ASSESSMENT OF THE MICROBIAL BIOMASS CARBON (MB-C), NITROGEN (MB-N) AND PHOSPHORUS (MB-P) IN SOIL SPIKED WITH PESTICIDES (CARBOFURAN AND PARAQUAT)

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ABSTRACT

This study aimed at determining the impact of Carbofuran and Paraquat use on soil microbial biomass and microbial population as soil health index. Pot experiment, set-up as a randomized block design with replicates were done, with both pesticides applied at recommended rates for eight weeks. Twenty-four (24) soil samples were taken from the pesticides polluted soil as well as the unpolluted soil. These samples were used to assess the effect of pesticides on microbial biomass carbon (MB-C), nitrogen (MB-N) and phosphorus (MB-P). Also, microbial population (determined by aerobic spread plate count) of the pesticide-polluted soils was used as health index. These assessments were done weekly. The microbial biomass values increased from 273.48 µg/g to 293.15 µg/g (MB-C), 17.275 µg/g – 18.52 µg/g (MB-N) and 10.605 µg/g – 11. 37 µg/g (MB-P) in carbofuran treated soil while increases from 277.26 µg/g to 288.365 µg/g (MB-C), 17.515 µg/g – 18.22 µg/g (MB-N) and 10.745 µg/g – 11.18 µg/g (MB-P) were observed in paraquat treated soil. The microbial counts in treated soils were within the ranges of 1.95 x 106 cfu/g to 1.03 x 107 cfu/g, 8.83 x 104 – 1.90 x 105 cfu/g, 1.08x 104 – 2.43 x 104, 1.15 x105 – 2.17 x 105 cfu/g, 1.38 x 105 – 2.22 x 105 cfu/g for total heterotrophic bacterial, fungal, actinomycetes, phosphate solubilizers, nitrifiers counts, respectively.

The pesticides had no negative effects on the MB-C, MB-N, MB-P and soil microorganisms at recommended field rates, hence their use must be strictly based on these rates. These findings indicate that the relationship between soil nutrients and microbial biomass is significant in facilitating the use of microbial biomass as an important soil quality indicator.

Keywords: Microbial biomass, soil quality, microbial counts, pesticides.

INTRODUCTION

In Nigeria, agriculture is the most fundamental economic activity and is faced with several problems. The activities of parasites, pathogens, fungi and weeds pose a serious challenge to the farmers' efforts. These pests are not only in constant competition with the farmers for space and food materials but also pose as agents of diseases to root crops, cereal crops, fibres, fruits, vegetables, stored grains and livestock. These pests reduce the crops yield and livestock productivity to the level that is uneconomical to the farmers such that farmers are forced to explore ways of combating them to reduce the losses. A major method of managing pests is the purchase and application of pesticides to farmlands, crops and stored grains to protect and prevent the farm produce from infestations of these unwanted organisms (1,2).

These toxicants not only affect the target organisms (pests) but also the microbial communities and these non-target effects may have negative impacts on the performance of important soil functions (3). Increased rates of pesticide misuse and resultant consequences on public health have become issues of great concern. (4).

Carbofuran belongs to the group of carbamates and most members are used as insecticides (5). Carbamates have fairly high insect and mammalian toxicities as cholinesterase inhibitors.

Paraquat, a bipyridinium compound is an example of quaternary ammonium herbicides with the trade name, Gramoxone. Paraquat is known to act on the Photosystem I within the photosynthetic membrane (6). On microorganisms, they have inhibitory effects, repressing effects reduces enzyme activity and mycelial growth (7).

Although, these pesticides play important roles in protecting crops from insect pests and weeds, and in controlling disease-transmitting vectors, they cause serious environmental pollution problems (8,9).

Soil quality is also known as Soil health is the capability of a specific type of soil to function, within managed or natural ecosystem boundaries, to be able to sustain biological productivity, enhance or maintain air and water quality as well as support human habitation and health (10). Soil health addresses the functionality of soil to promote environmental quality, preserve plant and animal health and sustain biological productivity while soil quality depicts the fitness of the soil for a specific purpose (11).

Soil quality is an indicator of sustainable management. A balanced correlation between soil function and quality is very important for optimal production of agricultural products. Sustainable soil management practices, as well as a dynamic indicator to monitor changes, are required. These indicators must be adequately diverse to give detailed information about the chemical, biological and physical processes and properties of the soil (12,13).

Microorganisms are biological indicators in the soil environment (14,15,16). Microorganisms act as an excellent measure of the quality and health of the soil. Numerous studies have reported the adverse impacts of pesticides on soil microorganisms and soil respiration (17,18). Furthermore, these compounds on the application may have inhibitory or lethal effects on a certain group of microorganisms and outnumber other groups by removing them from the competition in soil (19). Xie et al. (20) reported a 76% increase in bacterial biomass in response to endosulfan application and a decrease in the fungal biomass by 47%.

