PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (8) 2503-2514 August 2024

Synergistic Effect of Combining Animal Wastes for Bioremediation of Naphthalene, Chrysene and Pyrene in a Crude Oil Impacted Soil (An Ex-Situ Study)

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ABSTRACT: Synergistic properties usually produce combined effect greater than the sum of their separate effects. Hence, this paper assessed the synergistic effect of cow dung, goat droppings and poultry manure wastes for bioremediation of naphthalene, chrysene and pyrene in a crude oil-impacted soil at an experimental plot in a botanical garden, located at Abia State University, Nigeria, using appropriate standard methods. The result of the study showed about 96%, 93% and 90% removal of naphthalene, pyrene and chrysene respectively. This is in contrast with about <25% removal for the three contaminants using the natural attenuation method. It could also be seen that the amendment agents when combined performed better than when used singly, this shows that the combination of the animal manures has a synergistic effect on the removal of PAHs in a crude oil-impacted soil. The use of various animal wastes as supplements promotes microbial growth, accelerates the breakdown of contaminants, and improves soil fertility. In addition, combining multiple animal wastes produces a synergistic effect, leading to improved removal efficiency. This result highlights the potential of using a combination of animal wastes as an effective and sustainable strategy to remediate oil-contaminated PAH-contaminated soils.

DOI: https://dx.doi.org/10.4314/jasem.v28i8.28

Cite this Article as: NOSIRI, C. I; AGHALIBE, C. U; ONWUKA, K. E; IGWE, J. C. (2024). Synergistic Effect of Combining Animal Wastes for Bioremediation of Naphthalene, Chrysene and Pyrene in a Crude Oil Impacted Soil (An Ex-Situ Study). *J. Appl. Sci. Environ. Manage.* 28 (8) 2503-2514

Dates: Received: 04 June 2024; Revised: 27 June 2024; Accepted: 11 July 2024 Published: 05 August 2024

Keywords: bioremediation; pollution; kinetic analysis; crude oil; animal wastes.

The occurrence of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) in the soil is considered a major problem in modern environmental protection. Since most of these compounds are persistent, they have a significant impact on soils, and the presence of microbiota is key to the remediation of contaminated areas. These compounds fall into two groups: Low molecular weight PAHs (LMW-PAHs) with two or three fused rings and high molecular weight PAHs (HMW-PAHs) with four or more rings that impart persistence to these molecules (Wilcke, 2007, Lee *et al*., 2008). The main sources of these pollutants are incomplete emissions from the transport sector, organic matter combustion and petrochemical runoff in the land and marine environment (Evans *et al*., 2016; Bacosa *et al*., 2015). To create a healthier and greener environment, there is an urgent need to effectively prevent, mitigate, or degrade environmental pollutants that are emerging concerns (Liu *et al*. 2019). Though several remediation processes have been established and utilized against several environmentally-related toxic contaminants (Aleya *et al*., 2019a; Aleya *et al*., 2019b; Bilal *et al*., 2019; Ławniczak *et al*., 2020), however, none have

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been able to mitigate the pollutants completely. Major challenging issues that hinder the effective deployment of in-practice remediation processes or techniques include higher processing cost, excessive use of harsh chemicals, low cost-effective ratio, and generation of toxic by-products/residues or secondary environmental pollutants (Dasgupta *et al*., 2015; Liu *et al*., 2019). In this context, microbe-based bioremediation of toxic environmental pollutants offers numerous advantages, such as reaction efficiency, high cost-effective ratio, wide-ranging mineralization, and minimal/no side pollution generation issues (Garcia-Garcia *et al*., 2016; Rasheed *et al*., 2019). More specifically, numerous bacterial cultures, e.g., *Achromobacter*, *Dehalococcoides, Pseudomonas, Burkholderia, Rhodococcus, Comamonas, Alcaligenes, Sphingomonas*, and *Ralstonia* can alter contaminants into less/no toxic mixtures, consequently, diminish or remove pollutants from the aquatic atmosphere (Lloyd *et al*., 2003). First and foremost, microbial-facilitated bioremediation involves the secretion of catalytic enzymes that result in the efficient removal of harmful toxins (Dangi *et al*., 2019; Liu *et al*., 2019; Iqbal and Bilal, 2020). Animal wastes contain diverse microbial populations, including hydrocarbon-degrading bacteria and fungi that have the metabolic capacity to degrade PAHs (Verma and Jaiswal, 2016). In addition, nutrients contained in animal excreta, especially nitrogen and phosphorus, act as growth stimulators of native microorganisms and increase their metabolic activity. The use of animal wastes in bioremediation processes promotes the formation of favorable microbial communities and improves the bioavailability of PAHs. (Ayotamuno and Gobo, 2021). Combining excreta from different animals may enhance the biodegradation of PAHs due to the complementary microbial populations and trophic profiles provided by PAHs. (Zhang *et al*., 2021; Wang *et al*., 2021) Studies have shown that combining manure and poultry litter improves PAH removal efficiency compared to separate treatments. A synergistic effect may result from microbial interactions and the provision of a wide range of nutrients required for microbial growth and PAH degradation. (Su *et al*., 2016; Xu *et al*., 2017; Ni *et al*., 2019; Li *et al*., 2020). Therefore, the objective of this paper is to assess the synergistic effect of cow dung, goat droppings and poultry manure wastes for bioremediation of naphthalene, chrysene and pyrene in a crude oil impacted soil at an experimental plot in a botanical garden located at Abia State University, Uturu, Nigeria.

MATERIALS AND METHODS

The soil sample used for the study was collected from the top surface soil (0-15cm) of the botanical garden, Abia State University, Uturu, Abia State, Nigeria. The crude oil used for the ex-situ contamination is Bonny light crude obtained from the core analysis laboratory of the Nigeria National Petroleum Corporation (NNPC), Moscow Rd., Port Harcourt, Nigeria.

Animal Wastes: The cow dung was obtained from the cow market in Lopka, Abia State, Nigeria, the poultry droppings from a local poultry farm in Okigwe Imo State, Nigeria, and NPK fertilizer was obtained from Eke market in Okigwe, Imo State, Nigeria. All the different amendment agents were each air-dried for two weeks, ground, and sieved to obtain uniform-sized particles. Each amendment agent was stored in a polyethylene bag and kept in the laboratory prior to use.

