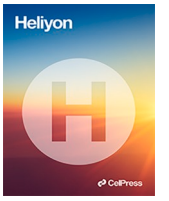
Heliyon 9 (2023) e23133

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Enhanced bioremediation of pesticides contaminated soil using organic (compost) and inorganic (NPK) fertilizers

Tega Lee-Ann Ataikiru [a,*], Chinyere Augusta Ajuzieogu [b]

[a] Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria
[b] Department of Microbiology, Federal University Otuoke, Bayelsa State, Nigeria

ARTICLE INFO

Keywords:
Bioremediation
Biostimulation
Compost
NPK
Pesticides
Soil microorganisms

ABSTRACT

This research examined the bioremediation of pesticides (Carbofuran and Paraquat) contaminated farmyard soil using compost and Nitrogen, Phosphorus, and Potassium (NPK) fertilizer. Microcosms representing each treatment were set-up in triplicates. Biostimulation was done using two concentrations (0.5 % and 1.0 % w/w) of NPK fertilizer and compost, following pesticides application at recommended rates [Carbofuran (1 g/kg) and Paraquat (5 ml/kg)] and four times the recommended rates. Two control soils were set-up; Abiotic control (sterile farmyard soil + pesticide) and Control (farmyard soil without treatment). Monitoring of the dynamics in microbial community abundance, and pesticide residues during the biostimulation period was done weekly for 28 days, using standard enumeration method, and High Performance Liquid Chromatography (HPLC), respectively. At the end of the monitoring period, considerable reduction in pesticide residues across the treatment set-ups was recorded. In Carbofuran-treated soils, there were no complete, but considerable losses in residual pesticide, however, in most of the Paraquat-treated soils, there were complete losses within 21 days. Lower pesticide residues were recorded in set-ups amended with compost than NPK, across both Carbofuran and Paraquat-treated soils. After pesticides application, decreases in microbial counts were recorded at Day 7 across all the treatments, followed by increases from Day 14–21, then decreases at Day 28. Microbial counts were lower in Carbofuran than in Paraquat-treated soils irrespective of nutrient (compost and NPK) amendments. Bacterial and fungal counts were in the magnitude of 10^6 and 10^5 CFU/g soil, respectively. Also, increased counts were recorded for Actinomycetes, Nitrifiers, Phosphate solubilizers across all treatments, and were in magnitude of 10^3–10^4 CFU/g soil. Soil microorganisms could breakdown and eliminate large concentrations of Carbofuran and Paraquat in compost-amended soils than in NPK-amended soils. This study suggests that bioremediation of pesticides contaminated soils can be achieved and enhanced by stimulating the indigenous microbial community with requisite nutrients (compost).

1. Introduction

One of the major global challenges is soil pollution. Soil pollution originates from both natural means and man-made activities like industrialization, urbanization and others. Increase in these man-made activities and food demand have led to increase in the demand and use of chemical substances and other compounds, which have consequently resulted to the age long environmental distribution

* Corresponding author.
E-mail address: ataikiru.tega@fupre.edu.ng (T. Lee-Ann Ataikiru).

https://doi.org/10.1016/j.heliyon.2023.e23133
Received 19 January 2023; Received in revised form 25 November 2023; Accepted 27 November 2023
Available online 1 December 2023

and accumulation of these pollutants (pesticides, hydrocarbons, heavy metals, and others) [1].

Specifically, pesticides (chemical or biological substances that weaken, incapacitate, repel and/or kill pests) serve as significant agro-agents that aid in reduction of economic losses resulting from pests (insects, weeds and others) and plant diseases, thus, enhancing the quality and quantity of global food production [1]. They act by interfering with an essential biological mechanism in the pests and because all living organisms have similar biological mechanisms, pesticides are never specific to a species. The ideal pesticide should be specific only to the target organism, be quick to acting, and degrade rapidly to harmless and inert materials in the environment [2]. However, long-term use and inadequate application of these pesticides results to adverse pollution of the environment and high health risk to public health, animals and plants, which happens to be non-target organisms [3]. In addition, even long after post-pesticide application, a portion of the pesticides remain (i.e. pesticide residue) in the environment (i.e. soil or sediment), and affect soil microorganisms negatively. Bound pesticide residue refers to the portion of pesticides that cannot be readily removed from the soil without modifying the chemical structure of the primary pesticides or its metabolites. Also, these pesticide residues can infiltrate groundwater or directly affect the food chain, resulting to health challenges [1]. Owing to these facts, there should be reduction in the concentration of pesticide residues available in the environment, and to achieve this, efficient remedial strategies have to be employed [1]. Thus, research to provide various methods for effective clean-up of these chemicals is ongoing.

The main chemical classes of pesticides are organochlorines, organophosphates, carbamates, pyrethroids, triazines and sulfonylureas [1]. These chemicals are classified based on type of pests they control, such as; those for controlling insects, herbs, fungus, rats, fishes, bacteria and nematodes called insecticides, herbicides, fungicides, rodenticides, piscicides, bactericides and nematicides, respectively [4].

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate [C12H15NO3]) is a carbamate insecticide that disrupts the nervous system by inactivating acetylcholinesterase. It is one of the mostly used insecticides in arable soils, gardening, horticulture, etc. [1]. Paraquat (1,1-dimethyl-4-4-bipyridinium) also known as Paraquat dichloride is a slightly toxic compound broadly used herbicide in arable and non-arable settings. It acts by inhibiting the photosynthetic process in plant and produces a highly reactive superoxide free radical [5].

Several biological and physico-chemical approaches have been employed in the clean-up of these toxicants. However, biological techniques are employed more often than the physico-chemical due to its cost-effectiveness, eco-friendliness, versatility, and the ability to reduce the toxicity and concentration of a broad spectrum of pollutants [6]. The application of biological methods relies on appreciating the mechanisms of biodegradation capabilities of the autochthonous microbial communities in transforming or completely mineralizing these organic compounds into water and carbon dioxide, and in some cases phosphates, halides, ammonium and others [6, 7]. The bio-transformed or bio-degraded pesticide is used as a carbon, nitrogen, mineral source or a final electron acceptor in electron transport chain reaction. Microorganisms can degrade several of these substances while others that cannot be degraded and remain in the environment are termed recalcitrant [6, 8]. Field application of bioremediation techniques have recorded success, thus gaining more acceptability and significance globally.

Bioremediation involves using naturally occurring soil microorganisms and/or plants to breakdown or degrade organic wastes into less toxic compounds. It employs strategies like bioaugmentation, biostimulation, biopiling, bioventing, bioreactors, biosparging, and land farming [9]. While the bioremediation process is ongoing, the microorganisms use the pesticides as co-substrates in their metabolism coupled with other nutrients, consequently eliminating the pesticides [1]. Bioremediation of pesticides utilizing biodegradation abilities of microorganisms includes the natural attenuation or enhanced natural attenuation process, either by addition of selected microorganisms (bioaugmentation) or by biostimulation, where nutrients are added [6]. Addition of organic and inorganic nutrients (biostimulants) have the capacity to improve soil nutrients, soil aeration and bioavailability of the pesticides, which are requisite factors for speedy microbial degradation of the pesticides and consequent bioremediation of the contaminated soil.

Composting is an eco-friendly biostimulation method that has been employed successfully in bioremediation of pesticides-contaminated environments [10]. It involves microbial degradation of organic matter under oxic conditions to obtain a stable substance (compost) utilized as organic fertilizer. Using compost for bioremediation, enhances and hastens the rate of pesticide breakdown by the pesticide-degrading organisms, due to the already established thermophilic temperatures during composting [11].

Several research frontiers have also demonstrated and reported success in biodegradation of contaminated soils using NPK fertilizer as a biostimulant [12]. Significant increase in degradation rates of herbicides and hydrocarbons are some of the positive effects recorded from the addition of NPK fertilizer as a biostimulant [12].

Biodegradation of carbofuran contaminated soils has been proven to be the most effective, eco-friendly and sustainable approach for the breakdown and detoxification of carbofuran [13], reported significant reductions in carbofuran and paraquat concentrations by the indigenous microbial population in the carbofuran and paraquat contaminated farmyard soil used in their study [14]. conducted a comparative bioremediation of carbofuran contaminated soil by bioaugmentation (*Burkholderia cepacia* PCL3) and biostimulation (using sludge from a production process of renewable energy) and revealed that combined bioaugmentation and biostimulation resulted to effective degradation of carbofuran, with the shortest half-live of 2.20–4.90 days in soil. Bacterial species from the genera *Enterococcus*, *Sphingomonas*, *Flavobacterium*, *Pseudomonas*, *Bacillus*, *Achromobacter*, and others, have been recovered from carbofuran-contaminated environments [15–17]. On the other hand, *Cupriavidus* sp. ISTL, *Enterobacter* sp. and *Sphingbium* sp. CFD-1 have been characterized as carbofuran degraders [17–19]. These bacterial species, alongside their fungal counterparts (*Tremetis versicolor*, *Aspergillus niger*, and others) [20] express excellent degrading potentials via an array of mechanisms including hydrolyzing enzymes, catabolic genes, metabolic pathways; all of which allow them evolve more efficient degradation characteristics [16].