The microorganisms which were sensitive to pesticide application serve as a reliable indicator of the biological value of soils while the resistant ones could be further studied for bioremediation purpose (6). Microbial biomass, potentially mineralized nitrogen and soil respiration are some of the indicators that are sensitive to different stressors over a given time.

There are scarce reports on soil biomass studies concerning pesticides used in the Niger- Delta region of Nigeria. Hence, the present study was designed to elucidate the effects of carbofuran and paraquat on microbial biomass-C, microbial biomass-N and microbial biomass-P with physicochemical properties of naturally spiked soil with pesticides which can provide a better understanding of the possible response of soil microorganisms to different pesticides.

2. materialS and methods

2.1 Study Area

The study area is located in the Federal University of Petroleum Resources, Effurun, Delta State, Nigeria (7° 23' N; 3° 51'E and 26.7m above mean sea level). The Niger Delta experiences tropical climate with distinct wet and dry seasons having a bimodal rainfall pattern with rainfall peaks mostly in June to September and the average temperature of 25.2°C (78.8°F) - 28°C (82.4°F). The soils were mostly sandy loam at the top, to the brown loamy sand subsoil and well-drained. Four different representative locations having similar ecological conditions were chosen for this study. The locations had no history of pesticides application.

2.2 Soil Sampling

The surface soil samples were collected from 0 - 15cm and mixed to form a composite sample at each location. Soil samples were sorted to remove stones, plant and root debris. All soil samples from the four locations were pooled together and stored.

2.3 Experimental Design

The experiment was conducted in pots set-up as a randomized block design. The experiment was laid out in four different blocks with replicates. Three kilograms (3kg) of unpolluted soil was weighed in 5L pots. The pesticides used in the experiment were commonly used in the agricultural fields by the local farmers and were purchased from a local agricultural store. The pesticides used were carbofuran and paraquat. The pots were sprayed with the individual pesticides individually for eight weeks at company recommended rates for carbofuran and paraquat (21). The control pots received no pesticides but deionised water. Samples were collected aseptically. Topsoil sample was collected from 0-5 cm depth from each pot (22). Ten soil samples were collected randomly from each pot and thoroughly mixed together to form a composite sample. The effect of different pesticides on soil was analyzed in response to microbial biomass carbon, microbial biomass nitrogen, microbial biomass phosphorus and microbial enumeration concerning control soil (without treatment) in replicates every week after the treatment period of eight weeks.

2.4 Physicochemical Analysis

Soil physical and chemical analyses were determined using standard methods. Soil particle size distribution was done by the hydrometer method (23). Analysis of pH, moisture content, electrical conductivity, total organic carbon (TOC), available phosphorus, total nitrogen was assessed according to the standard methods of APHA (24). Mercury, arsenic, cadmium, lead, calcium, magnesium, potassium and sodium were detected by the flame analysis method 7000B using the Atomic absorption spectrophotometer following the protocol described by the American public health association (24).

2.5 Analysis of Soil Microbial Biomass

2.5.1 Microbial Biomass Carbon (MB-C)

Soil samples were stored at 28±2℃ for a week to stabilize respiration before analysis. Microbial biomass-C in pesticide-treated soil and control were determined by fumigation extraction method

(25). Four (4) millilitres of 0.5M K_2SO_4 was added to 1g of soil to extract organic carbon by dichromate oxidation. The soil microbial biomass was calculated thus:

Microbial biomass-C = ΔOrganic-C/KEC

Using a KEC factor of 0.33 and ΔOrganic-C is the difference in organic-C content between the fumigated and unfumigated sample.

2.5.2 Microbial Biomass-Nitrogen (MB-N)

A ninhydrin assay for biomass α-amino-N and ammonium-N was used to estimate microbial N. Microbial biomass nitrogen was calculated as:

Microbial biomass-N = ΔNinhydrin reactive-N/KninhN

Using a KninhN factor of 0.20 (26) and ΔNinhydrin reactive-N is the difference in the ninhydrin-N between the fumigated and unfumigated sample.

2.5.3 Microbial Biomass-Phosphorus (MB-P)

Soil biomass phosphorus was evaluated using the method of Martin and Correll (27) by estimating phosphorus before the addition of liquid chloroform (biocide) and after addition of liquid chloroform ($CHCl_3$). Ten grams of wet soil homogenized with 100mg of powdery material evenly labelled with 33P (radioisotope) was incubated at 250C for 28 days to get the 33P-labelled soil microbial biomass.

Subsequently, extraction of the soil was done for 2 hours using 200ml of 0.01M ethylene diamine tetraacetic acid (EDTA) a measure (aliquot) of each extract was taken for analysis, then 40ml of liquid $CHCl_3$ was introduced to soil suspensions and another portion of extract was taken for analysis after an additional 2 hours extraction. Another batch of soil was treated similarly, excluding the introduction of $CHCl_3$ to soil suspension. Biomass phosphorus was calculated as the difference in phosphorus extracted from soil treated with $CHCl_3$ and untreated soil.