Experimental Design and Soil Treatment: The method of Agarry *et al.*, (2013) with slight modification was adopted. About I.5 kg each, of the oil sample was measured out, placed in nine (9) plastic containers, and labeled A-I. The soil in each container was spiked with 10 % (w/w) bonny light crude oil to simulate severe crude oil contamination. The soil in each container was thoroughly mixed together to achieve complete artificial contamination. Forty-eight (48) hours after contamination, the different amendment agents were applied as shown in Table 1.

Table 1. Application of different amendment agents to the contaminated soil

A	No amendment agent
B	1.5 kg of Soil + 150 g of CD
C	1.5 kg of Soil + 150 g of PM
D	1.5 kg of Soil +150 g of GD
E	1.5 kg of Soil + 150 g of NPK
F	1.5 kg of Soil + 75 g CD + 75 g PM
G	1.5 kg of Soil + 75 g CD + 75 g GD
H	1.5 kg of Soil + 75 g PM + 75 g GD
I	1.5 kg of Soil + 50 g PM + 50 g PM + 50 g GD

*** CD – Cow dung, PM – Poultry manure, GD – Goat droppings.*

Each container was made up to 50% volume with distilled water for proper percolation. The contents of each container were tilled every 2 days to ensure homogenization and adequate aeration. This experiment was set up in triplicates. Sample A was without an amendment agent and thus used as a control.

Determination of Total Petroleum Hydrocarbon: About 10 g of the soil sample was weighed into an extraction bottle and 20 cm^3 of extraction mixture (DCM: n-Hexane: acetone) in a ratio of 2:2:1 was added. The mixture was sonicated for 1 h and the organic layer was decanted. The extracted organic phase was dried using anhydrous sodium sulfate and concentrated using a rotary evaporator to about 10 cm^3. About 10 cm^3 of the final extract was injected into already calibrated Gas Chromatography (HP 5890, USA) equipped with a capillary column. The peak areas were used in the quantifications.

RESULTS AND DISCUSSION

The levels of Naphthalene, Chrysene and Pyrene during the remediation period are presented in Figures 1 to 3 showed a significant reduction in the concentration of naphthalene, pyrene and chrysene within the 12 weeks of bioremediation. In a study by Liu *et al*. (2017), the combination of chicken and cow manures significantly enhanced PAH degradation in a crude oil-contaminated soil. The synergistic effect was attributed to the complementary microbial communities and nutrient profiles of the two manures. Jiang *et al.* (2019), also examined the use of swine and horse manures in combination for PAH remediation. The study demonstrated improved PAH removal efficiency compared to the individual manure treatments, highlighting the synergy between different animal manures. This also agrees with the field-scale investigation by Smith *et al*. (2021) evaluating the efficacy of a blend of chicken, cow, and horse manures for PAH remediation in a large-scale crude oil-impacted site. The results indicated a substantial reduction in PAH concentrations, underscoring the potential for combining animal manures in real-world applications.

The calculated percentage degradation are shown in Figures 4 to 6. Percentage naphthalene removal was found as 29.05, 95.64, 92.31, 94.01, 92.97, 93.98, 94.71, 95.80 and 92.04 for NA, CD, GD, CD, CDPM, CDGD, GDPM, CDGDPM and NPK respectively. Obviously, combined animal waste (CDGDPM) effectively removed naphthalene, compared to the other amendments, although GD was also quite effective. Similar results of 97.30% and 94.64% was obtained for chrysene and pyrene respectively. This high PAH removal efficiency displayed by combined AMENDMENT, may be due to synergestic interactions among microbial communities as well as improved energy profile.

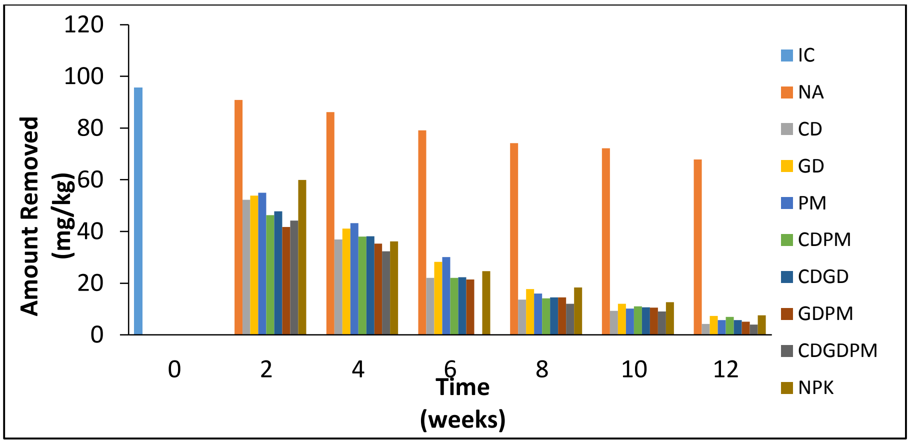

Fig 1. Percentage degradation of Naphthalene by the different remediation treatments during the remediation period.
Key: IC=Initial Concentration, CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings.

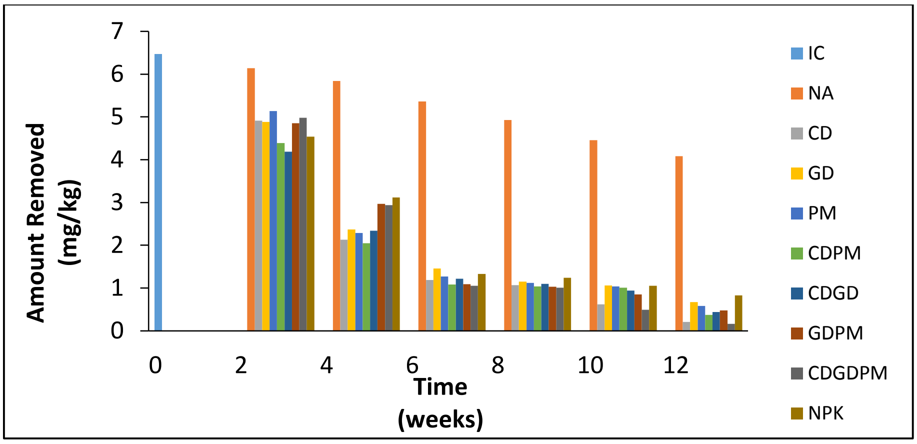

Fig 2. Percentage degradation of Chrysene by the different remediation treatments during the remediation period.
Key: IC=Initial Concentration, CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings.