Also, bacterial species like *Sporohalobacter orenetal* BCK-3, *Oscillospira* sp. BCK1, and *Clostridium prazmowski* BCK-2, have been reported to efficiently degrade paraquat up to 86.22, 79.35, and 80.26 %, respectively, after 3 days of treatment [21].

In Nigeria, several factors like paucity of studies on pesticide residue analysis with appropriate state-of-the-art equipment in order

to effectively monitor the biodegradation of pesticides in the environment, among others, have seriously hindered the effective control of pesticides pollution including the West-African sub-region resulting in instances of pesticide poisoning and pesticide related deaths, soil degradation and pollution of the environment [22]. Hence, this study focused on assessing the bioremediation of pesticides-contaminated soils via the biostimulation approach, by effectively monitoring the dynamics in microbial community abundance and pesticide residues during the biodegradation period.

2. Materials and methods

2.1. Study area

The study was conducted at the Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria, with coordinates (7° 23' N; 3° 51'E) and lies within 26.7 m above mean sea level.

2.2. Sample collection

Farmyard topsoil samples were obtained from three different locations with no history of pesticide application, at the study area, as shown in Fig. 1. These locations had similar ecological conditions. The soils were mostly sandy loam at the top, to brown loamy sand sub soil and well drained. The soil samples were pooled together, homogenized properly and transported to the laboratory for physico-chemical and preliminary microbiological analysis. Pesticides (Carbofuran and Paraquat) were purchased from local retailers in Warri, Delta State. The pesticides were chosen because of their popular use in potato, maize, groundnuts farming and flower lawns in the Niger Delta region.

2.3. Physicochemical characterization of soil and pesticides

Physical and chemical properties (pH, particle size, soil organic matter, soil organic carbon, total nitrogen, phosphorus, electrical conductivity, total exchangeable bases, calcium, magnesium, sodium and potassium) of freshly homogenized soil sample was characterized before the addition of pesticides, following the standard methods.

The pH of the soil samples was determined using pH meter (Metler Toledo Seven compact series). Soil particle size was determined according to the modified hydrometer method of [23]. Soil moisture content was measured by dry oven method [24]. Five grams (5 g) of sample was measured into an already weighed Petri dish (a). The sample was transferred in a Petri dish into the oven and allowed to stand for 1 h at 105 °C. After this, the sample was allowed to cool in a desiccator and weighed (b). Percentage moisture is given as:

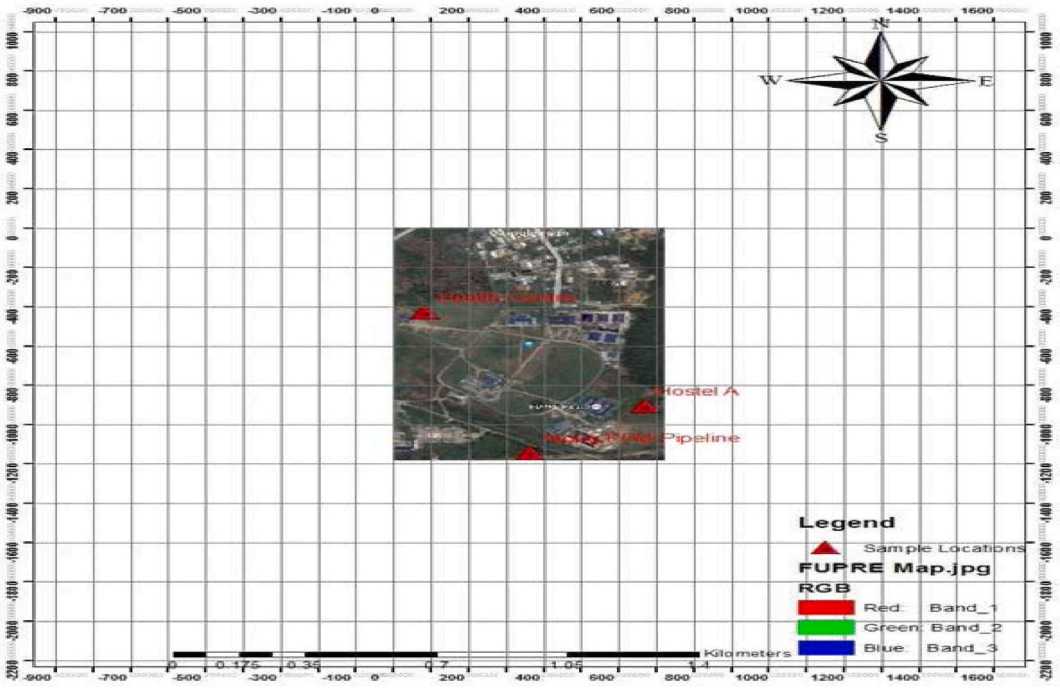


Fig. 1. Soil sample collection sites.

$$\% \text{ Moisture} = \frac{a - b}{Sample\ weight}$$

Soil Electrical conductivity (EC) was ascertained by weighing 5 g of each sample into a clean beaker, adding 50 ml of distilled water and homogenizing properly using a stirrer or glass rod. The calibrated conductivity meter was submerged in the samples to determine the conductivity.

Soil percentage carbon and organic matter were determined by Ref. [25]. Soil organic carbon (SOC) was estimated by oxidation with potassium dichromate and titration with ferrous sulphate using diphenylamine as indicator. Soil organic matter (SOM) was calculated from SOC from the equation: % SOM = % OC × 1.724. At end point of the titration, the color of the indicator will change from violet to green.

The total nitrogen content was measured by the Kjeldahl method [26]. One gram (1 g) of the soil sample was weighed and introduced into the Kjeldahl flask. A selenium catalyst was added and 20 ml of concentrated H_2SO_4 was also added to the mixture. The digestion flask with the mixture was heated in the DK20 heating digester block beginning with a temperature of 80 °C and later 350 °C. The contents of the digestion vials were heated until the volume was reduced to 3–4 ml. The contents of the digestion flask were cooled and the volume made up to 100 ml in a volumetric flask. Ten (10) milliliters of each sample extract was pipetted into a Kjeldahl, to which 20 ml of 40 % NaOH was added and distilled. The distillate was collected (in a 250 ml conical flask) over 10 ml of 4 % boric acid and three drops of indicator added and allowed to stand for 5 min. The presence of nitrogen gave a light blue color. Two hundred milliliters (200 ml) of the distillate was titrated with 0.1 N HCl until the color changed from light blue to gray and suddenly turned pink. A blank titration was conducted on the sample solution. The weight of nitrogen was calculated as follows: 14 g of nitrogen contained in a milliequivalent weight of ammonia (Black, 1965). The percentage of nitrogen in the sample was calculated as follows:

$$\text{Total N}\% = \frac{14\ (A - B) \times acid\ conc \times 100}{1000\ \times\ l}$$

Where, A = volume of standard HCl used in the titration of the sample

B = volume of standard HCl used in the blank titration

14: atomic weight of nitrogen

l = mass of the sample in grams.

Available phosphorus was determined by weighing soil sample (5 g) was into the plastic bottle and adding 40 ml of the extracting solution. This was homogenized for 1 min and filtered with Whatman filter paper No. 42. Three to five drops of conc. H_2SO_4 was added and left to stand for about 2 h. The clear supernatant was decanted. The filtrate or supernatant was then measured for available phosphorus.

The organic carbon content was measured using the wet dichromate oxidation method. A portion of soil (0.05 g) was measured in an Erlenmeyer flask. Ten milliliters of concentrated sulfuric acid, 10 ml of 0.1667 M K2Cr207 and 10 ml of orthophosphoric acid were added. After the addition of 200 ml of distilled water, the solution was left to stand for 30 min on an asbestos sheet and titrated again with solutions of FeSO 0.333 M with the diphenylamine indicator [27].

For total exchangeable bases, 5 g of air-dried soil was weighed into a plastic bottle, 100 ml of neutral 1 M ammonium acetate was added. The mixture was shaken mechanically for 30 min and filtered into a 100 ml volumetric flask using a No 42 Whatman filter paper. This was made up with the acetate to the mark. Na (589-nm wavelength) and K (766.5 nm wavelength) were determined with a Flame Photometer, while Ca and Mg by Atomic Absorption Spectrophotometer. Ten to 20 mL of soil saturation extract was pipetted, having not more than 1.0 meq Ca, into a 250-ml Erlenmeyer flask. This was diluted to 20–30 ml with distilled water; and 2–3 ml 2 N NaOH solution was added; and about 50 mg ammonium purpurate indicator. This was titrated with 0.01 N EDTA. The color change was from red to purple. Near the end point, EDTA was added, one drop every 10 s, since the color change was not instantaneous. For Magnesium measurement, 10–20 mls soil saturation extract was pipetted into a 250-ml flask, and diluted to 20–30 ml with distilled water. Then 5 ml buffer solution was added, and a few drops of Eriochrome Black Indicator. This was titrated with 0.01 N EDTA until the color changed from red to blue. For measurement of calcium, 50 mls of the sample filtrate was taken and 0.1 N HCI was added to decompose bicarbonates. It was then boiled to expel CO_2 after which it was cooled and 2 ml of NaOH was added to produce a pH of 12–13, Miroxide indicator was added and then the solution was slowly titrated with EDTA and continous stirring to the proper end point. The solution changed from pink to purple. The concentration of calcium calculated.