2.6 Microbial Counts

The population counts of microorganisms were carried out by traditional viable cell counts using selective media. One (1) gram of each soil sample was suspended in 9 ml of sterile distilled water. Serial dilution was done aseptically. Aliquots (0.1ml) of the dilutions were plated out using appropriate media for the enumeration of microorganisms. Rose-Bengal chloramphenicol agar was used for the enumeration of fungi (22). Plate count agar (PCA) was used for the enumeration of total heterotrophic bacteria (28). Actinomycetes were enumerated using starch-casein agar (29) and Pikovskaya's medium for phosphate solubilizing microbes (30). Ashby culture medium was used to enumerate nitrogen fixers (31) and individual colonies were recorded as colony forming units (cfu).

2.7 Statistical Analyses

Two way-ANOVA was used to test the effects of the different agro pesticide use with time on microbial biomass carbon MB-C, microbial biomass nitrogen MB-N and microbial biomass phosphorus MB-P.

3. results and discussion

3.1 Physico-Chemical Properties of the Pesticides Treated Soils

The physicochemical characteristics of the different pesticide-treated soil are shown in Table 1.

Table 1: Physico-chemical characteristics of the different pesticide-treated soil

Parameter	Control	Carbofuran	Paraquat
pH	6.17	6.19	6.12
Electrical conductivity (µs/cm)	147	173	467
Total organic carbon (%)	1.25	1.66	1.62
Total nitrogen (mg/kg)	0.04	0.04	0.09
Available phosphorus (mg/kg)	2.33	2.42	9.39
Calcium (meq/100g soil)	6.11	6.67	5.12
Magnesium (meq/100g soil)	0.01	0.01	0.004
Sodium (meq/100g soil)	11.52	11.03	10.79
Potassium (meq/100g soil)	4.71	8.42	2.58

3.2 Microbial Biomass-Carbon (MB-C), Microbial Biomass Nitrogen (MB-N) and Microbial Biomass Phosphorus (MB-P)

The microbial biomass carbon values estimated from the different pesticides treated soil are shown in Fig.1. There were increases in MB-C values in all the pesticides treated soil throughout the study while the reverse was seen in the unpolluted soil. The MB-C values were highest in unpolluted soil at day 7(285.055 µg/g) and 14 (279.685 µg/g) and in the carbofuran treated soil at day 21(287.735 µg/g) and 28 (293.15 µg/g). The values increased from 273.48 µg/g to 293.15 µg/g and 277.26 µg/g to 288.365 µg/g for carbofuran and paraquat, respectively. A decrease from 285.055 µg/g to 272.725 µg/g was seen in the unpolluted soil. A two-way ANOVA showed that there was statistical significance in MB-C values concerning the different pesticides treated soil at *P=0.05* value.

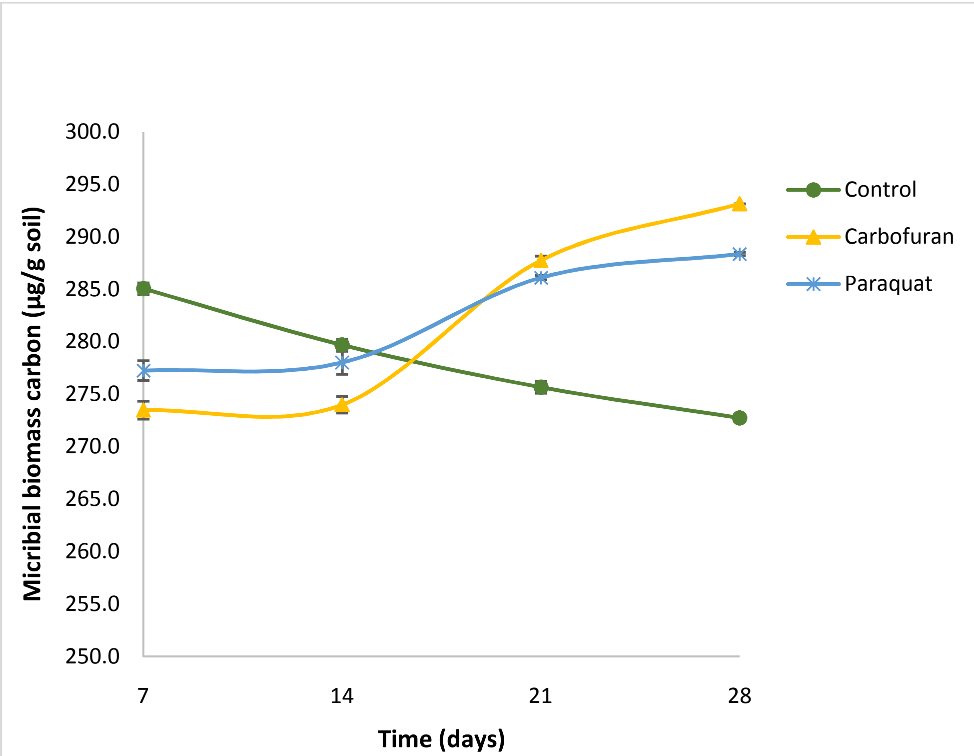

Fig.1: Microbial biomass carbon

Microbial biomass nitrogen (MB-N) analysis also showed variation concerning the different treatments (Fig.2). The MB-N values constantly decreased for the control samples but increases were observed for the pesticides treated the soil. This study recorded the least MB-N value at day 7 and the highest value at day 28 for carbofuran treated the soil. The MBN value increases were 17.275 µg/g – 18.52 µg/g for carbofuran and 17.515 µg/g – 18.22 µg/g paraquat treated soils. At *P=0.05* there was a significant difference in microbial biomass nitrogen values using the two-way ANOVA.