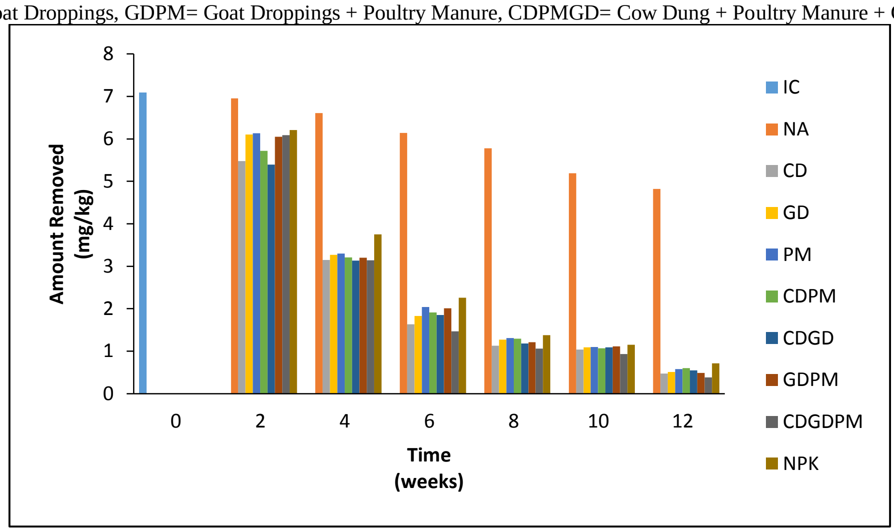

Fig 3. Pyrene removal by the different remediation treatments during the remediation period.
Key: IC=Initial Concentration, CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings.

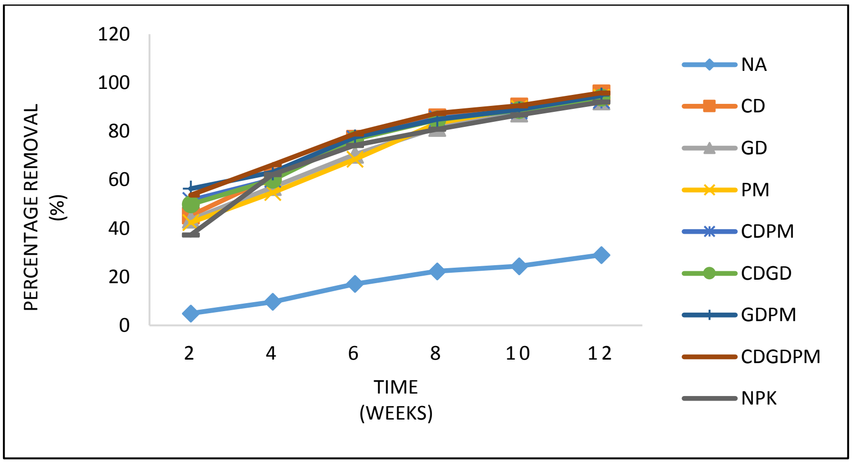

Fig 4. Percentage degradation of Naphthalene by the different remediation treatments during the remediation period.
Key: IC=Initial Concentration, CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings.

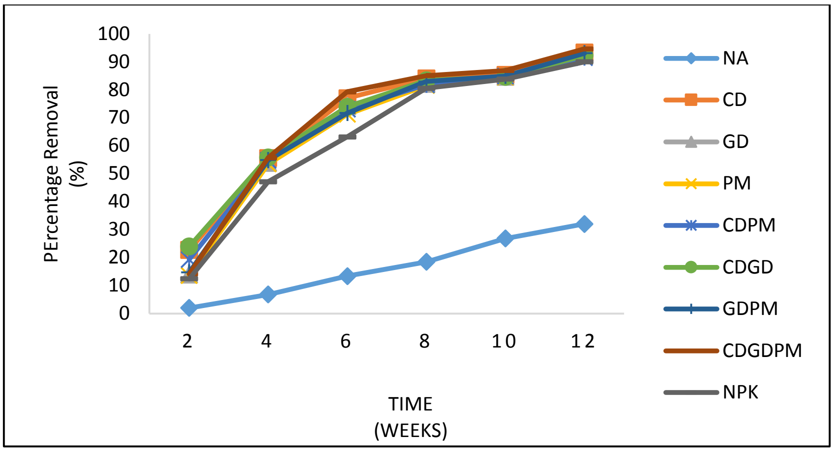

Fig 5. Percentage degradation of Chrysene by the different remediation treatments during the remediation period.
Key: IC=Initial Concentration, CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings.

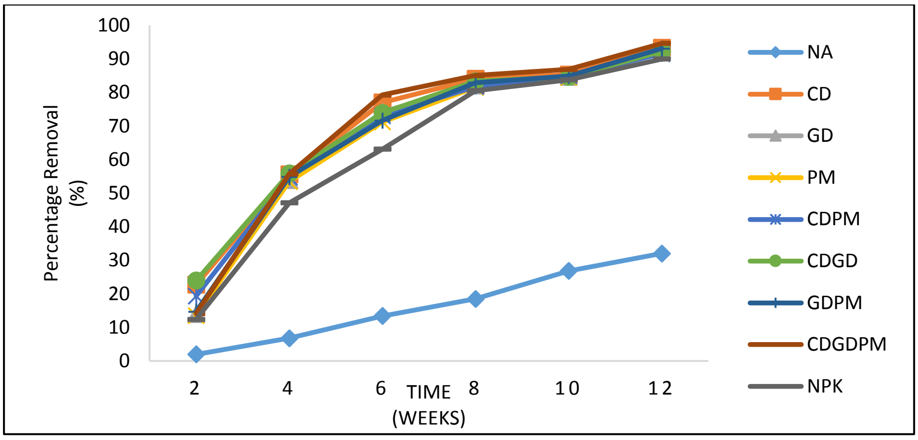

Fig 6. Percentage degradation of Pyrene by the different remediation treatments during the remediation period.
Key: IC=Initial Concentration, CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings.