For heavy metals analysis, soil samples were oven-dried at 70–80 °C for 24 hrs, to remove all moisture. Dried samples were milled into a fine powder of 80 μm. Following this, 1.0 g of the dried sample was measured into a digestion tube and 10 ml of 98 % nitric acid was added. This was then placed in a water bath and allowed to boil for about 72 h after which it was allowed to cool and the content transferred into a 100 cm^3 volumetric flask and made-up to the volume mark with water. The solution was used for determination of mineral elements. The solution was also analyzed for arsenic, cadmium, lead, and mercury using Atomic Absorption Spectrophotometer (AAS, PerkinElmer model 2130).

The pesticides (Carbofuran and Paraquat) were characterized using a gas chromatography-mass spectroscopy (GC-MS) [28]. The different compounds were identified after preparing and digesting samples using the gas chromatography-mass spectrophotometer Model 7820 (Agilent instruments, USA) based on mass to charge ratio.

2.4. Preliminary microbiological analysis of soil

Population of the indigenous total heterotrophic bacteria, fungi, actinomycetes, phosphate solubilizers and nitrifying bacteria were

enumerated before treatment (pesticide and nutrient application) was done. Serial dilution was done using one (1) gram of soil sample suspended in 9 ml of sterile physiological saline. Aliquots (0.1 ml) of the dilutions were plated out using appropriate media for the enumeration of microorganisms. Rose-Bengal (Sigma Aldrich) fortified with chloramphenicol agar (0.1g/1 L) was used for the enumeration of fungi [29]. Plate count agar (PCA) (Sigma Aldrich, Steinheim, Germany) fortified with griseofulvin (50 μl/ml) was used for the enumeration of heterotrophic bacteria [30]. Actinomycetes were enumerated using starch-casein agar (Sigma Aldrich) [31] and Pikovskaya's medium for phosphate solubilizing microbes [29]. Ashby agar was used to enumerate nitrogen fixers [32]. Plate Count Agar plates were incubated at room temperature for 24 hrs, Fungi plates were incubated at room temperature for 3–5 days, Actinomycetes plates were incubated at similar temperature for 48 hrs, Phosphate solubilizers plates were incubated at similar temperature for 5days and nitrogen fixers were incubated at similar temperature for 7 days. After incubation, inoculated plates were observed for growth and cultural characteristics of the isolates were recorded. All discrete were enumerated and expressed in colony forming units per gram (CFU/g).

2.5. Biostimulation studies and biodegradation monitoring

The modified methods of [33,34] were adopted for biostimulation and modified method of [35] was adopted for biodegradation. Pot experiment (using nursery bags) in replicates was used for the study. One (1) kg of homogenized farmyard soil was weighed into each nursery bag, spiked with pesticides at recommended rates [Carbofuran (1 g/kg) and Paraquat (5 ml/kg)] and four times the recommended application rates. The soils were further amended with compost (organic fertilizer) and NPK (inorganic fertilizer), both at 0.5 % and 1.0 % w/w. Also, an abiotic control containing sterile soil with pesticides was set up. Abiotic control set-up received biocide (15 g silver nitrate) weekly. Sample codes and descriptions for soil treatments are displayed in Tables 1 and 2. The nursery bags were perforated and tilled weekly for soil aeration, and watered weekly for soil moisture. The set-ups were left under shade at 28±2 °C for 28 days. Sampling was done weekly to monitor pesticides degradation by soil microorganisms using the High Performance Liquid Chromatography (Dionex HPLC Model). Dynamics in microbial community abundance was ascertained via culture dependent method.

2.6. Pesticide residues analysis during biodegradation

Pesticide residue in soil after pesticides' application at recommended and four times recommended rates were assessed using High Performance Liquid Chromatography (HPLC) [36]. The soil samples were dried to a constant weight in an oven at 40 °C. Twenty grams (20 g) of Carbofuran-contaminated soil was weighed into a 100 ml beaker. Twenty milliliters (20 ml) of hexane was subsequently added to the sample and extraction was done for 25 min ultrasonic extraction. After that, the extract was decanted and filtered through a piece of Whatman filter paper (filled with approximately 1 g of anhydrous sodium sulphate [Na_2SO_4]) into a 20 ml vial. The filtrate was gently evaporated to dryness using nitrogen gas and reconstituted with 1 ml acetonitrile. Concentration of the pesticide was determined using a Dionex HPLC. Ten grams (10 g) of Paraquat-contaminated soil was weighed into a 100 ml beaker. Ultrasonic extraction was done by adding 10 ml of methanol:water (80:20) to soil for 25 min. After that, the extract was decanted and separated with a piece of Whatman filter paper into a 20 ml vial. Paraquat concentration was determined using Dionex HPLC. Aside this analysis, spiking was done to check for accuracy of the method. This was done by adding standards with concentrations between 1 and 200 μg/ml the percentage of the recovery studies was calculated for each sample thus;

$$\%Recovery = \frac{Cs_2 - Cs_1 \times 100\%}{C_5}$$

Where Cs_1 is the concentration of pesticide in soil sample

Cs_2 is the concentration of pesticide in soil sample + Standard

C_5 is the concentration of pesticide present in the original standard

Table 1
Sample codes and description for Carbofuran-treated soils.

S/N	Sample code	Description
1.	T1 (Control)	Farmyard soil without treatment
2.	CT2 (Abiotic control)	Sterile farmyard soil + carbofuran
3.	CT3	Farmyard soil + 1 g/kg carbofuran
4.	CT4	Farmyard soil +4 × (1 g/kg) carbofuran
5.	CT5	Farmyard soil + 1 g/kg carbofuran +0.5 % w/w compost
6.	CT6	Farmyard soil + 1 g/kg carbofuran +1 % w/w compost
7.	CT7	Farmyard soil +4 × (1 g/kg) carbofuran + 0.5 % w/w compost
8.	CT8	Farmyard soil +4 × (1 g/kg) carbofuran + 1 % w/w compost
9.	CT9	Farmyard soil + 1 g/kg carbofuran +0.5 % w/w NPK
10.	CT10	Farmyard soil + 1 g/kg carbofuran +1 % w/w NPK
11.	CT11	Farmyard soil +4 × (1 g/kg) carbofuran + 0.5 % w/w NPK
12.	CT12	Farmyard soil +4 × (1 g/kg) carbofuran + 1 % w/w NPK

Table 2
Sample codes and description for Paraquat-treated soils.

S/N	Sample code	Description
1.	T1 (Control)	Farmyard soil without treatment
2.	PT2 (Abiotic control)	Sterile farmyard soil + paraquat
3.	PT3	Farmyard soil + 1 g/kg paraquat
4.	PT4	Farmyard soil +4 × (1 g/kg) paraquat
5.	PT5	Farmyard soil +1 g/kg paraquat +0.5 % w/w compost
6.	PT6	Farmyard soil +1 g/kg paraquat +1 % w/w compost
7.	PT7	Farmyard soil +4 × (1 g/kg) paraquat + 0.5 % w/w compost
8.	PT8	Farmyard soil +4 × (1 g/kg) paraquat + 1 % w/w compost
9.	PT9	Farmyard soil + 1 g/kg paraquat +0.5 % w/w NPK
10.	PT10	Farmyard soil + 1 g/kg paraquat +1 % w/w NPK
11.	PT11	Farmyard soil +4 × (1 g/kg) paraquat + 0.5 % w/w NPK
12.	PT12	Farmyard soil +4 × (1 g/kg) paraquat + 1 % w/w NPK

2.7. Microbiological analyses during biodegradation monitoring

Enumeration of viable microbial counts from the pooled soil sample was done and recorded first, before pesticide application and nutrient amendments at Day 0. Subsequently, after pesticide application and nutrient amendment, viable microbial counts were done weekly from Day 7 to Day 28 via conventional methods. One gram (1 g) of each treated soil sample was suspended in 9 ml of sterile distilled water, and serial dilutions were done. Aliquots (0.1 ml) of the dilutions were plated out in duplicates using appropriate media for the enumeration of microorganisms. Rose-Bengal (Sigma Aldrich) fortified with chloramphenicol agar (0.1g/1 L) was used for the enumeration of fungi [29]. Plate count agar (PCA) (Sigma Aldrich, Steinheim, Germany) fortified with griseofulvin (50 μl/ml) was used for the enumeration of heterotrophic bacteria [30]. Actinomycetes were enumerated using starch-casein agar (Sigma Aldrich) [31] and Pikovskaya's medium for phosphate solubilizing microbes [29]. Ashby agar was used to enumerate nitrogen fixers [32]. Plate Count Agar plates were incubated at room temperature for 24 hrs, Fungi plates were incubated at room temperature for 3–5 days, Actinomycetes plates were incubated at similar temperature for 48 hrs, Phosphate solubilizers plates were incubated at similar temperature for 5days and nitrogen fixers were incubated at similar temperature for 7 days. After incubation, inoculated plates were observed for growth and cultural characteristics of the isolates were recorded. All discrete were enumerated and expressed in colony forming units per gram (CFU/g).