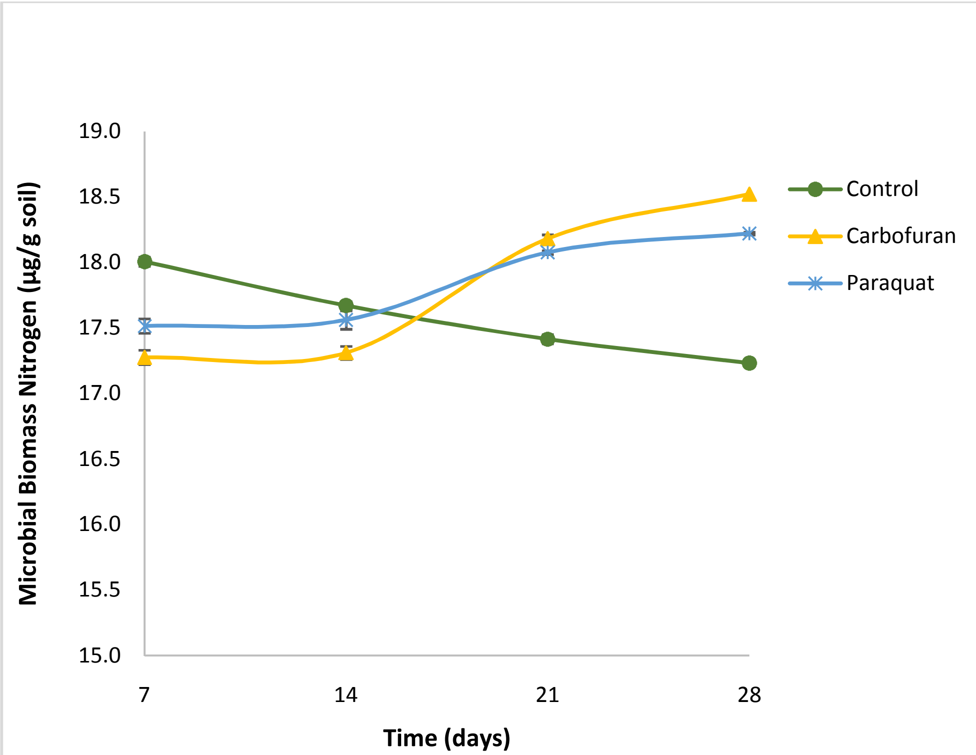

Fig.2: Microbial biomass nitrogen

The microbial biomass phosphorus (MBP) showed a declining trend from day 7 to day 28 in the unpolluted soil (control). The effect of carbofuran on MBP was highest at day 28 as seen in Fig.3. Similar increases in MBP values were exhibited by the different pesticides treated the soil. The value increased from 10.605 µg/g – 11. 37 µg/g and 10.745 µg/g – 11.18 µg/g for carbofuran and paraquat, respectively. A two-way ANOVA showed that there was statistical significance between MB-P values at *P=0.05*.