The results of analysis of variance (ANOVA) for the percentage removal of Naphthalene, Chrysene and Pyrene are displayed in Table 2(a-c). Statistical analysis of variance was carried out in order to checkmate if the process parameters are statistically significant or not. The F-value for the removal amount of each PAH by the amendments (Ravikumar et al., 2006) indicates which of the PAH was effectively removed by the amendments and also which of either CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings performed best. Usually, the larger the F-value, the greater the effectiveness of the amendment agent (CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings) for PAHs removal. The influence of various parameters and their interaction on the removal percent was decided with the help of ANOVA analysis and its performance characterized. The results of ANOVA for the removal of Naphthalene, Chrysene and Pyrene are given in Table 2. The F-values obtained for the removal of all the PAHs were found as 7.396171, 3.1493 and 2.420503 for Naphthalene, Chrysene and Pyrene indicating that significant amount of both Naphthalene, Chrysene and Pyrene were removed during the entire process and also suggesting the order of PAH removal as Naphthalene< Chrysene < Pyrene for the entire removal process. Furthermore results from cluster analysis shown in figures confirms synergistic behaviour in the removal of the individual PAH. For instance, at the linkage distances before 20, 2 and 1 for Naphthalene, Chrysene and Pyrene respectively interactions between the different amendments are evident, except for natural attenuation (NA).

Table 2a: Results for ANOVA a= Naphthalene, b= Chrysene, c= Pyrene

ANOVA(a)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17652.49	8	2206.561	7.396171	3.11E-06	2.152133
Within Groups	13425.22	45	298.3383			
Total	31077.71	53				

ANOVA(b)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	14184.03	8	1773.003	3.1493	0.006467	2.152133
Within Groups	25334.25	45	562.9834			
Total	39518.28	53				

ANOVA(c)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	13922.61	8	1740.326	2.420503	0.028801	2.152133
Within Groups	32354.71	45	718.9937			
Total	46277.32	53				

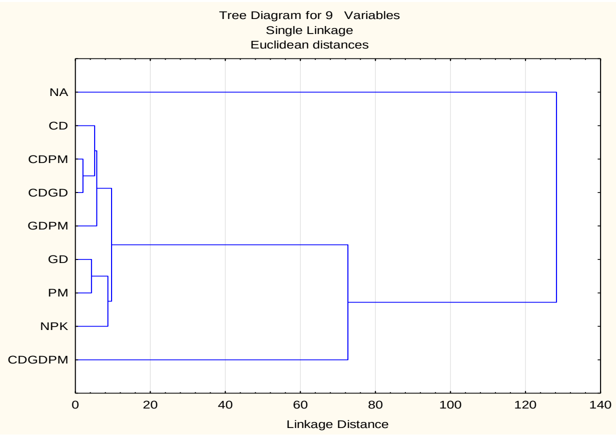

Fig 7. Dendograms showing the synergistic interactions of the amendment agents for Naphtalene during the remediation period. *Key: IC=Initial Concentration, CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings.*

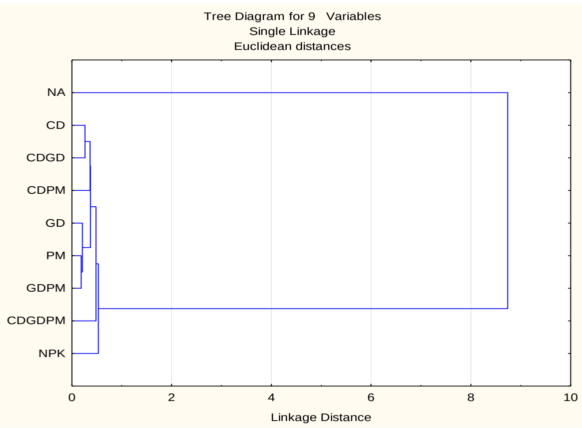

Fig 8. Dendograms showing the synergistic interactions of the amendment agents for Chrysene during the remediation period. *Key: IC=Initial Concentration, CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings.*

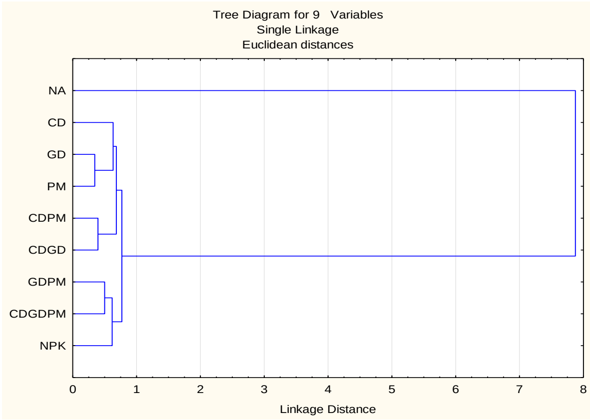

Fig 9. Dendograms showing the synergistic interactions of the amendment agents for Pyrene during the remediation period. *Key: IC=Initial Concentration, CD=Cow Dung, PM=Poultry Manure, GD=Goat Droppings, CDPM=Cow Dung +Poultry Manure, CDGD= Cow Dung +Goat Droppings, GDPM= Goat Droppings + Poultry Manure, CDPMGD= Cow Dung + Poultry Manure + Goat Droppings.*

Kinetic Analysis: Kinetic studies helps to determine the rate at which contaminants are degraded by microorganisms. By measuring degradation rates, researchers can assess the efficiency and effectiveness of bioremediation strategies. This information is important for estimating the time required to achieve the desired level of contaminant removal and for determining the feasibility of using bioremediation as a remedial approach. (Zhou *et al*., 2016; Lladó *et al*., 2017; Ma *et al*., 2020). It also enables prediction of the fate and persistence of contaminants in the environment. By quantifying the degradation rate, researchers can estimate the duration and extent of contaminant removal. This information is important for assessing the long-term efficacy of bioremediation and predicting potential risks associated with residual contaminants. (An *et al*., 2018; Carucci and Rossetti, 2018; Sutha and Murugesan, 2019; Xu *et al*., 2020; Liu *et al*., 2021). Biodegradation kinetic data for the biodegradation of Naphthalene, Pyrene and Chrysene was fitted to zeroth and first-order kinetic equations. These kinetic equations are:

Zeroth order:

$$[A]t = [A]_0 - k_0 t \qquad (1)$$

First order:

$$\ln[A]\, t = lnA_O - k_1 t \qquad (2)$$

Where $[A]_0$ and $[A]_t$ are amounts of contaminants present at the beginning of the experiment and at various time intervals, k is the ith order rate constant and t stands for the various time intervals.