Table 3
Physicochemical characteristics of soil.

Physicochemical Parameters	Value
Electrical conductivity (μs/cm)	144
pH	6.70
Total organic carbon (TOC) (%)	3.316
Total nitrogen (%)	0.3029
Nitrates (mg/kg)	36.28
Phosphates (mg/kg)	26.32
Moisture content (%)	19.41
Calcium (meq/100 g)	61.83
Magnesium (meq/100 g)	22.15
Sodium (meq/100 g)	0.97
Potassium (meq/100 g)	1.03
Arsenic (mg/kg)	0.215
Mercury (mg/kg)	<0.001
Lead (mg/kg)	19.25
Cadmium (mg/kg)	0.725
Soil particle size distribution (%)	
Silt	2.65
Sand	97.192
Clay	0.158
Microbiological analysis	
Microorganisms	Microbial counts (CFU/g)
Total heterotrophic bacteria	$6.3 \times 10^7 \pm 8.48$
Fungi	$1.41 \times 10^5 \pm 2.82$
Actinomycetes	$1.99 \times 10^4 \pm 4.24$
Nitrifying bacteria	$1.45 \times 10^4 \pm 2.82$
Phosphate solubilizers	$1.52 \times 10^4 \pm 4.24$

3. Results and discussion

3.1. Physical, chemical and microbiological characteristics of soil, and pesticides

The physico-chemical and microbiological characteristics of the pooled soil are shown in Table 3, while the chemical properties of the pesticides used in this study are presented in Table 4. Soil pH (6.7) was normal (6–8.5), with an Electrical conductivity of 144 μs/cm. Total organic carbon content was 3.316 %, nitrates, phosphates and moisture content were 36.28 mg/kg, 26.32 mg/kg and 19.41 % respectively. The soil had appreciable concentrations of exchangeable cations (Ca^{+}, Mg^{+}, Na^{+}, K^{+}) and lead (19.25 mg/kg), and slight concentrations of cadmium (0.725 mg/kg) and arsenic (0.215 mg/kg).

Population counts for total culturable heterotrophic bacteria, fungi, actinomycetes, nitrifying bacteria and phosphate solubilizers were $6.3 \times 10^{7} \pm 8.48$, $1.41 \times 10^{5} \pm 2.82$, $1.45 \times 10^{4} \pm 4.24$, and $1.52 \times 10^{4} \pm 4.24$ CFU/g respectively.

Paraquat contained 2-amino - 1- propanol, 4,4'- bipyridine, 3, 3'-bipyridine, paraquat dichloride, benzene, bromo-benzene, neopentane and dimethyl-diazene.

Carbofuran contained over thirty (30) different compounds including tetradecane, oxalic acid, allyl pentadecyl ester, 2-aminono-nadecane, 2,6- pyrazinediamine, n-nonadecanol-1, 1-ecosanol, nonadecane, isobutyl nitrite, heptanonitrile, carbofuran and others.

3.2. Pesticide residues during biodegradation monitoring

Results of pesticides residues during biodegradation are displayed in Fig. 2. There were decreases in Carbofuran concentrations in treatments without nutrient amendment, from 108.6 ± 0.69 μg/kg (at day 0) to 39.2 ± 3.8 μg/kg (at day 28) at the recommended rate (1 g/kg) while it reduced from 301.4 ± 1.29 μg/kg (at day 0) to 241.4 ± 2.83 μg/kg (at day 28) at four times the recommended rate. In set ups with nutrient amendment, carbofuran concentrations decreased from 108.6 ± 0.69 μg/kg (at day 0) to 22.00 ± 0.66 μg/kg (at day 28) in 0.5 % compost amended soils with recommended rates (1 g/kg) of carbofuran application, whereas it decreased from 108.6 ± 0.69 μg/kg (at day 0) to 34.60 ± 4.07 μg/kg (at day 28) in 0.5 % NPK amended soils with recommended rates (1 g/kg) of carbofuran application. At day 28, in 1 % compost amended soils at recommended and four times recommended carbofuran rates, biodegradation rates of the pesticide were more intensive than in 0.5 % compost amendment. Also, in 0.5 % and 1 % NPK amended soils at recommended and four times the recommended carbofuran rates, similar biodegradation trend was observed as with compost, however, treatments with compost enhanced greater biodegradation rates than with NPK.

On the contrary, in most treatments with recommended rate of Paraquat, there were total losses from soil within 21 days of

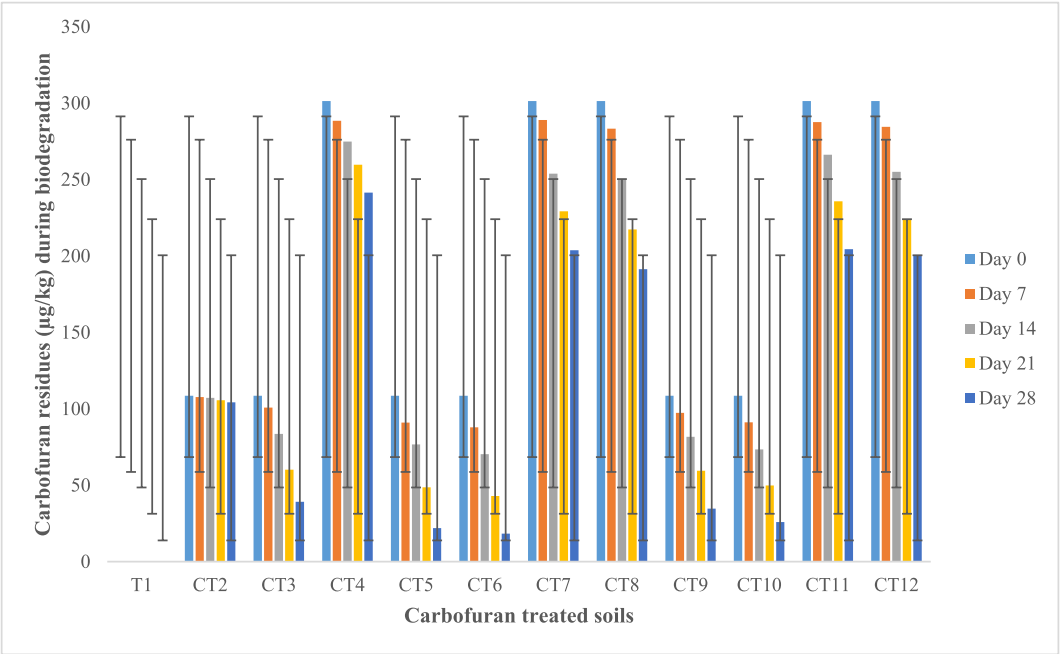


Fig. 2. Carbofuran residues (μg/kg) during biodegradation
Key: T1 - control, CT2 - abiotic control, CT3 - FS + RCP, CT4 - FS + 4RCP, CT5 - FS + RCP + 0.5 % compost, CT6 - FS + RCP + 1 % compost, CT7- FS + 4RCP + 0.5 % compost, CT8 - FS + 4RCP + 1 % compost, CT9 – FS + RCP + 0.5 % NPK, CT10 – FS + RCP + 1 % NPK, CT11 – FS + 4RCP + 0.5 % NPK and CT12 - FS + 4RCP + 1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * Treatments were done in duplicates. * **Results are in mean ± standard deviation**.

biodegradation monitoring, irrespective of the nutrient amendment used. However, in treatments with four times recommended rate, paraquat residues were observed, as shown in Fig. 3.

3.3. Microbiological analyses during biodegradation

After the application of pesticides, decreases in microbial counts for all the classes of microorganisms enumerated (total culturable heterotrophic bacteria, fungi, actinomycetes, phosphate solubilizers and nitrifiers) were recorded at day 7, followed by increases from day 14–21, then decreases at day 28 in all treatments. The microbial counts in paraquat-treated soils were greater than those in Carbofuran-treated soils, irrespective of the nutrient amendments. These results are displayed in Figs. 4–12 13.

4. Discussion

This study focused on assessing the bioremediation of pesticides-contaminated soils via the biostimulation approach, by effectively monitoring the dynamics in microbial community abundance and pesticide residues during the biodegradation period.

Results obtained from this study suggests that a level of biodegradation of the pesticides occurred in the contaminated soils without nutrient amendments, however, greater biodegradation rates of both Carbofuran and Paraquat were enhanced by stimulation of the indigenous microbial population with organic (compost) and Inorganic (NPK) nutrients. The level of biodegradation recorded without nutrient amendment could be as a result of natural attenuation, which is a natural process (that involves volatilization, biodegradation, dispersion, dilution, sorption and others) where pollutants are degraded by indigenous soil microorganisms [1]. The report of [37], on the microbial mineralization of two metabolites of insecticide endosulfan (endosulfan diol and endosulfan sulphate) in an endosulfan-contaminated soil, corroborates this finding. The contaminated soil was left to natural attenuation, thus resulting in biodegradation reported from the study. Also [38], in their study, reported that despite natural attenuation resulting in the biodegradation of atrazine (herbicide), the process was slow compared to the process of biostimulation and bioaugmentation. Successful biodegradation via the biostimulation approach has been reported for atrazine (after 35 days), tebuconazole, dichlorvos, and other organochlorine pesticides by Refs. [38–40].