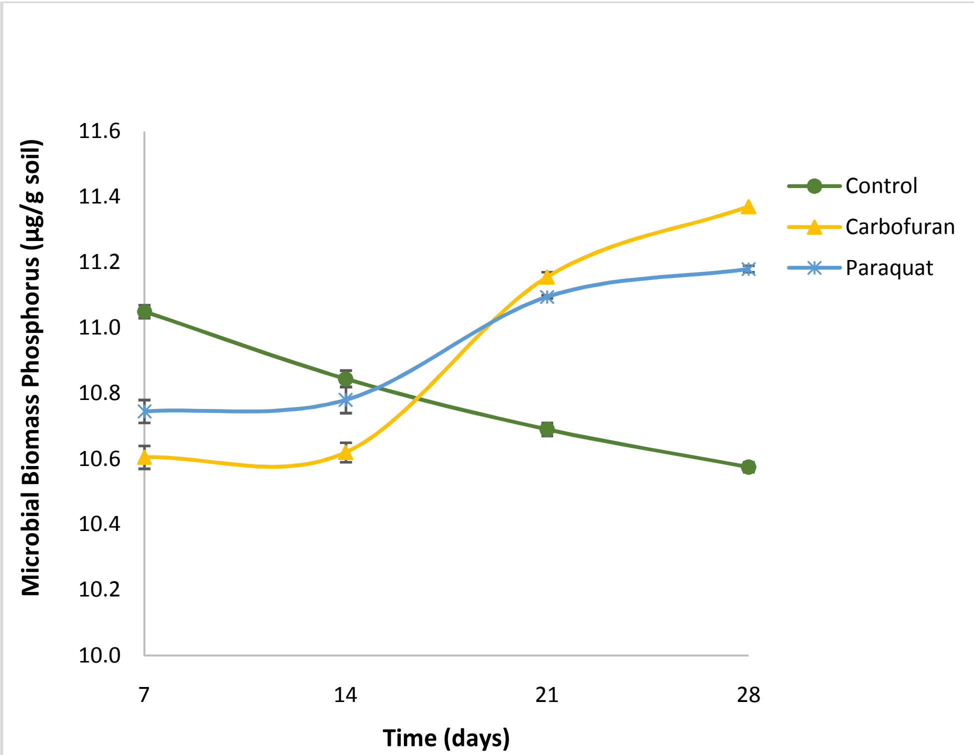

Fig.3: Microbial biomass phosphorus

The analysis of microbial biomass (MB) is an important tool during ecological studies as indicators of microbial activity and soil health. A major effect of these pesticides is the immediate response of the microbial activities due to the imbalances on both biological and chemical characteristics of the soils; sometimes suppressing their activities. Some researchers have reported that the reduction in microbial biomass could be attributed to the adsorption of the small amount of these toxicants on the organic matter present in the soil causing the lysis of microbial cells (32,22). These chemicals have effects on different microbial activities and processes, inhibiting their break down based on the type and application rates, hence altering the microbial biomass quantitatively and qualitatively in both short and prolonged use. More so, these pollutants can have adverse effects on the non-target microbial populations influencing important processes like respiration, cell growth and division, photosynthesis and others (3). On the contrary, there has been a report that these pesticides may stimulate an increase in microbial biomass values and have been traced to the fact that they are metabolized as nutrient sources by the soil microbial populations leading to their multiplication in the environment (33). Bhagobaty and Malik (34) from their study reported that bacteria isolated from wastewater irrigated agricultural soil was capable of utilizing chlorpyriphos as a carbon source for their growth. Nevertheless, these pesticides only cause temporary and little changes when compared with natural and spatial variation in soil microbial biomass.

3.4 Microbial counts

The enumeration of the different microbial populations was carried out to show the distribution and abundance in respect to the pesticide treatments. Figure 4 shows the total heterotrophic bacterial counts throughout the study. The total heterotrophic bacterial counts increased in all the pesticides treated soil after the initial decline in numbers. THB counts in carbofuran treated soils increased, ranging from 2.14 x 106 cfu/g (day 7) to 6.9 x 107 cfu/g (day 21) and decreased to 4.53 x 107 cfu/g soil (day 28). The counts increased from 2.38 x 106 cfu/g to 1.03 x 107cfu/g and reduced to 8.57 x 107 cfu/g soil at day 7, 21 and 28, respectively for paraquat treated soil. A reverse trend was obtained in the counts from the control soil. Cycon and Piotrowska-Seget (45) reported an initial drop in the population of heterotrophic bacteria and fungi but was stimulated at higher doses of an organophosphate insecticide, diazinon, in the soil which we also observed from our study. Several researchers (4,33) have reported the same from their studies after the application of different pesticides to the soil. A two-way ANOVA showed that the variation in the total heterotrophic bacterial count concerning different pesticides and days was significant at *P=0.05*.