The kinetic plots are as shown in Fig 10 to 36 below:

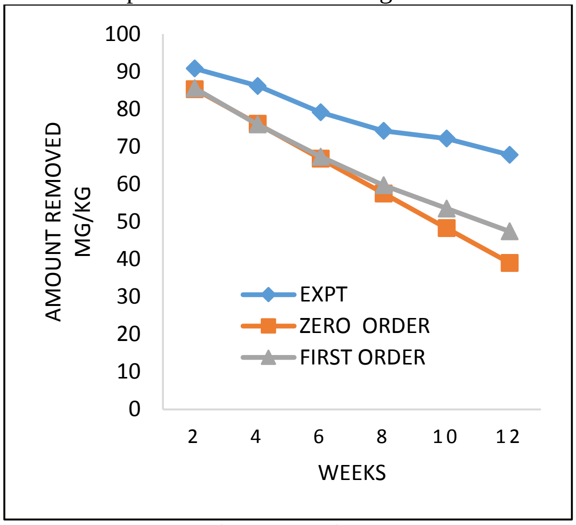

Fig 10. Kinetic plots for removal of Naphthalene by natural attenuation during the remediation period.

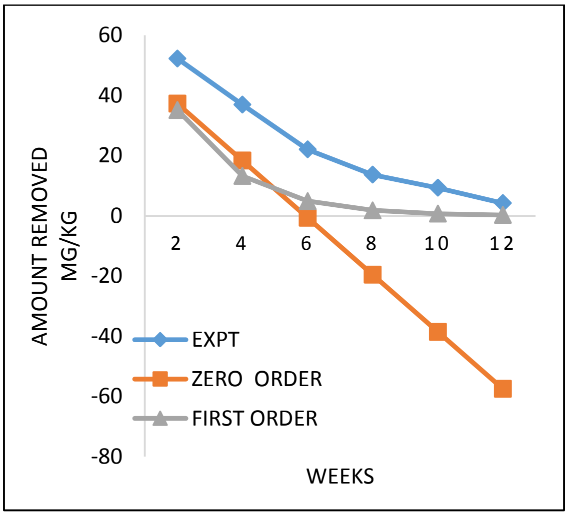

Fig 11. Kinetic plots for removal of Naphthalene by cow dung during the remediation period.

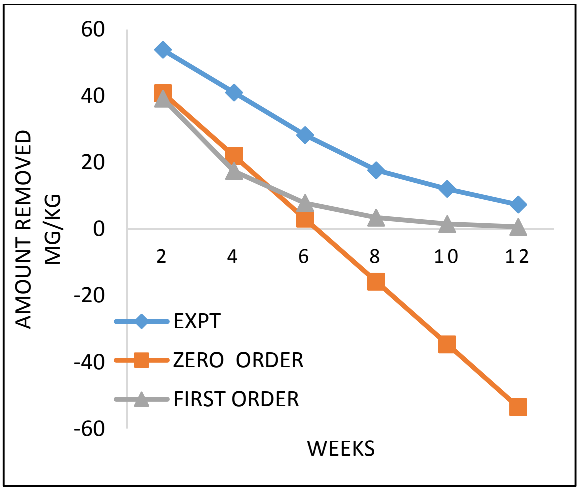

Fig 12. Kinetic plots for removal of Naphthalene by goat droppings during the remediation period.

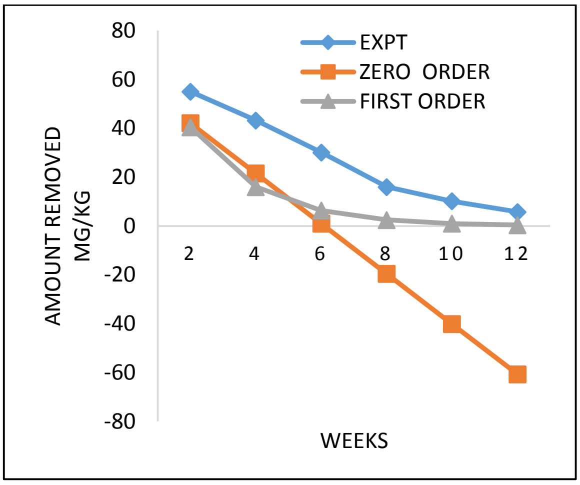

Fig 13. Kinetic plots for removal of Naphthalene by poultry manure during the remediation period.

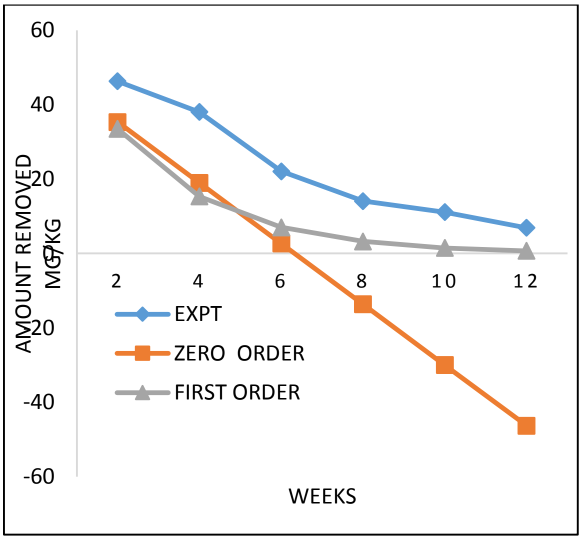

Fig 14. Kinetic plots for removal of Naphthalene by cow dung and poultry manure during the remediation period.

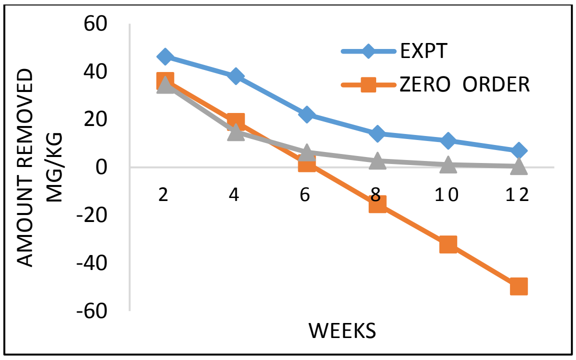

Fig 15. Kinetic plots for removal of Naphthalene by cow dung and goat droppings during the remediation period.

