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ACCELERATED BIODEGREDABILITY OF CHLORPYRIFOS (PESTICIDE) RESIDUE IN CONTAMINATED SAVANNAH SOIL WITH *MORINGA OLEIFERA* LEAF POWDER

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Abstract
The residue of organophosphate pesticides (chlorpyrifos) present in the sandy-loam soil of the Guinea Savannah Nigeria is a threat to the soil ecosystem health. This study evaluated the efficacy of *Moringa oleifera* leaf powder (MOLP) as a bio-stimulant for the remediation of chlorpyrifos-contaminated farmland in Zango, Adavi, Kogi State, Nigeria. Samples of the soil were treated with varying concentrations of MOLP low dose, 5% and high dose, 10% over 90 days study period. High-performance liquid chromatography (HPLC) analysis revealed that the higher-dose MOLP treatment achieved 81% removal efficiency, following first-order kinetics with a significantly reduced half-life of unamended controls. The amendment stimulated a "microbial bloom," increasing from $1.2 \times 10^{6,}$ to 5.2×10^{6} cfu/g. These findings suggest that Moringa leaf amendment served dual functions: detoxifying pesticide residues and improving soil microbial population. The study concludes that MOLP is a viable, sustainable alternative to conventional chemical oxidation, achieving effective decontamination without compromising soil ecological integrity.

Keywords: Bioremediation, Bio-stimulation, Chlorpyrifos, *Moringa oleifera,* Microbial count.

Introduction

Soil contamination by pesticides has become a major environmental concern due to the widespread use of chemical pesticides in modern agricultural practices. Pesticides, including herbicides, insecticides, and fungicides, are applied to crops to control pests and diseases, but their persistent nature often leads to the accumulation of toxic residues in agricultural soils (Kumar *et al*., 2019. Chlorpyrifos (O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) amongst many other pesticides stands out as one of the most widely used and most problematic organophosphates (Gara *et al*., 2018). Farmers prize it for its broad-spectrum efficacy against soil-borne insects and foliage pests. However, its chemical stability presents a significant environmental challenge. Chlorpyrifos is characterized by low water solubility and a high soil sorption coefficient (Zhang *et al.,* 2020). Rather than washing away, it binds stubbornly to soil particles, resisting degradation. Ishag *et al*.

(2016) had noted that in typical agricultural soils, chlorpyrifos can pose a significant risk to food safety and human health.

There is a gap with the use of chemicals for soil remediation and fallow decontamination which is slow in active farming. So, in recent years, sustainable and eco-friendly approaches have been explored. Bioremediation that involves the use of organisms is not only effective, but less expensive and environmentally friendly (Ali *et al;* 2019*)*. By harnessing the natural metabolic processes of these organisms, contaminants such as heavy metals and agrochemicals can be converted into simpler, non-toxic end products like carbon dioxide, water and biomass (Mekonnen *et al*., 2024). *Moringa oleifera* plant is culturally abundant in Kogi State, particularly as an adopted leguminious vegetable tree in Zango. It thrives exceptionally well across the southern and northern guinea savannah vegetational zones of Nigeria (Zaku, *et al*., 2015).

The potential of *M.oleifera* in environmental engineering cannot be over emphasized the scientific premise for using Moringa leaves lies in their unique biochemical profile: The leaves are rich in nitrogen, phosphorus, and potassium. Moringa, a fast-growing and highly resilient plant, has shown potential in environmental remediation due to its bioactive compounds, which possess adsorptive and degradative properties against pollutants (Leone *et al.,* 2016). When applied to contaminated soil, they act as a high-quality nutrient source (bio-stimulant), triggering a population explosion of indigenous soil bacteria, which are empowered to degrade pesticide residues (Agboun *et al*., 2016).