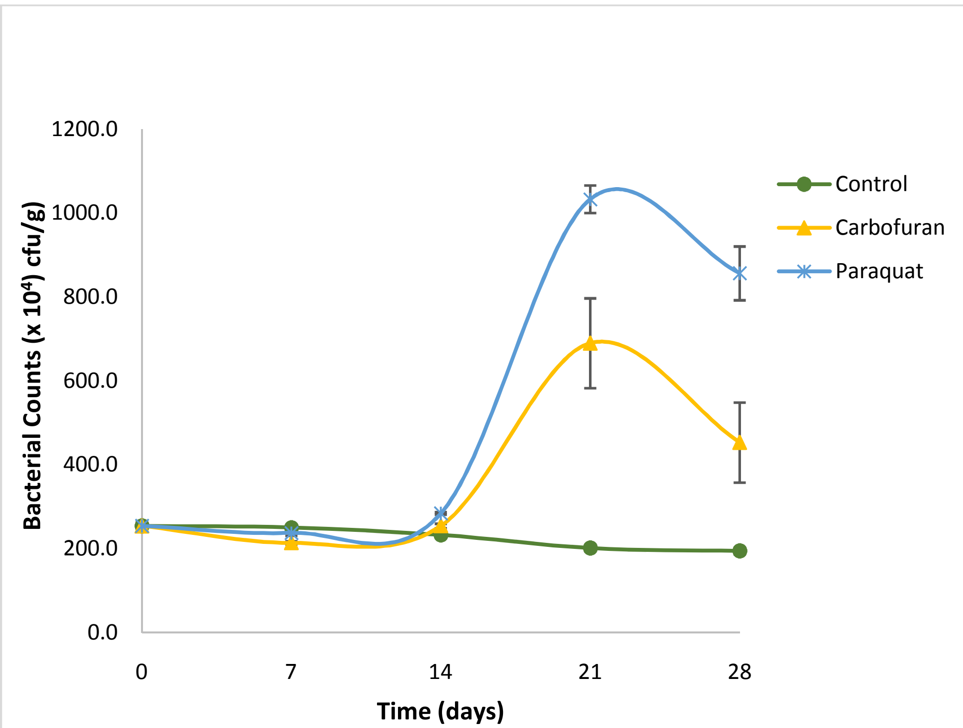

Fig.4: Total heterotrophic bacteria (THB) counts

There was a general decrease in fungal counts in the different treatments initially except the control soil. Fungal counts increased in all the treatments from day 7 to day 21 (Fig.5). The fungal counts were in the range of 8.83 x 104 to 1.44 x 105 cfu/g soil for carbofuran and 1.22 x 105 to 1.79 x 105 cfu/g soil for paraquat, respectively. There were increases in the fungal population throughout the study from 1.50 x 105 to 1.90 x105 cfu/g soil for the control. A two-way ANOVA showed that the variation in the fungal count concerning different pesticides and days was significant at *P=0.05*.

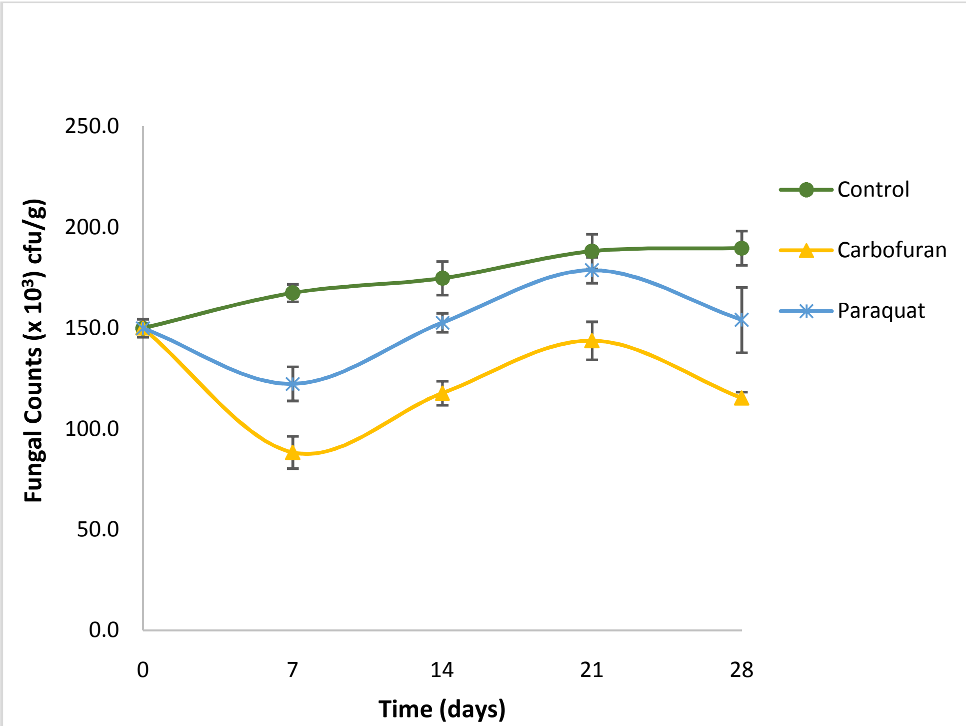

Fig.5: Fungal counts during the study

The actinomycetes count increased for both treatments from day 14 to day 21 and a decrease was observed at day 28 (Fig.6). Counts increased from 1.08 x 104 cfu/g to 2.11 x 104 cfu/g for carbofuran and 1.12 x 104 cfu/g to 2.43 x 104 cfu/g for paraquat. The reverse was the trend for the control soil from 1.99 x 104 to 1.27 x 104 cfu/g. A two-way ANOVA showed that the variation in actinomycetes count for different pesticides and days was significant at *P=0.05*.

