RESPONSE OF SOME MICROORGANISMS, EARTHWORMS AND SNAILS TO PESTICIDES (CARBOFURAN AND PARAQUAT) UNDER TROPICAL CONDITIONS

ABSTRACT

Aim: determine the response of bacteria, earthworms and snails to pesticides under tropical conditions.
Study design: soil experiment with a controlled lighting system of 12 hour of light and 12 hour of darkness was adopted.
Place and Duration of Study: Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Nigeria/ three months.
Methodology: Acute effect of pesticides on earthworms and snails was assessed using a 14 day soil bioassay test as recommended by Organization for Economic Cooperation and Development (OECD) and International Organization for Standardization (ISO). Effect of pesticides application on microorganisms in soil was evaluated via standard microbial counts.
Results: Toxicity of Carbofuran to the earthworms and snails was higher relative to Paraquat with a median lethal concentration (LC_{50}) of 10.7 mg/kg; 159571 mg/kg (earthworms) and 23.22 mg/kg; 759000mg/kg (snails), respectively. Microbial counts increased in pesticides treated soil after the initial decline in numbers. Total heterotrophic bacterial counts in Carbofuran and Paraquat treated soils increased from 1.59×10^6 CFU/g to 2.41×10^6 CFU/g and 1.46×10^6 CFU/g to 2.08×10^6 CFU/g, respectively for day 14 to 21. A reverse trend was observed in control soil. Fungal counts increased in Carbofuran (1.18×10^5 CFU/g to 1.42×10^5 CFU/g); Paraquat (1.53×10^5 CFU/g to 1.79×10^5 CFU/g) from day 14 to day 21. Actinomycetes counts were in magnitude of 10^4 CFU/g soil for both Carbofuran and Paraquat treated soils, while population of phosphate solubilizers and nitrifiers were in 10^5 CFU/g soil.
Conclusion: Toxicity test estimates the possibility that antagonistic environmental impacts/influence might take place or are taking place due to susceptibility to sole or additional pesticides. The pesticides had no negative effects on the soil microorganisms at recommended field rates, hence their used must be strictly based on these rates.

Keywords: lethal toxicity, pesticides, microorganisms, snails, earthworms, tropics

1. INTRODUCTION

Pests refer to biotic constituents of the environment like fungi, insects, birds, other mammals, microorganisms and weeds that cause injuries to plants and animals that man invest in and rely on for food material to maintain life [1,2,3]. These organisms compete with man for food and are means through which diseases attack his root crops, fibres, cereals, fruits, vegetables, stored grains and his livestock. Their overall effect on crops and livestock is the decrease of yields to an uneconomical point for the agriculturalist to remain in the business [4,5,6].

High demand is placed on the supply of food to support the ever increasing world population. This has shifted the global attention on soil biological processes and the agro ecosystem to better understand the complex processes and interaction to stabilize agricultural land. In other to increase food production to meet the alarming increase population has led to the introduction of pesticides [7,8].

Pesticides are formulations used to kill or manage pests at tolerable levels. They consist of different products with different functions. They can be natural or synthetic. The use of pesticides has its positive and negative effects. It is an advancement to improve food supply for the teaming population and the prevention of human diseases. On the other hand, the continuous use has led to contamination of food and natural resources with substances that are toxic to life, many of these pesticides are carcinogenic. The indiscriminate use of these substances has led to contamination of soil, ground water, lakes and man [9]. Pesticides are classified based on their mode of application and action, target organisms, desired goal, chemical composition among others [10,11].

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl-N-methyl carbamate) is a broad spectrum, systemic, non-ionic, acaricide/insecticide and nematicide included in the general group of the carbamate derivative pesticides. It is widely used for the control of soil dwelling and foliar feeding insects including wireworms, white grubs, weevils, stem borers, aphids and several other insects [12]. Its effectiveness, as a soil-applied pesticide, however, depends upon the physico-chemical properties of the soils, environmental conditions, and its ability to reach the target organisms in an adequate concentration for a certain period of time [10].

