), 145-158.

[2] Ebulue, M.M., and Ebulue, C.S. (2022). Physicochemical properties of soil ecosystem polluted with spent engine oil. *ASEAN Journal for Science and Engineering in Materials, 1*(2), 59-66.

[3] Ebulue, M.M. (2023). Metabolic disequilibrium: A review in the indication of soil ecosystem insulted with xenobiotics. *ASEAN Journal for Science and Engineering in Materials, 2*(1), 1-8.

[4] Chaineau, C.H., Morel, J.L., and Oudot, J. (1997). Phytotoxicity and plant uptake of fuel oil hydrocarbons. *Journal of Environmental Quality, 26*, 1478-1482.

[5] Salanitro, J.P., Dorn, P.B., and Moore, K.O. (1997). Crude oil bioremediation and soil ecotoxicity assessment. *Environmental Science & Technology, 31*(6), 1769-1774.

[6] Atlas, R.M. (1981). Biodegradation of petroleum hydrocarbons: An environmental perspective. *Microbiological Reviews, 45*, 180-209.

[7] Atashgahi, S., Sanchez, I., Heipieper, H.J., van der Meer, J.R., Stams, A.J.M., and Smidt, H. (2018). Prospects for harnessing biocide resistance for bioremediation and detoxification. *Science, 360*, 743-746.

[8] Atlas, R.M., and Bartha, R. (1983). Biotechnology of petroleum pollutant biodegradation and microbial ecology. *Microbial Ecology, 12*, 155-157.

[9] Ibekwe, V.I., Ubochi, K.C., and Ezeji, E.U. (2006). Effect of organic nutrient on microbial utilization of hydrocarbons on crude oil contaminated soil. *African Journal of Biotechnology, 5*(10), 983-986.

[10] Falsario, M.N.J.S., Rabut, B.J.F., Gonzales, L.B., Tayuan, J.B.M., Kinazo, M.N.E.C., and Valdez, A. (2022). Citrullus lanatus (Watermelon): Biofertilizer for eggplants. *ASEAN*

Journal of Agricultural and Food Engineering, 1(1), 19-22.

[11] Achuba, F.I., and Peretiemo-Clarke, B.O. (2008). Effect of spent engine oil on soil catalase and dehydrogenase activities. *International Agrophysics, 22*, 1-4.

[12] Margesin, R., Schinner, F., and Zimmerbauer, A. (2000). Monitoring of bioremediation by soil biological activities. *Chemosphere, 40*, 339-345.

[13] Casida, L.E. Jr. (1977). Microbial metabolic activity in soil as measured by dehydrogenase determinations. *Applied and Environmental Microbiology, 34*(6), 630-636. This study specifically evaluates dehydrogenase determination as an indicator of soil microbial metabolic activity.

[14] Dick, W. A., Cheng, L., & Wang, P. J. S. B. (2000). Soil acid and alkaline phosphatase activity as pH adjustment indicators. *Soil Biology and Biochemistry*, *32*(13), 1915-1919.