Biodegradation rates in both pesticides-treated soils were better enhanced in soils amended with compost than those amended with NPK, resulting in presence of less pesticide residues in compost amended soils than in NPK amended soils. This agrees with the report of [10]. They reported that concentrations of carbamate and organophosphate pesticides are lower after composting [41]. also reported increased biodegradation of tebuconazole via compost amendment.

Furthermore, results from this study showed there were complete losses in most treatments with Paraquat within 21 days, however, significant but not complete losses were observed in Carbofuran treatments. This could be attributed majorly to the chemical

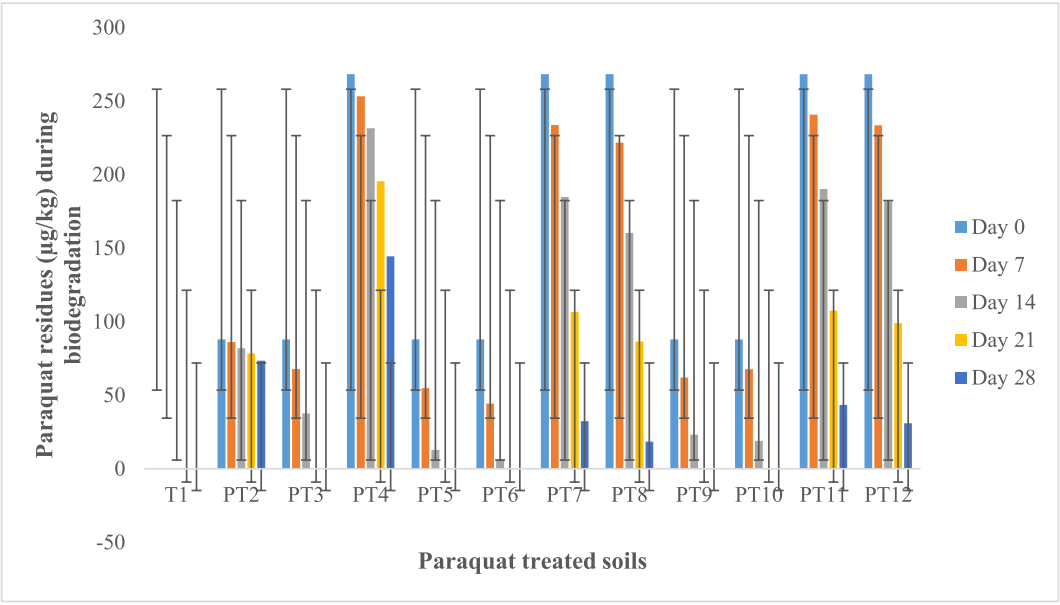


Fig. 3. Paraquat residues (µg/kg) during biodegradation
Key: T1 - control, PT2 - abiotic control, PT3 - FS + RCP, PT4 - FS + 4RCP, PT5 - FS + RCP + 0.5 % compost, PT6 - FS + RCP + 1 % compost, PT7 - FS + 4RCP + 0.5 % compost, PT8 - FS + 4RCP + 1 % compost, PT9 – FS + RCP + 0.5 % NPK, PT10 – FS + RCP + 1 % NPK, PT11 – FS + 4RCP + 0.5 % NPK and PT12 - FS + 4RCP + 1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * MDL = Minor Detection Limit (<0.00). *Treatments were done in duplicates. *** Results are in mean ± standard deviation.**

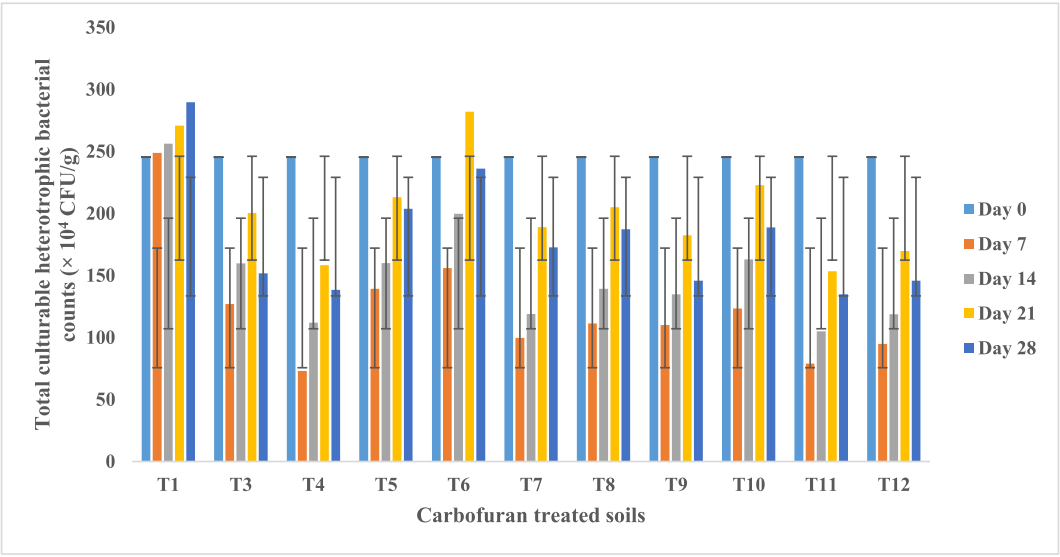


Fig. 4. Total culturable heterotrophic bacterial counts in Carbofuran-treated soil
Key:T1 - control, T3 - FS + RCP, T4 - FS + 4RCP, T5 - FS + RCP +0.5 % compost, T6 - FS + RCP +1 % compost, T7- FS + 4RCP +0.5 % compost, T8 - FS + 4RCP +1 % compost, T9 – FS + RCP +0.5 % NPK, T10 – FS + RCP +1 % NPK, T11 – FS + 4RCP +0.5 % NPK and T12 - FS + 4RCP +1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * **Results are in mean ± standard deviation**.

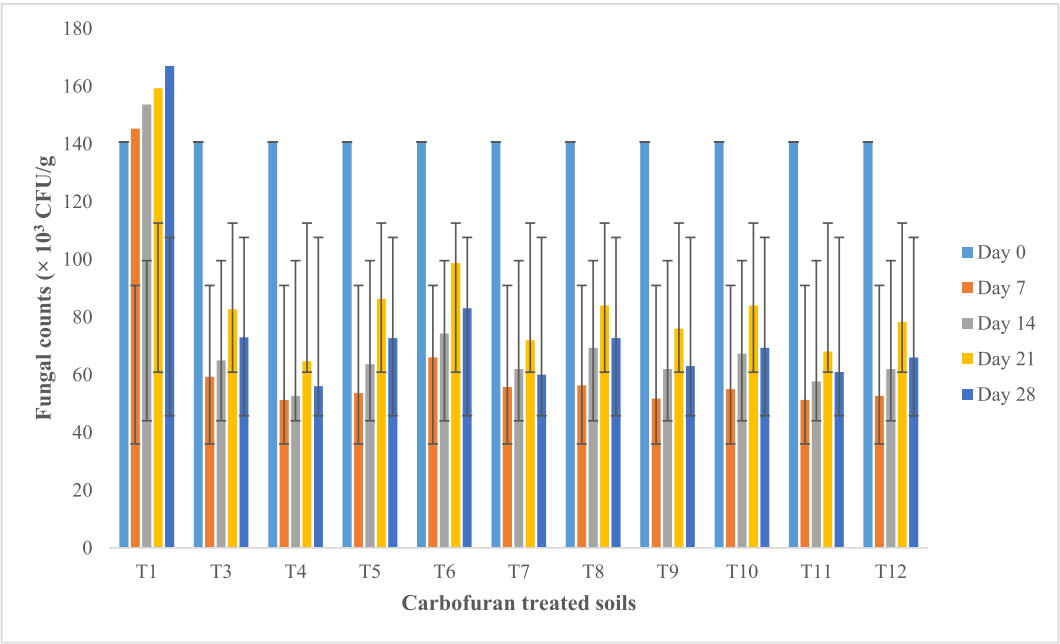


Fig. 5. Fungal counts in Carbofuran-treated soil
Key: T1 - control, T3 - FS + RCP, T4 - FS + 4RCP, T5 - FS + RCP +0.5 % compost, T6 - FS + RCP +1 % compost, T7- FS + 4RCP +0.5 % compost, T8 - FS + 4RCP +1 % compost, T9 – FS + RCP +0.5 % NPK, T10 – FS + RCP +1 % NPK, T11 – FS + 4RCP +0.5 % NPK and T12 - FS + 4RCP +1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * Treatments were done in duplicates. * **Results are in mean ± standard deviation**.

properties of carbofuran and paraquat, water solubility, and others [9]. Paraquat is slightly toxic compound and has less complex chemical constituents compared to Carbofuran (Table 4). Paraquat is also highly soluble in water which increases its availability to microorganisms for degradation, however, carbofuran (carbamate) has a complex chemical composition and is moderately soluble in water, which limits its availability to microorganisms for degradation [9, 42].