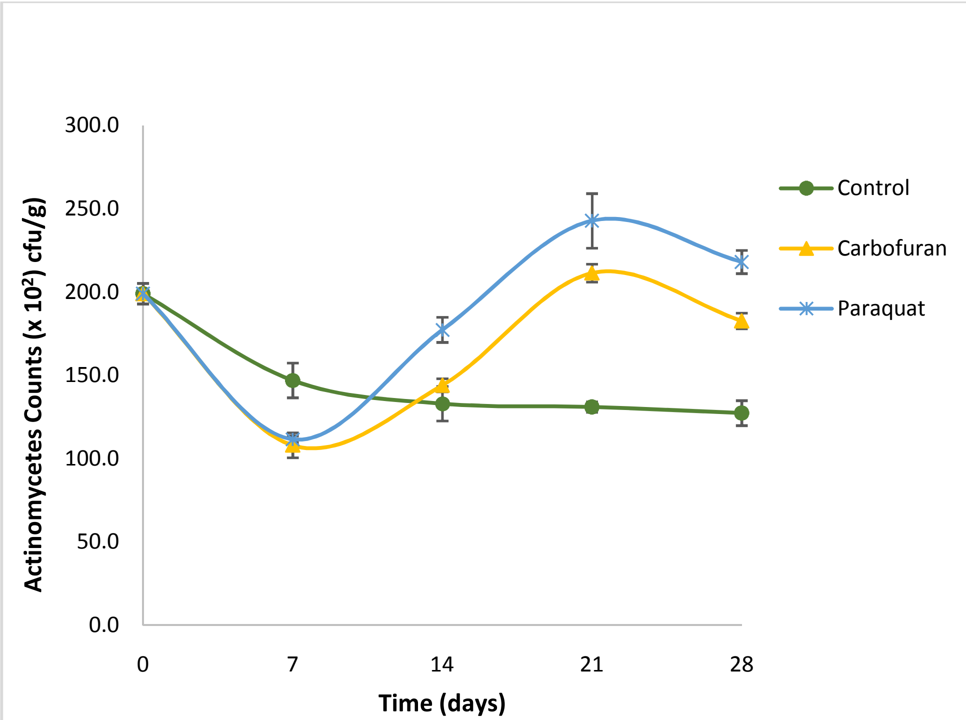

Fig.6: Actinomycetes counts during the study.

There was a decrease in phosphate solubilizers' counts throughout the study. In carbofuran treated soil, the phosphate solubilizers increased from 1.15 x 105 cfu/g to 1.98 x 105 cfu/g and in paraquat from 1.60 x 105 cfu/g to 2.11 x 105 cfu/g (Fig.7). A two-way ANOVA showed that the variation in phosphate solubilizers count for different pesticides and days was significant at *P=0.05* value.

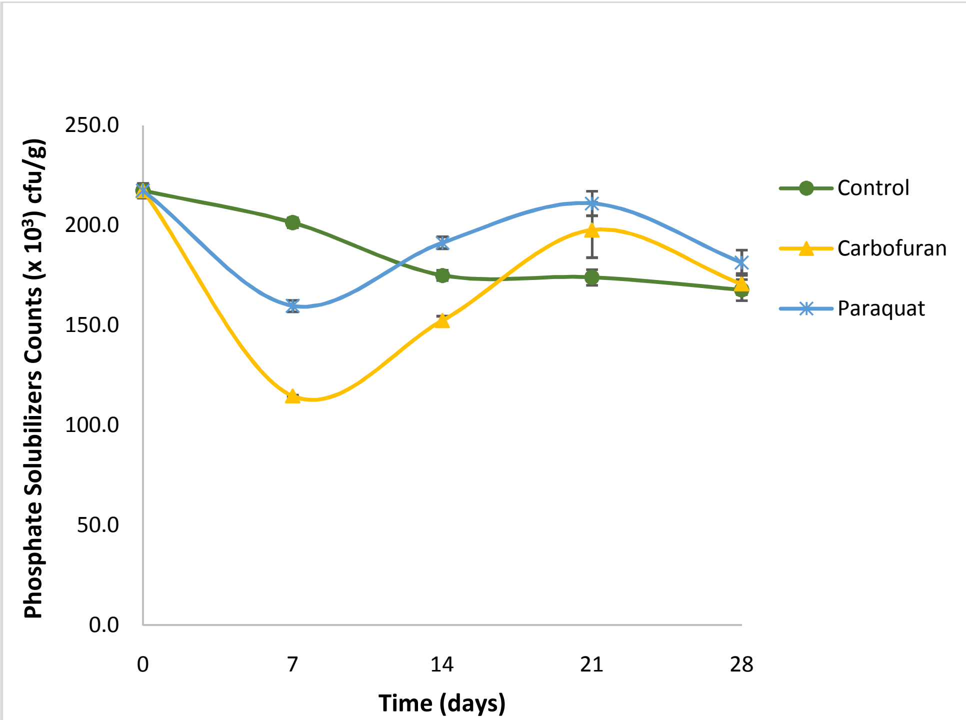

Fig.7: Phosphate solubilizers counts during the study.