Moringa contains natural bioactive compounds (saponins and proteins) that function as bio-surfactants, suggesting that these compounds can help detach hydrophobic pesticides like chlorpyrifos from soil particles, making them more accessible for microbial breakdown (Culver *et al*., 2019). Moringa leaves contain phytochemicals such as flavonoids, polyphenols, and alkaloids which are known to enhance the breakdown of toxic substances in the environment (Adeyemi and Fagbenro, 2021). The ability of Moringa to improve soil health by enhancing microbial activity and nutrient availability makes it a promising candidate for the remediation of pesticide-contaminated farmlands. This study therefore aims to evaluate the effectiveness of Moringa leaves in reducing chlorpyrifos pesticide residues and improving soil quality in Zango Adavi, Local Government Area (LGA) Kogi State, Nigeria.

Materials and Methods

Study Area

The research was carried out in Zango, a rural agrarian community located in the Adavi Local Government Area (LGA) of Kogi State, Nigeria. Zango lies within the Guinea Savanna ecological zone of central Nigeria, an area characterized by mixed grassland vegetation interspersed with deciduous trees. The coordinates of the study area are latitude 7.45° N and longitude 6.84° E, with an average elevation of approximately 350 m above sea level (Nigerian Meteorological Agency, 2023).

The climate is tropical with two distinct seasons: rainy from April to October and dry season, from November to March. Mean annual rainfall ranges from 1,200 mm to 1,500 mm, and mean annual temperature fluctuates between 24 °C and 33 °C, with peak values observed in March. Relative humidity averages around 70–80 % during the wet months but drops to 50 % during the dry season. The soils are classified as ferruginous tropical soils (Acrisols) according to the FAO–UNESCO system (FAO, 2015). They are predominantly sandy-loam, slightly acidic (pH 5.5), and generally low in organic matter, nitrogen, and phosphorus. These characteristics make the soils highly responsive to organic inputs such as *Moringa oleifera* leaves.

Majority of the 15,000 estimated population are small holder farmers, cultivating maize, cassava, rice, yam, and vegetables. The area has been reported to experience moderate pesticide accumulation due to indiscriminate use of organophosphates and carbamates (Agbo *et al*., 2022).