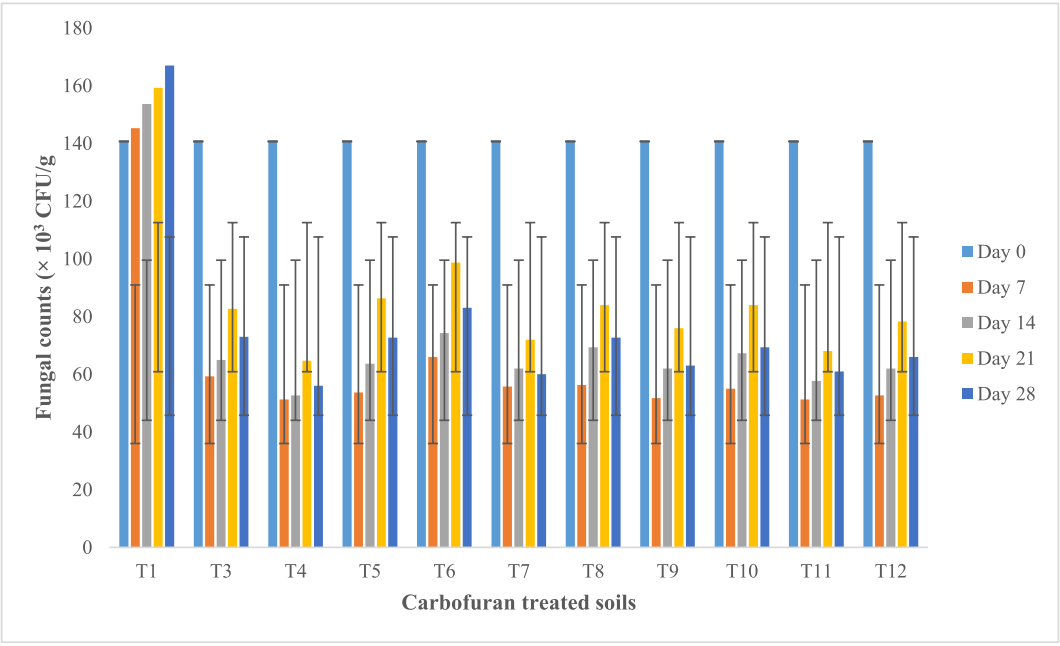


Fig. 6. Actinomycetes counts in Carbofuran-treated soil
Key: T1 - control, T3 - FS + RCP, T4 - FS + 4RCP, T5 - FS + RCP +0.5 % compost, T6 - FS + RCP +1 % compost, T7- FS + 4RCP +0.5 % compost, T8 - FS + 4RCP +1 % compost, T9 – FS + RCP +0.5 % NPK, T10 – FS + RCP +1 % NPK, T11 – FS + 4RCP +0.5 % NPK and T12 - FS + 4RCP +1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * Treatments were done in duplicates. * **Results are in mean ± standard deviation**.

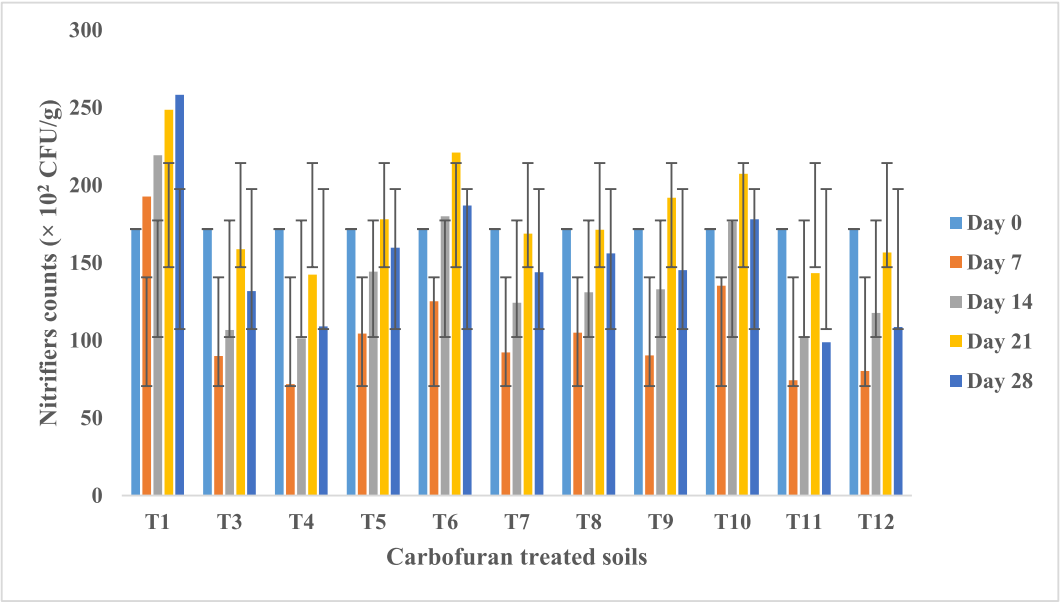


Fig. 7. Nitrifiers counts in Carbofuran-treated soil
Key: T1 - control, T3 - FS + RCP, T4 - FS + 4RCP, T5 - FS + RCP +0.5 % compost, T6 - FS + RCP +1 % compost, T7- FS + 4RCP +0.5 % compost, T8 - FS + 4RCP +1 % compost, T9 – FS + RCP +0.5 % NPK, T10 – FS + RCP +1 % NPK, T11 – FS + 4RCP +0.5 % NPK and T12 - FS + 4RCP +1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * Treatments were done in duplicates. * **Results are in mean ± standard deviation**.

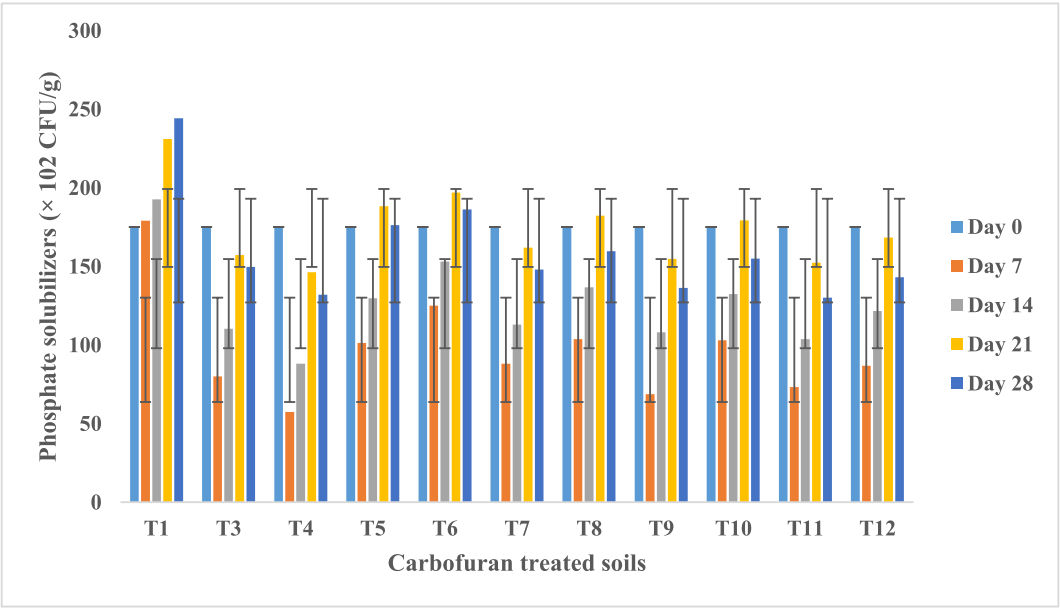


Fig. 8. Phosphate solubilizers in Carbofuran-treated soil
Key: T1 - control, T3 - FS + RCP, T4 - FS + 4RCP, T5 - FS + RCP +0.5 % compost, T6 - FS + RCP +1 % compost, T7- FS + 4RCP +0.5 % compost, T8 - FS + 4RCP +1 % compost, T9 – FS + RCP +0.5 % NPK, T10 – FS + RCP +1 % NPK, T11 – FS + 4RCP +0.5 % NPK and T12 - FS + 4RCP +1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * Treatments were done in duplicates. * **Results are in mean ± standard deviation**.