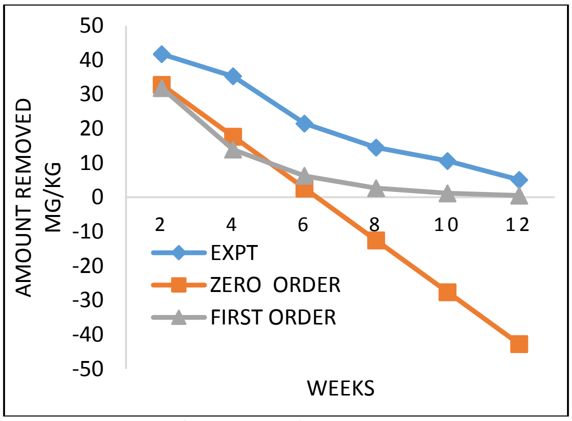

Fig 16. Kinetic plots for removal of Naphthalene by goat droppings and poultry manure during the remediation period.

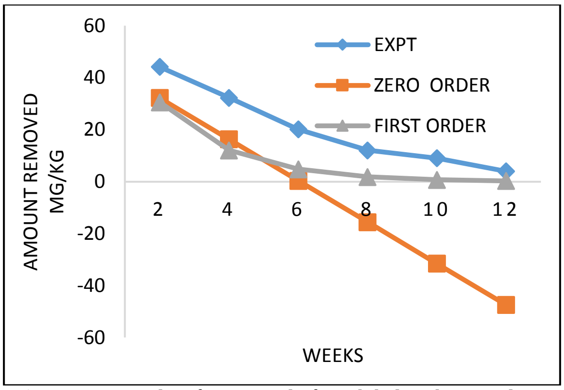

Fig 17. Kinetic plots for removal of Naphthalene by cow dung, goat droppings and poultry manure during the remediation period.

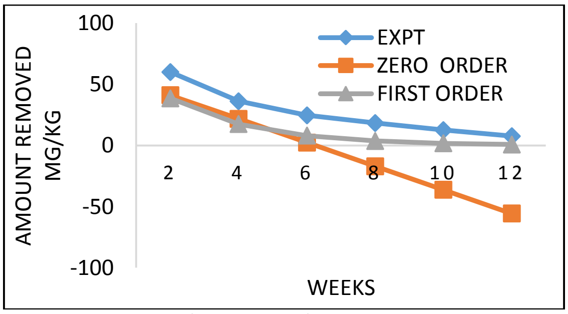

Fig 18. Kinetic plots for removal of Naphthalene by NPK during the remediation period.

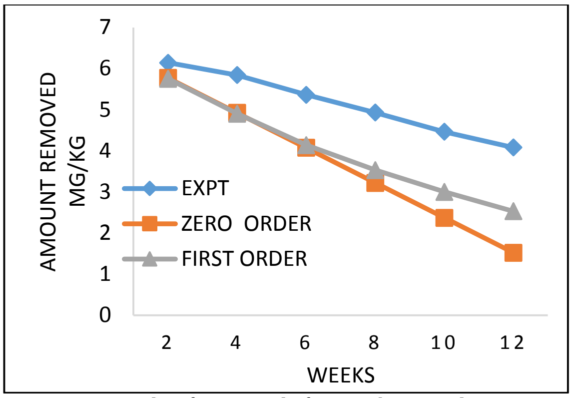

Fig 19. Kinetic plots for removal of pyrene by natural attenuation during the remediation period.

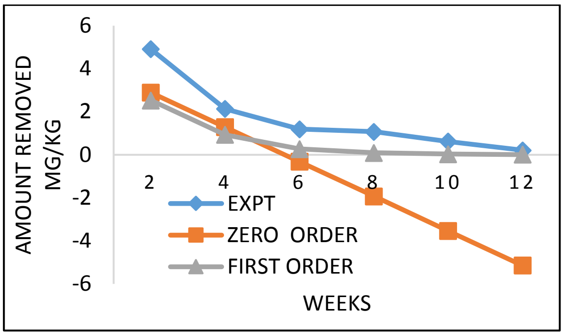

Fig 20. Kinetic plots for removal of Pyrene by cow dung during the remediation period.

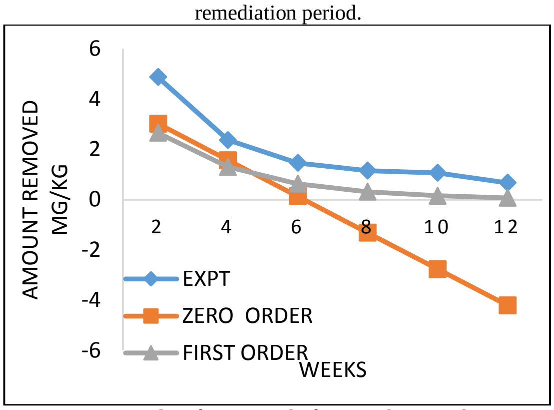

Fig 21. Kinetic plots for removal of Pyrene by goat droppings during the remediation period.

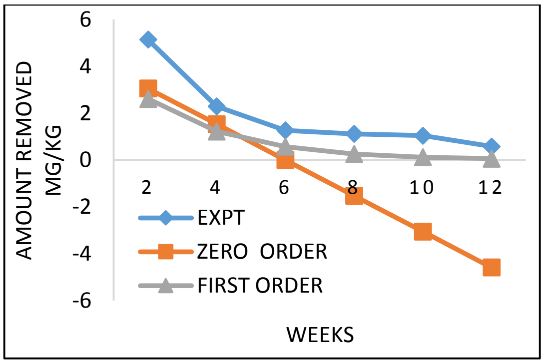

Fig 22. Kinetic plots for removal of Pyrene by poultry manure during the remediation period.