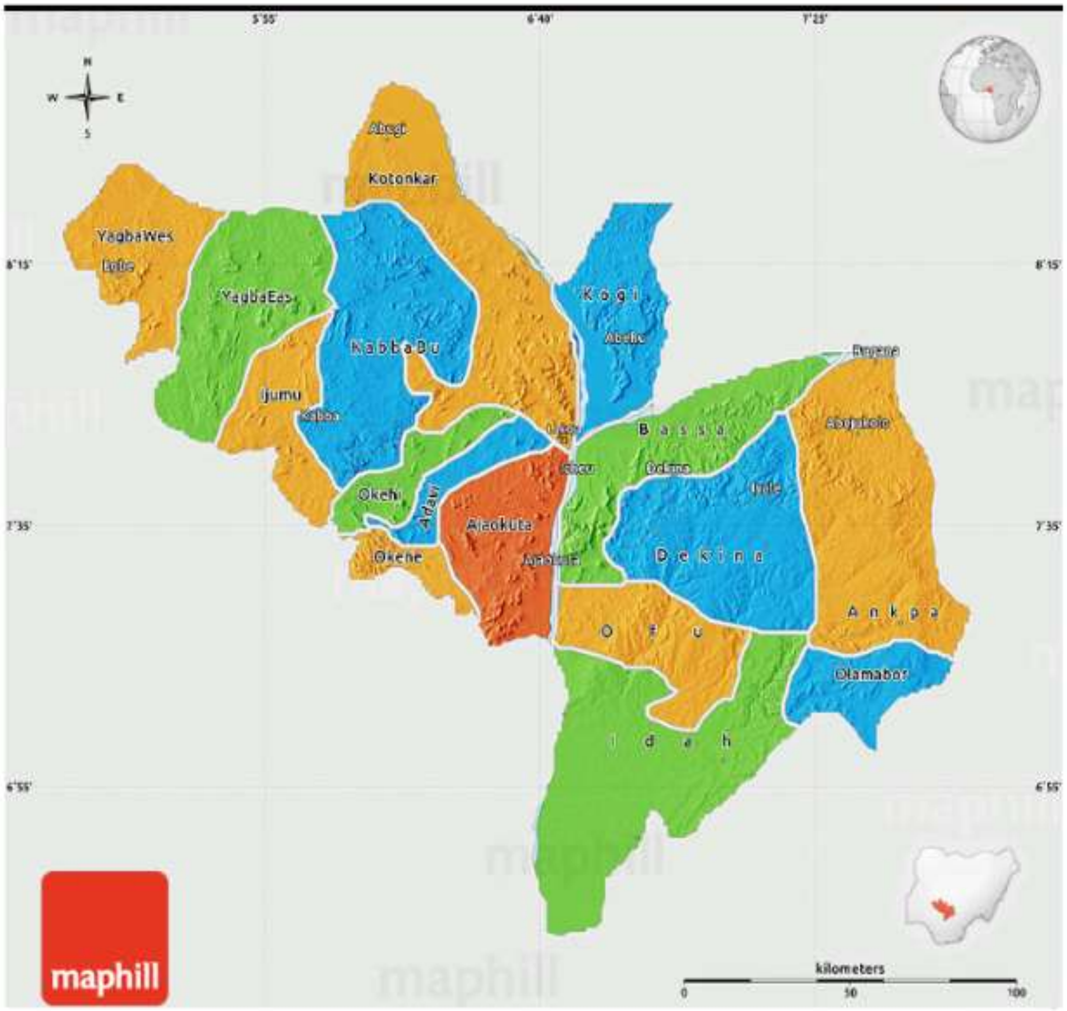

Figure 1: Map of Kogi State, inclusive of Adavi Local Government Area, with arrow indicating the sampling point (Zango).

Soil Sampling Technique

A purposive sampling technique was used to select the farmland sites with a history of chlorpyrifos application. A systematic zig-zag sampling technique was employed across the identified farms, to obtain soil samples at 0-15 cm depth using an auger. These sub-samples were thoroughly bulked and packed in sterile labelled polythene bags and transported to the laboratory. The soil was air-dried at room temperature (27 ± 2°C) to remove excess moisture, until constant weight. Crushed and broken up soil was passed through a 2 mm sieve to ensure uniformity (AOAC, 2019). Sampling tools were disinfected with 70% ethanol.

Preparation of Moringa oleifera Leaf Powder

Fresh leaves of *Moringa oleifera* were harvested from the plant. The leaves were washed under running tap water and rinsed with distilled water, drained and shade dried at room temperature (27± 2^{o}C) for 7 days. Once dried, the leaves were pulverized with a blender (SE-505 R) into fine powder, sieved through a 2mm mesh to obtain a uniform texture (MOLP) and stored in airtight container until needed for experimentation.

Treatment Application

Contaminated soil samples (1 kg each of three replicates) using a randomized block design (RBD) were thoroughly mixed with Moringa leaf powder at the prescribed rates (T_1 and T_2). Each mixture was moistened to approximately 60 % field capacity. Moisture was maintained by periodic addition of deionized water after every two days in the laboratory. The experimental treatments were designed as follows;

T_0 (Control) contaminated soil without amendment

T_1 (Low Dose Moringa) contaminated soil + 5 % or 50g Moringa leaf powder.

T_2 (High Dose Moringa) contaminated soil + 10% or 100g of Moringa leaf powder.

T_3 (Chemical Remediation) contaminated soil + 30% Hydrogen peroxide solution

Determination of Chlorpyrifos Residues

The initial pesticide residue levels in the soil were determined using gas chromatography-mass spectrometry (GC-MS) agilent 8890 GC system with 7010B MS following the EPA 8081B method for organo phosphate pesticides (USEPA, 2016), fitted with an electron-capture detector (ECD). Extraction was done with 10 mL of acetone and hexane (1:1) followed by rotary evaporation and clean-up on a Florisil row. Quantification used external calibration standards detection limit = 0.01 mg kg^{-1}. GC-MS (Santo *et al*., 2021). Ten gram of soil was mixed with 20 ml acetonitrile and stirred for one hour and agitated to extract the pesticide residues. Measurement was repeated after 30, 60 and 90 days of the application of Moringa powder and Chemical treatments. Percentage degradation of the chlorpyrifos was calculated by measuring the residual pesticide concentration at the end of the study to the initial concentration using the formula;

% Degradation = $\frac{Ci-Cf}{Ci} \times 100$

where:

Ci = the initial

Cf = the final concentration (USEPA, 2015).