Also, there was an initial decrease in the nitrifiers counts at day 7 and thereafter increases from day 14 to day 21 as shown in Fig.8. The counts increased from 1.49 x 105 to 1.67 x 105 cfu/g for carbofuran treated soil and 1.67 x 105 cfu/g to 2.22 x 105 cfu/g. There was a gradual decline in counts from 1.82 x 105 to 1.38 x 105 cfu/g from the control soil. A two-way ANOVA showed that the variation in the nitrifying bacterial count for different pesticides and days was significant at *P=0.05*

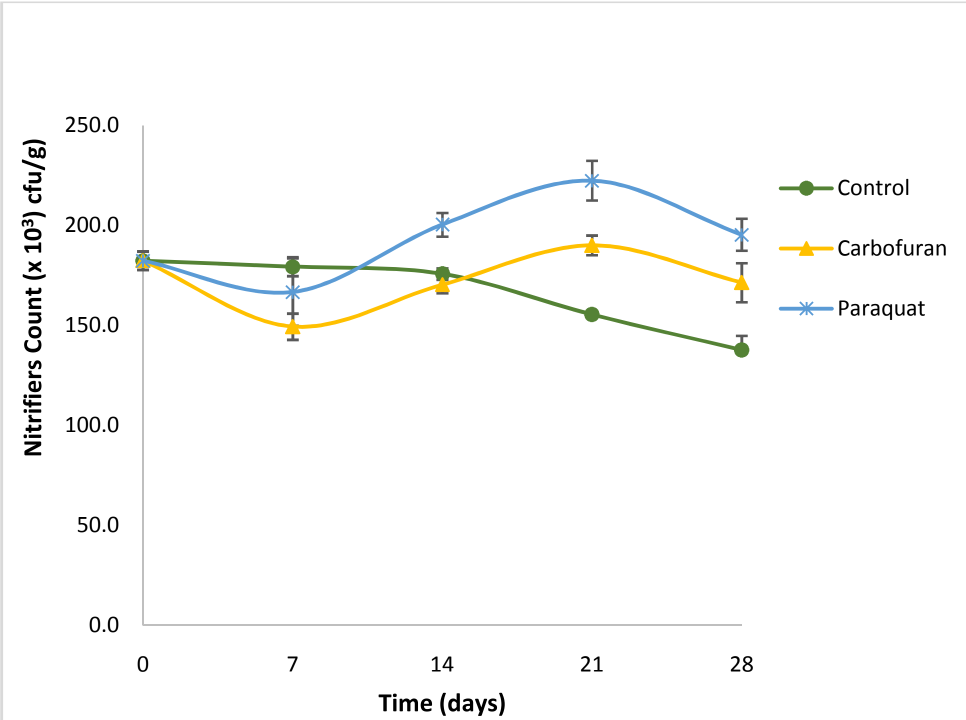

Fig.8: Nitrifiers count during the study.

Soil is one of the major components of the environment inhabited by a variety of microorganisms including bacteria, fungi, algae, viruses and protozoa (36). Soil microorganisms are an important part of the ecosystems and are involved in energy flow adjustments and the cycle of matter by digesting animal, plant and oil residues. These microorganisms play a major role in the growth and development of crops, the balance of the soil ecosystem, organic matter transfer and bioremediation. Furthermore, the diversity of the microbial community in the soil is closely related to the function and structure of the ecosystem and is one of the components to maintain soil productivity (37).

The xenobiotics (pesticides) vary in their toxicity depending on their composition, concentration, environmental factors and the biological state of the organisms at the time of the contamination. Hence, their removal from the ecosystem by natural populations of microorganisms is the main process in the depuration of a polluted environment (38). The ability to enumerate these microbes from the pesticides-polluted environment is commonly taken as evidence that these microorganisms may be the active degraders of these pollutants in the environment.

From this research, there was a general decrease in total heterotrophic aerobic bacteria, actinomycetes fungi, phosphate solubilizers as well as nitrifiers counts in different pesticides treated soil at day 7 followed by a gradual increase as the days progressed. The rise in microbial counts in pesticides treated soil may be due to their ability to metabolize these pesticides as an energy source. However, the initial decreases in microbial counts in the pesticide-treated soils may be attributed to the cidal or lethal effects of these stressors on microbial populations that were tolerant of the pesticides (2,22,39). Also, the decline in microbial counts observed at day 28 in the pesticide-treated soils could be as a result of depletion of nutrients necessary for microbial metabolism, which is typical of a ''batch culture" or ''closed system".

4. Conclusion

The study confirmed that the pesticides (carbofuran and paraquat) may alter the microbial populations for different days after treatment, and thereby affects the different soil microbial biomass. The study proved that the negative effect of pesticides towards soil MB-C, MB-N, MB-P and microbial populations decreased with time. This present-day study has shown the positive response of MB-C, MB-N and MB-P to the pesticides used but this can only be true at recommended rates based on our findings.

Ethical: NA

Consent: NA

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Competing interests

Authors have declared that no competing interests exist.

Authors' Contributions

This study was done in collaboration between all authors. ‘Author A' (Okpokwasili, G.S.C.) designed the study and wrote the protocol. ‘Author B' (Ataikiru T.L.) performed the laboratory analyses, statistical analysis and wrote the first draft of the manuscript. ‘Author C' (Okerentugba, P.O.) managed the literature searches. All authors read and approved the final manuscript.