Paraquat (N, N′-dimethyl-4,4′-bipyridinium dichloride) is termed a chemical flame gun, in so much as it kills the above-ground parts of plants whilst not damaging the roots. Paraquat inhibits photosynthesis and applied post emergence to leaf tissues [13]. It inhibits CO_2 fixation by inhibiting the variable chlorophyll fluorescene by decreasing oxygen evolution. Its activity is irreversible. It is non selective, quick and lethal to man and animals. Paraquat is a non - selective herbicide used to control most yearly weeds and several broad leaf weeds in field corn, potatoes, rice, cotton, soybeans, tobacco, peanuts and sunflowers [14]. It is administered in pre-emergence and early post-emergence weed control. Paraquat is classified in category 111 as “slightly toxic”. It inhibits CO_2 fixation by inhibiting the variable chlorophyll fluorescene by decreasing oxygen evolution. Its activity is irreversible.

Soils are termed polluted when the concentration of the contaminant exceeds the level defined by the application regulations. In most developed countries, environmental regulation on water and air pollution are common however, only a few countries have such for soil contamination. Data from chemical analysis of soil, water and food samples are not enough indication of the degree and source of pollution [15]. Hence, there’s need for bioassays to provide useful and important information on the risks posed by these pesticides to the biotic population [16].

Microorganisms are biological indicators in the soil environment. They are very sensitive to manmade or foreign disturbances and climate change. Microorganisms act as excellent measures of soil integrity. Understanding the reaction of soil microbial community composition to farm management practices over time will help to determine the influence of such practices on soil integrity. The conventional method of evaluating the diverse nature of microorganisms relies on their potential to grow and form visible colonies on solid media. Several selective media and direct viable cell counts on media plates are performed in this technique [17]. It is a quick and inexpensive method. The method provides useful information on the active and heterotrophic microbial diversity within the population.

Earthworms make up over 80% of the biomass of soil invertebrates and are important in structuring and nutrient cycling in the soil. Earthworms are seen as suitable bio-indicators of anthropogenic contamination. Earthworms are used in ecological risk assessment because they ingest large quantities of decomposed organic matter in soil converting it to rich topsoil. Also, their skin is a significant route of toxicant uptake. Mortality has been the most frequently used indicator in assessing chemical toxicity on earthworms [16,18,19,20,21,22,23,24].

Earthworms are common soil organisms in majority of ecosystems that are severely influenced by agrochemicals. They function essentially in improving texture, structure, soil aggregation, and physico-chemical constituents of the soil with improved fertility [25]. Earthworms are responsive, hence predisposed to agro-chemicals due to non-existence of hard cuticle around their body. This makes them appropriate bioindicators of soil pollution, and could be employed to offer thresholds for pesticide usage [26]. It has been proven from studies that increased amounts of pesticide deposits in farm soils impose a greater threat to soil fauna and these harmful impacts on soil fauna may disrupt the processes they drive, including those related to organic matter decomposition, nutrient cycling, and preservation of soil structure [27]. Acute toxicity of earthworm is an efficient tool in ecological risk assessment of polluted soils and endpoint is mortality [28]. According to Bhandari et al. [29], earthworms are among the first receptors affected by soil pollution and are highly responsive to metals and pesticides than any other group of soil invertebrates. Data on toxicity of earthworm are key in assessing the “safe levels” for stressors in soil.

The Organization for Economic Cooperation and Development (OECD) method has two approaches to the analysis of toxicants; the filter paper test and soil test method. The former is useful for screening while the latter is a better depiction of the natural environment as the earthworms absorb these pesticides through their guts [30]. Hence, the toxic impact of pesticides can be adequately determined using the soil test.

According to Yasmin & D’Souza [31], earthworms are considered as important bioindicators of chemical toxicity in the soil ecosystem and compose over 80% of terrestrial invertebrates. Their use as bioindicators depends on their ability to ingest large amounts of decomposed litter, manure and other organic matter converting it into rich topsoil. Also, their skin is an essential route for these chemicals uptake [21]. Such study is vital because earthworms are common prey of many vertebrates like birds and small animals, thus are fundamental in the biomagnification process of many soil pollutants.