Determination of the soil Microbial Population

The measurement of bacteria microbial population in the soil was taken as indication of soil health. A decontaminated soil would have increased bacteria population. Total heterotrophic Bacterial count was determined using the pour plate method on nutrient Agar. Three replicate plates of nutrient agar medium were inoculated from 1 x $10-^{5}$ serially diluted mixtures. The agar plates were incubated at room temperature (27 ± 2 ^{0}C).The agar medium was incorporate with 1% nystatin to prevent the growth of fungi (Keta and Suberu 2026). The bacteria colonies were counted, with 30 – 300 colonies using the fomula cfug/g = 50 x 10^{5}= 5x10^{6}Cfu/g (Kagan *et al*., 2024).

Data Analysis

To ensure the result was not just due to chance all quantitative data obtained were subjected to a one- way Analysis of Variance (ANOVA) using statistical software (International Business Machines Statistical Package for the Social Sciences (IBM SPSS) Version 25). Significant differences were observed at ($P<0.05$), means were separated using Duncan multiple range test. It was hence, possible to specifically compare if the Moringa treatment performed significantly better or worse than chemical treatments.

Results

Effect of Moringa Leaf Amendment on the Degradation of Chlorpyrifos Residue in Soil

Chlorpyrifos in soil progreesively reduced over the 90 days of evaluation. At 30 days of treatment the pesticide reduced from 12.50 mg/ kg to 11.80 mg/kg, 8.40 mg/kg, 7.10 mg/kg and 6.20 mg/kg respectively for control, 5*% Moringa oleifera* Powder (MOLP), 10% MOLP and hydrogen peroxide amendments, implying a significant reduction with the treatments than the control. The same trend was recorded at days 60 and 90. Calculated respective percentage reductions in the chlorpyrifoS were 21.6%, 74.4%, 80.8% and 91.2% (Table 1). It thus appear like hydrogen peroxide is most effective for reducing the chlorpyrifos in the soil.

Table 1: Effect of Moringa Leaf Amendment on the Degradation of Chlorpyrifos Residue in Soil

Day	Chlorpyrifos Residue (mg/kg) T_0 (Control)	T_1 (5% MOP)	T_2 (10% MOP)	T_3 (30% H_2O_2)
0	$12.50{\pm}0.40^{a}$	$12.50{\pm}0.40^{a}$	$12.50{\pm}0.40^{a}$	$12.50{\pm}0.40^{a}$
30	$11.80{\pm}0.35^{a}$	$8.40{\pm}0.30^{b}$	$7.10{\pm}0.28^{bc}$	$6.20{\pm}0.25^{c}$
60	$10.90{\pm}0.30^{a}$	$5.60{\pm}0.20^{b}$	$4.20{\pm}0.22^{bc}$	$3.10{\pm}0.18^{c}$
90	$9.80{\pm}0.28^{a}$	$3.20{\pm}0.15^{b}$	$2.40{\pm}0.12^{bc}$	$1.10{\pm}0.10^{c}$
% Degradation (Day 90)	21.6%	74.4%	80.8%	91.2%

The results are presented as mean ± standard deviation. Values with different superscripts within the same row are significantly different at $p < 0.05$.