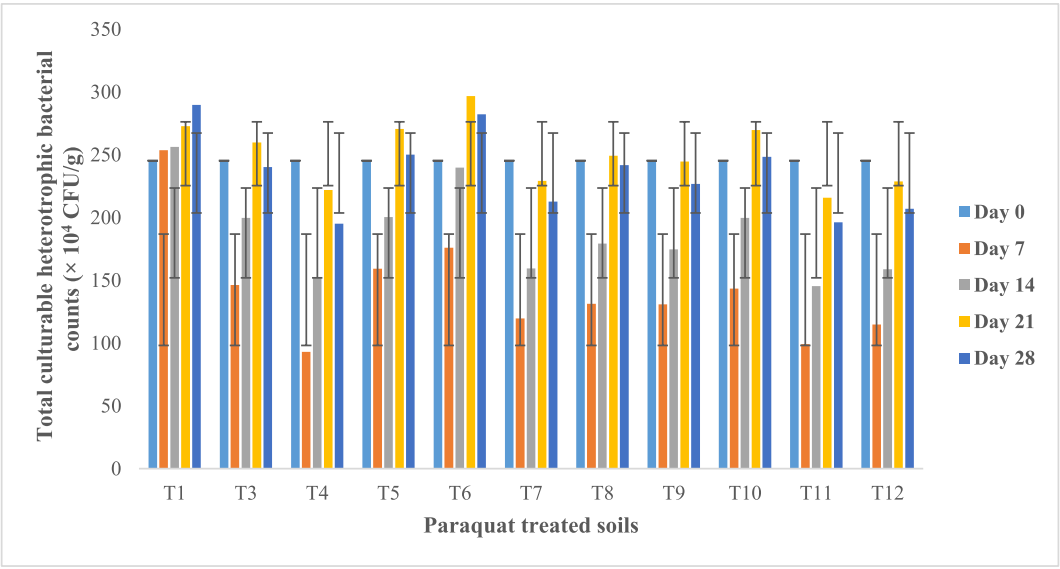


Fig. 9. Total culturable heterotrophic bacterial counts in Paraquat-treated soil Key: T1 - control, T3 - FS + RCP, T4 - FS + 4RCP, T5 - FS + RCP +0.5 % compost, T6 - FS + RCP +1 % compost, T7 - FS + 4RCP +0.5 % compost, T8 - FS + 4RCP +1 % compost, T9 – FS + RCP +0.5 % NPK, T10 – FS + RCP +1 % NPK, T11 – FS + 4RCP +0.5 % NPK and T12 - FS + 4RCP +1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * Treatments were done in duplicates. * **Results are in mean ± standard deviation**.

It is important to note that the physical and chemical properties of pesticides (viz; soil mineral particle sizes, organic matter chemical composition and structure, volatility, octanol/water partition coefficient, diffusivity in both air and water, and soil adsorption coefficient) are critical, as they influence the pesticide application process, its efficiency, its retention and *in situ* remediation/degradation potential [43,44]. Soils high in organic matter or clay are more partly adsorptive than coarse, sandy soils. Also, the extent to which clay minerals contribute to sorption is dependent on the nature of the pesticides and the quotient of clay mineral to

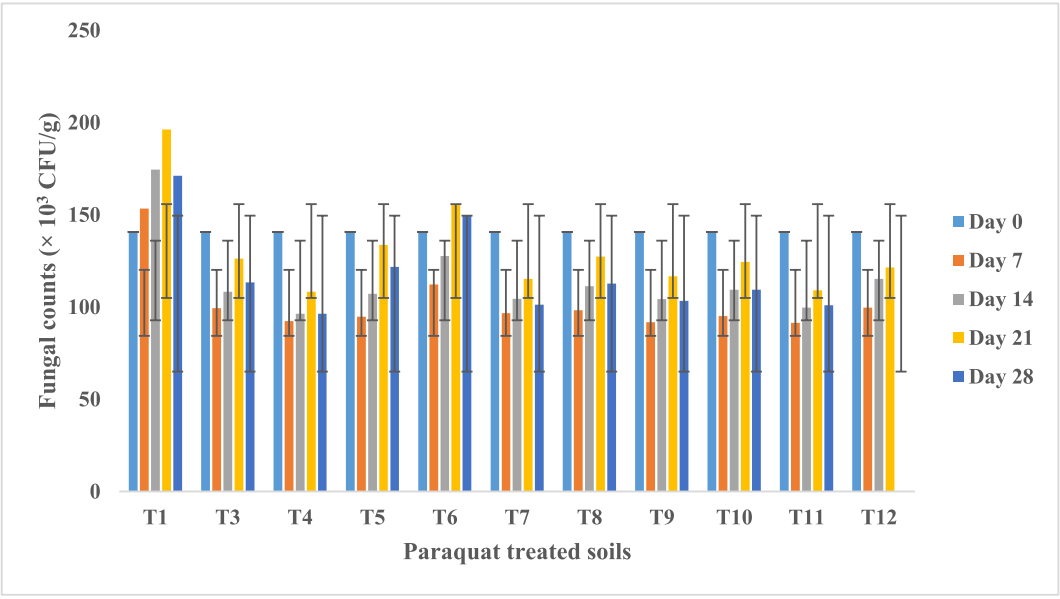


Fig. 10. Fungal counts in Paraquat-treated soil
Key:T1 - control, T3 - FS + RCP, T4 - FS + 4RCP, T5 - FS + RCP +0.5 % compost, T6 - FS + RCP +1 % compost, T7- FS + 4RCP +0.5 % compost, T8 - FS + 4RCP +1 % compost, T9 – FS + RCP +0.5 % NPK, T10 – FS + RCP +1 % NPK, T11 – FS + 4RCP +0.5 % NPK and T12 - FS + 4RCP +1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * Treatments were done in duplicates. * **Results are in mean ± standard deviation**.

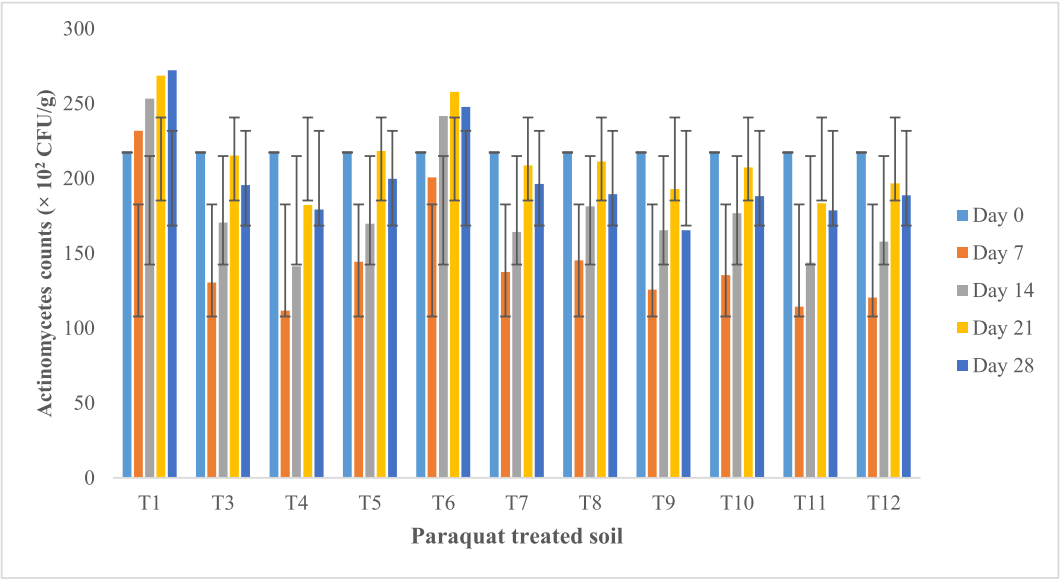


Fig. 11. Actinomycetes counts in Paraquat-treated soil
Key: T1 - control, T3 - FS + RCP, T4 - FS + 4RCP, T5 - FS + RCP +0.5 % compost, T6 - FS + RCP +1 % compost, T7 - FS + 4RCP +0.5 % compost, T8 - FS + 4RCP +1 % compost, T9 – FS + RCP +0.5 % NPK, T10 – FS + RCP +1 % NPK, T11 – FS + 4RCP +0.5 % NPK and T12 - FS + 4RCP +1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * Treatments were done in duplicates. * **Results are in mean ± standard deviation**.

organic carbon fractions of the soil [45]. Pesticides that do not adsorb (bind) to the soil particle will be degraded to produce less toxic metabolites, while adsorbed pesticides will persist in the surrounding for years and may accumulate into food chains so many years after use [46]. Carbofuran is soluble in water and highly mobile. It exhibits a moderate sorption to soil, depending on both clay material and organic matter content. It was reported that its sorption potential is weaker in sandy soils than in clay soils [47]. The soil sampled in this study belonged to the "sandy soil" textural class, therefore, is expected to have a weaker sorption to the soil particles,