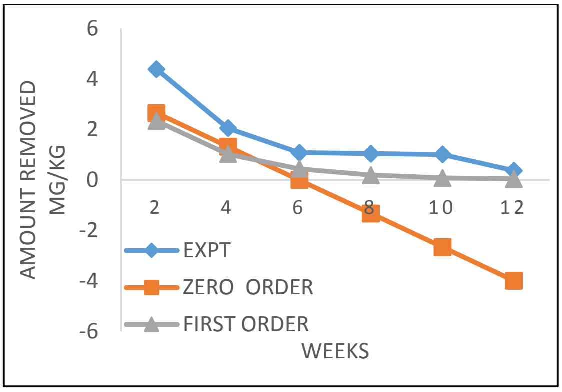

Fig 23. Kinetic plots for removal of Pyrene by cow dung and poultry manure during the remediation period.

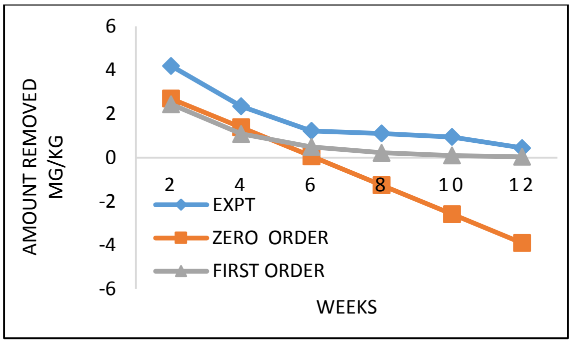

Fig 24. Kinetic plots for removal of Pyrene by cow dung and goat droppings during the remediation period.

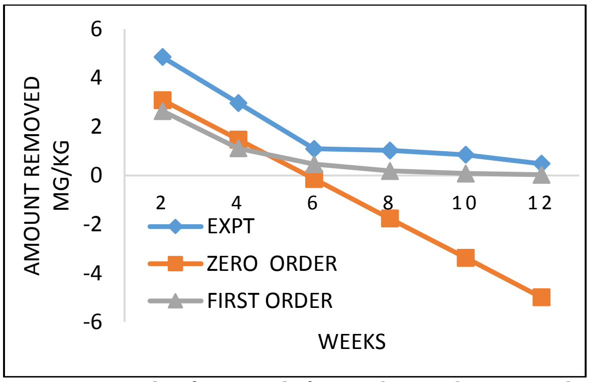

Fig 25 Kinetic plots for removal of Pyrene by goat droppings and poultry manure during the remediation period.

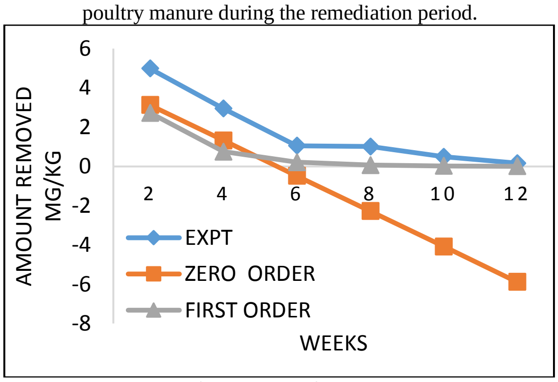

Fig 26. Kinetic plots for removal of Pyrene by cow dung, goat droppings and poultry manure during the remediation period.

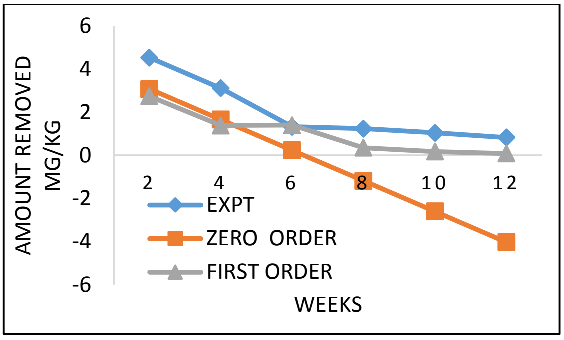

Fig 27 Kinetic plots for removal of Pyrene by NPK during the remediation period.

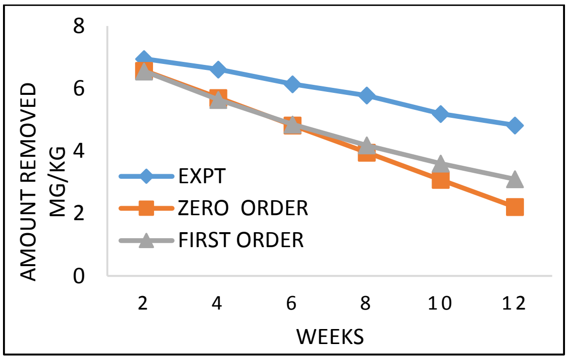

Fig 28. Kinetic plots for removal of Chrysene by natural attenuation during the remediation period.

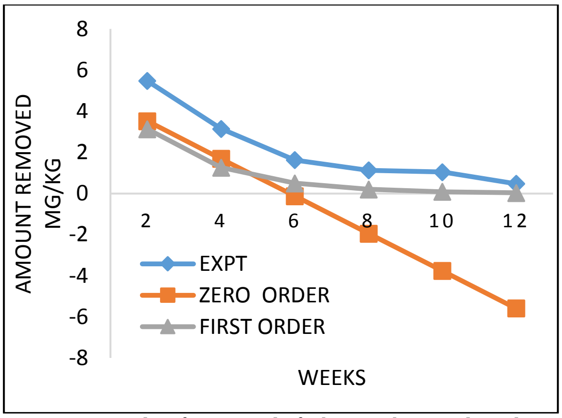

Fig 29. Kinetic plots for removal of Chrysene by cow dung during the remediation period.

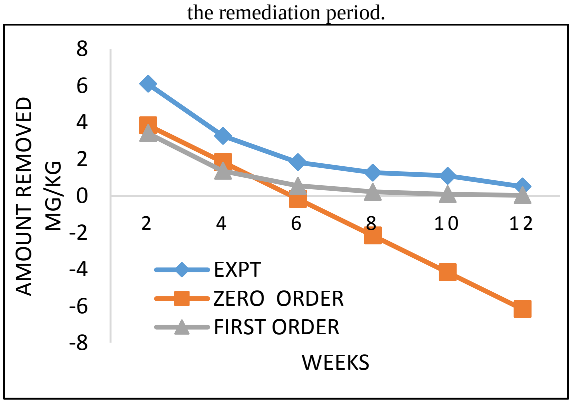

Fig 30. Kinetic plots for removal of Chrysene by goat droppings during the remediation period.