Key

MOLP = *Moringa oleifera* leaf powder,
H_2O_2 = Hydrogen Peroxide
T_0 = Untreated soil

Effect of Moringa Leaf Powder on Soil Microbial Population

Microbial population changed over time (0–90 days) under different treatments: untreated control (T0), 5% *Moringa oleifera* leaf powder (T_1), 10% *Moringa oleifera* leaf powder (T_2), and chemical treatment (T_3). At day zero, microbial counts were 1.2×10^6 cfu g^{-1} across the groups. By day 30, microbial population increased slightly in the control (1.4×10^6 cfu g^{-1}), suggesting natural microbial recovery over time. Much higher microbial counts were recorded with soils treated with Moringa leaf powder (MOLP). T_1 increased to 2.3×10^6 cfu g^{-1}, while T_2 increased to 3.0×10^6 cfu g^{-1}. In contrast, the chemical treatment reduced microbial counts to 1.0×10^6 cfu g^{-1}, suggesting an inhibitory effect on soil microorganisms. At day 60, this trend became more pronounced. The control showed a modest increase (1.6×10^6 cfu g^{-1}), while 5% and 10% recorded substantial increases to 3.0×10^6 cfu g^{-1} and 4.0×10^6 cfu g^{-1}, respectively. Meanwhile, microbial activity in the chemically treated soil declined further to 0.8×10^6 cfu g^{-1}, indicating continued microbial suppression. By day 90, microbial population reached its highest values in the Moringa treated soils. 5% MOLP increased to 3.6 × 10^6 cfu g^{-1}, and 10% MOLP peaked at 5.2 × 10^6 cfu g^{-1}. The control showed only a gradual increase to 1.8 × 10^6 cfu g^{-1}, reflecting limited natural recovery. The chemical treatment recorded the lowest value (0.7 × 10^6 cfu g^{-1}) (Table 2).

Table 2: Effect of Moringa Leaves Powder on Soil Microbial population at cfu x 10^6 g^{-1}
Microbial Counts

	Microbial Population in the Contaminated Soil			
Day	T_0 (Control)	T_1 (MOLP 5%)	T_2 (MOLP 10%)	T_3(30% H_2O_2)
0	$1.20±0.10^a$	$1.20±0.10^a$	$1.20±0.10^a$	$1.20±0.10^a$
30	$1.40±0.12^c$	$2.30±0.20^b$	$3.00±0.25^a$	$1.00±0.10^d$
60	$1.60±0.15^c$	$3.00±0.25^b$	$4.00±0.30^a$	$0.80±0.10^d$
90	$1.80±0.15^c$	$3.60±0.30^b$	$5.20±0.35^a$	$0.70±0.08^d$

The results are presented as mean ± standard deviation. Values with different superscripts within the same rows are significantly different at $p < 0.05$.

Key
MOLP = *Moringa oleifera* leaf powder,
H_2O_2 = Hydrogen Peroxide
T_0 = Untreated soil

Discussion
The chlorpyrifos residue of the soil before treatment were uniform contamination level at 12.5mg/ kg. Over the course of the study, natural attenuation in the control showed slow decline in the pesticide level, reducing by 21% after 90 days. This suggest that without remediation, chlorpyrifos remains a problem in the soils. However, But, the application of MOLP significantly accelerated the breakdown of the pesticide. There was a dose - dependent response; 5% amendment achieved a 74% reduction, and 10% amendment achieved a remarkable 80.8% reduction. The exponential degradation rate in the treated soil is attributed to bio-stimulation. Moringa leaves are rich in limiting nutrients (nitrogen and phosphorus) and bioactive compounds like glucosinolates (Agubosi *et al*., 2024). When introduced to the soil, these nutrients alleviated the indigenous microbes, allowing them to proliferate and produce enzymes like phosphotriesterase, which hydrolyzes the ester bonds in chlorpyrifos. The rapid degradation of chlorpyrifos in the Moringa

–treated soils suggest that the organic amendment successfully stimulated biological activity acting as a catalyst for breaking down the complex pesticide structure.