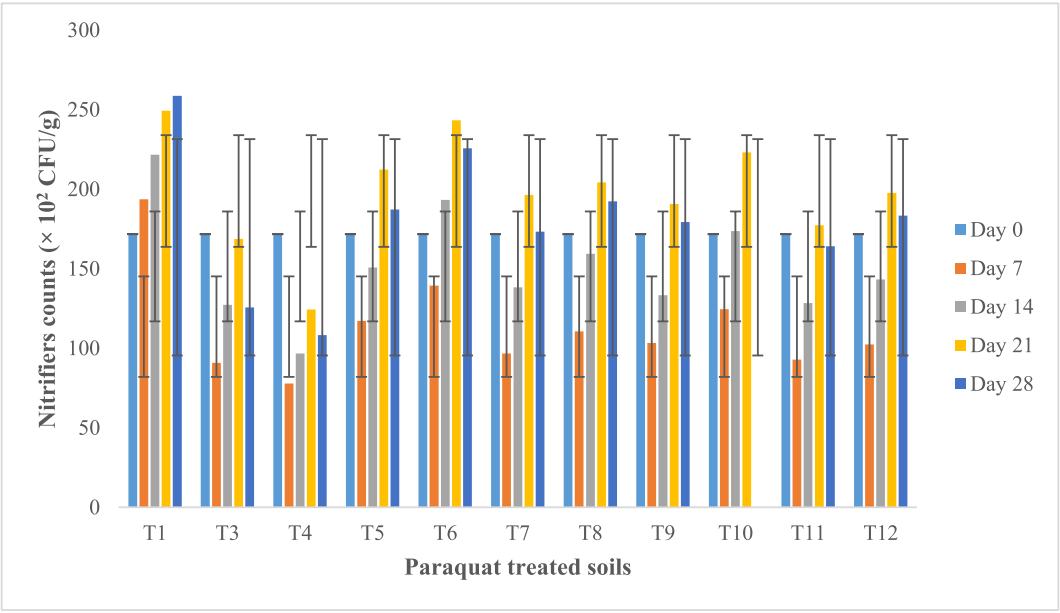


Fig. 12. Nitrifiers counts in Paraquat-treated soil
Key: T1 - control, T3 - FS + RCP, T4 - FS + 4RCP, T5 - FS + RCP +0.5 % compost, T6 - FS + RCP +1 % compost, T7- FS + 4RCP +0.5 % compost, T8 - FS + 4RCP +1 % compost, T9 – FS + RCP +0.5 % NPK, T10 – FS + RCP +1 % NPK, T11 – FS + 4RCP +0.5 % NPK and T12 - FS + 4RCP +1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * Treatments were done in duplicates. * **Results are in mean ± standard deviation**.

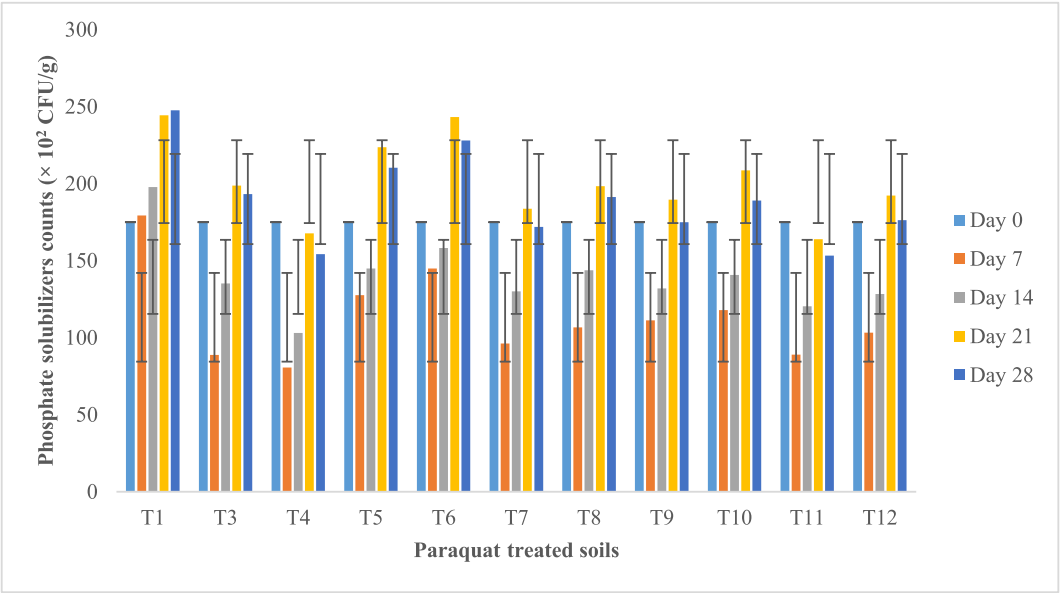


Fig. 13. Phosphate solubilizers counts in Paraquat-treated soil
Key: T1 - control, T3 - FS + RCP, T4 - FS + 4RCP, T5 - FS + RCP +0.5 % compost, T6 - FS + RCP +1 % compost, T7- FS + 4RCP +0.5 % compost, T8 - FS + 4RCP +1 % compost, T9 – FS + RCP +0.5 % NPK, T10 – FS + RCP +1 % NPK, T11 – FS + 4RCP +0.5 % NPK and T12 - FS + 4RCP +1 % NPK. FS- Farmyard soil, RCP - Recommended concentration of pesticide, 4RCP - four times the recommended concentration of pesticides, NPK - nitrogen, phosphorus and potassium fertilizer. * Treatments were done in duplicates. * **Results are in mean ± standard deviation**.

and should be available for microbial degradation. This could be the reason for the low levels of carbofuran residues observed in this study at the end of the monitoring period.

With respect to the influence of pesticides treatment and nutrients amendment on the dynamics in microbial population counts

representing the diverse microbial classes (total culturable heterotrophic bacteria, fungi, actinomycetes, nitrifying bacteria and phosphate solubilizers) enumerated during the biodegradation monitoring, results showed a general decrease in microbial counts at day 7, followed by increases from day 14–21, then decreases at day 28, across both pesticide treatments and nutrient amendments. However, from day 14–21 when increases were recorded, microbial counts in paraquat-treated soils were greater than those in Carbofuran-treated soils, irrespective of the nutrient amendments. Similar trend was recorded at day 7 and 28 when decreases in microbial counts were recorded. Also, from day 14–21, microbial counts in compost amendments were greater than those in NPK amendments across the pesticides-treated soils. On the contrary, control soil which had zero pesticide treatment and nutrient amendment experienced increased microbial counts from day 7–28.

The general decrease in microbial counts across both pesticide treatments and nutrients amendment recorded at day 7 could be attributed to the toxicity of the pesticides to the indigenous microbial population. Increased counts recorded across the treated and nutrient amended soils from day 14–21 is a consequent effect of the nutrient (compost and NPK) addition to the pesticide treated soils which provided the requisite limiting nutrients/nutrients (nitrogen, phosphorous, carbon) for microbial growth, activity and subsequent biodegradation of the pesticides. Reports from several authors align with findings from this study [1, 38, 39, 48, 49].

The decreases recorded at day 28 across all treatments could be attributed to resultant effect of low nutrient concentration in the treated soils, marked by the one-off nutrient amendment performed in this study, and typical of the dynamics in microbial growth observed in batch (closed) systems. The low nutrient concentration reduces microbial growth rate which results to decrease in pesticide uptake by indigenous microorganisms [6,8]. stated that at low contaminant concentration in a less nutrient environment, microbial cells may degrade the pollutant but the low nutrients in the environment reduce their growth rate which leads to reduced pesticide uptake by microbes and consequently slows down bioremediation process.

The greater microbial counts recorded in compost amendments compared to counts in NPK amendments confirm report that organic fertilizers which are slow releasing, make better nutrient sources due to amount of plant essential and trace nutrients they contain, which are readily biodegradable and increase heterotrophic bacterial biomass [50].

5. Conclusion

In Nigeria, as it is in other countries, the use of pesticides is quite beneficial to agricultural practices, however, their toxicity to public health, soil health and persistence in the environment has led to research on cost-effective and eco-friendly ways to completely or partially remediate soils contaminated with them. This study evaluated the biodegradation and subsequent bioremediation of pesticides (carbofuran and paraquat) in a pesticide contaminated soil, by applying the biostimulation approach using compost and NPK fertilizer. The pesticides application was a one-off treatment at recommended rate and four times the recommended rates. Nutrients amendment was also done at 0.5 and 1 % for compost and NPK. The addition of nutrients provided limiting nutrients to the indigenous microbes, thus, increased pesticides biodegradation rates, resulting to low pesticides residues (significant loses) for the duration of biodegradation monitoring (28 days). Actinomycetes, fungi, nitrifying bacteria and others stimulated for enhanced biodegradation in this study, have been implicated as chief pollutant-degrading microrganisms found in soil and indicators of soil health and quality. Composts have also been reported as facilitators and major drivers or mediators in the remediation of contaminated soils. Results from this study proves bioremediation of pesticide-contaminated soils can be achieved using the biostimulation approach and that greater biodegradation rates will be achieved if nutrient re-amendment of contaminated soils is done at intervals. This will ensure the indigenous pesticide-degrading organisms in constant growth and activity, resulting in better biodegradation/bioremediation outcomes.

Conflicting interest

Authors have declared that no competing interests exist.

Data availability

Data associated with this study have not been deposited into a publicly availabe repository. Data from this study is incuded in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Tega Lee-Ann Ataikiru: Writing – original draft, Investigation, Data curation, Conceptualization. **Chinyere Augusta Ajuzieogu:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to acknowledge Dr. (Mrs) J. Ukpebor, University of Benin, Benin City, Edo State and the laboratory technologists of Environmental Management and Toxicology Laboratory, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23133.

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