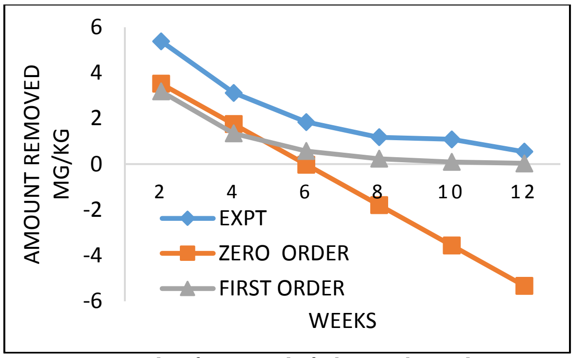

Fig 31. Kinetic plots for removal of Chrysene by poultry manure during the remediation period.

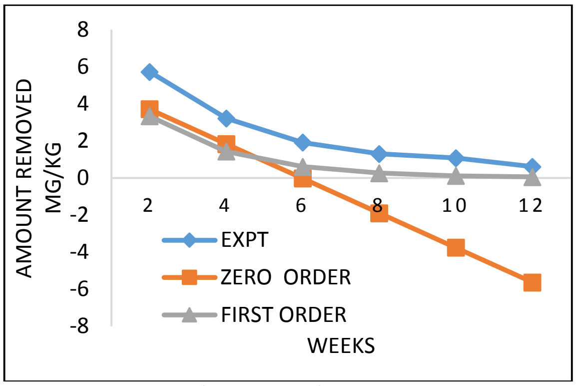

Fig 32. Kinetic plots for removal of Chrysene by cow dung and poultry manure during the remediation period.

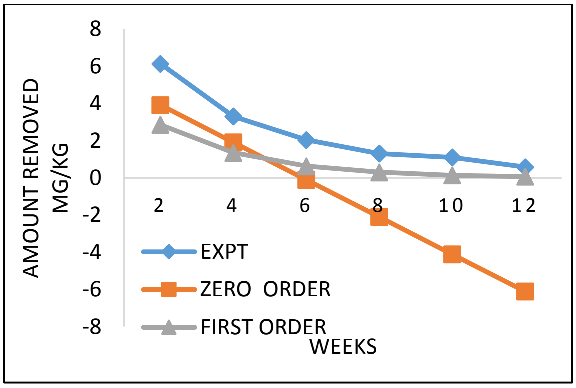

Fig 33. Kinetic plots for removal of Chrysene by cow dung and goat droppings during the remediation period.

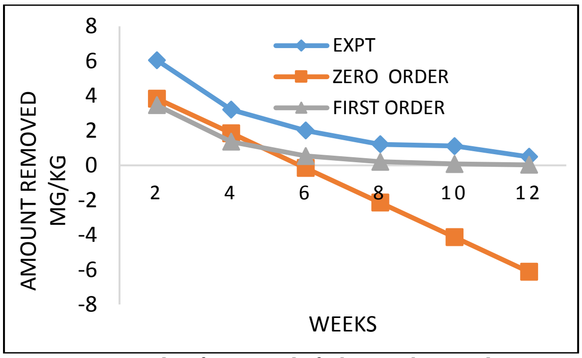

Fig 34. Kinetic plots for removal of Chrysene by goat droppings and poultry manure during the remediation period.

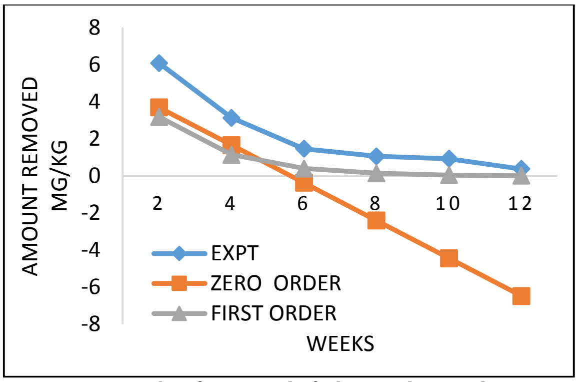

Fig 35. Kinetic plots for removal of Chrysene by cow dung, goat droppings and poultry manure during the remediation period.

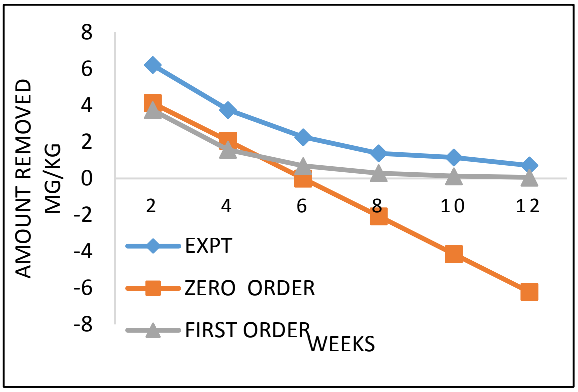

Fig 36. Kinetic plots for removal of Chrysene by NPK during the remediation period.

The kinetics of the bioremediation process showed a first-order reaction. This is important because a first-order kinetic bioremediation process allows for the prediction of contaminant degradation rates over time. By determining the first-order rate constant (k), one can estimate how quickly contaminants will be removed from the soil (Werner *et al*. 2009). It is also essential for designing remediation strategies. It helps in selecting appropriate microbial consortia, optimizing environmental conditions (e.g., temperature, pH), and determining the duration of treatment required to achieve cleanup goals. Furthermore, First-order kinetics provides a quantitative measure of the efficiency of bioremediation processes. Comparing the observed rate constant ($k_{observed}$) with the literature values ($k_{literature}$) for specific contaminants allows for evaluating the effectiveness of a bioremediation approach. (Suthersan *et al*. 2013)

Conclusion: The synergistic effect of combining various animal manures represents a sustainable and environmentally friendly approach to enhance the removal of PAHs from crude oil-impacted soil. However, successful implementation requires site-specific assessments, careful monitoring, and adherence to environmental regulations. Further research and field-scale studies are needed to validate the efficacy of combined animal manures in addressing PAH contamination, offering a potential solution for mitigating its negative environmental impacts.

Acknowledgements: The authors gratefully acknowledge the financial support provided by the Tertiary Education Trust Fund (TETFUND/ABSU/RP.2018).

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the first author or corresponding author.

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