Agboun *et al*. (2016) observed a similar trend where Moringa leaf extract accelerated the degradation of total petroleum hydrocarbons (TPH) in the Niger Delta. They reported that the "nutrient shock" from the leaves reduced TPH half – life by 65%, paralleling the rapid pesticide loss seen in this study. Furthermore, also, Briceno *et al*. (2012) in their work on diazinon (another organophosphate), they found that organic amendments increased degradation rates by 3 - fold compared to unamended soils. They argued that the organic matter acts as an "analogue substrate," priming bacteria to attack the pesticide structure. Ishag *et al*. (2016) working specifically with chlorpyrifos, they noted that without biostimulation, the pesticide persists due to high soil sorption. Their control groups showed <30% degradation after 60 days, which perfectly corroborates the slow natural attenuation (22%) observed in the control samples of this study. Rayu *et al*. (2017) demonstrated that the addition of nutrient-rich organic waste accelerated the breakdown of organophosphates by stimulating the Proteobacteria phylum, further validating the biological mechanism proposed here.

One of the most profound differences observed was in the biological life of the soil. Chlorpyrifos is known to inhibit soil microbes (Dutta *et al*., 2010) and this was evident in the control, where bacterial counts remained low and stagnant. The introduction of Moringa powder triggered a "microbial bloom." The powder served as a nutrient for the native soil bacteria and fungi. .The total microbial population is as an indicator of the overall health of the soil ecosystem. The sandy soils of Adavi are typically carbon-limited (Adeleye *et al*., 2021). The Moringa powder provided a readily available carbon and nitrogen, shifting the soil ecosystem from a dormant state to an active growth phase. The increased surface area of the powdered leaves also provided physical micro-habitats for bacterial colonization.

Total Heterotrophic Bacterial Counts (THBC) in 10% Moringa amended soil increased by over 300% compared to the control. This finding confirms that Moringa does not just passively bind the pesticide; it actively feeds the specific microbes responsible for degrading the pesticide.

Rani *et al*. (2008) investigating soil enzyme activity, found that organic amendments increased dehydrogenase activity (a proxy for microbial life) by over 150%. They concluded that the amendments serve as a metabolic "catalyst," exactly as the Moringa powder did in this experiment. Abbas *et al*. (2023) demonstrated that the addition of bio-waste to soil significantly increased the population of *Pseudomonas* and *Bacillus* species, which are known chlorpyrifos degraders. Their genetic sequencing of soil samples confirms the "population explosion" that was counted via traditional plating. Gomes *et al*. (2020) in a review of bioremediation strategies, highlighted that plant-based bio-stimulants promote a "rhizosphere-like effect", even without living plants, sustaining higher microbial diversity than unamended bulk soil. Madaban (2021) specifically studying Nigerian soils, found that Moringa leaf extract acted as a prebiotic for soil microflora, increasing bacterial counts by 2 logs (100-fold) within 21 days, which supports the magnitude of growth observed in this study. Hydrogen peroxide amended soil where the lowest microbial population was recorded, indicated that while the chemical cleaned the soil, it also significantly reduced the population of microorganisms.

Conclusions

Moringa oleifera leaf powder is an effective bio-stimulant for the rapid degradation of chlorpyrifos in savannah soils. The capability of this local plant resource to eliminate over 80% of pesticide residues within ninety days demonstrates that expensive, imported synthetic remediators are not a prerequisite for effective environmental cleanup in the Agriculture region. Also, it may be proposed that the mechanism driving this remediation is biological stimulation. The Moringa leaves act as a catalyst, waking up the invisible biological workforce of the soil. This confirms that the indigenous microbes in soils have the genetic potential to degrade pollutants if they are provided with the right nutritional support, which Moringa supplies in abundance.

Declarations of Authors Contribution

AF Conceptualized the study. AF designed the study, AF participated in the fieldwork and data collection. AF performed the data analysis. AF and SHA interpreted the data. AF and SHA prepared the first draft of the manuscript, reviewed by SHA and AKL. All authors contributed to the development of the final manuscript and approved its submission.

Conflict of interest

None

Ethics Approval and Informed Consent

This study did not involve human or animal subjects. Therefore, ethical consideration was not applicable.

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