Among the lower tier earthworm tests are the acute toxicity and chronic toxicity test. The acute toxicity test detects qualitative effects and lethality of the toxicants while the chronic detects the sub-lethal impacts like retarded development, reduced fertility and teratogenic effects. The chronic can detect the type and level of alterations in the worms' population even in the nonexistence of mortality. These testings are advantageous as they are fast, cost-effective and sometimes are higher in sensitivity relative to other toxicity test [23,32].

These tests are standardized, recognized and promoted at international levels by Organization for Economic Cooperation and Development (OECD) and International Organization for Standardization (ISO). Alves et al. [18], reported that only a few researches have been performed on the effects of pesticides application on earthworms under tropical scenerios. Moreover, a risk assessment based on temperate data could be less appropriate for tropical conditions, hence, this study.

Snails are important indicators of terrestrial ecological risk assessment; they are soil surface dwellers because major stages of their life-cycle rely on the soil (laying, hatching, preliminary/early growth stages and hibernation). They get nutrition from decomposing organic matter and plants [33,34]. These invertebrates are in a continuous contact with soil till late phases of growth by feeding on soil or via the moist pedal/foot used for exchange of materials (water, mineral salts, waste and other organic materials).

They are vital in the environment due to their capacity to concentrate materials by ingestion and other metabolic activities/processes which presents them perfect tools for environmental monitoring. They are readily available, identified and abundant all round yearly with well-known functional and environmental characteristics for easy breeding under controlled/simulated conditions. These terrestrial animals were chosen because they are eaten by man and other higher animals as good source of protein [35]. Exposure of these fauna to toxicants could lead to dangerous impacts on them and several species higher the food-chain/web [29].

Ogeleka et al. [16], carried out an investigation on the lethal and sub-lethal impact of a non-selective herbicide, Grassate on snails (*Achachatina margarita*) and reported a median lethal concentration LC_{50} toxicity of 1.73 ± 0.05 mg/kg. This result was a pointer that Grassate was "extremely toxic" and such ought not to be administered indiscriminately. Also, they noticed a reduction in biomass (26 - 65% reduction in weight) with increased concentrations for the species. Furthermore, a risk assessment based on other types of pesticide could be appropriate for tropical conditions. Hence, the essence for this study.

2. MATERIALS AND METHODS

2.1 Study area

The study area was 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 average temperature of 25.2°C (78.8°F) - 28°C (82.4°F). The soils were mostly sandy loam at the top, to brown loamy sand sub soil and well drained. Three different representative locations having similar ecological conditions were selected. The locations had no history of pesticide applications.

2.2 Test pesticides

The pesticides used for this study were Carbofuran and ParaQ. Pesticides (Carbofuran and Paraquat) were purchased from local retailers in Warri, Delta State. These pesticides were chosen because of their popular use in potato, maize, groundnuts farming and flower lawns in the Niger Delta region. The Carbofuran formulation contained (5%) and ParaQ formulation contained 200g/l Paraquat. These formulations were used as stock and different concentrations were prepared for the range finding and definitive tests.

2.3 Test species

The test organisms used in this research were recommended by Organization for Economic Development (OECD) and International Organization for standardization (ISO). The organisms were obtained from their natural habitat with no history of pesticides use at Federal University of Petroleum Resources (FUPRE), Effurun, Delta State. The test organisms were earthworms (*Lumbricus terrestris*) and snails (*Helix aspersa*). Healthy test species were collected by hand sorting from cultured farms in FUPRE.

2.4 Earthworm and snail ecotoxicity (acute toxicity test)

The 14 day acute toxicity test was carried out in accordance with the protocol adopted from [36] and [16] for earthworms (*Lumbricus terrestris*) and the International Organization for Standardization [35] protocol for snails. Garden snails, *Helix aspersa* were collected from farms with no pesticide history. The selected snails and earthworms were well cleaned; kept in large plastic containers and acclimatized for 7 days before the experiments. The natural topsoil was gotten from the field from which the test animals were collected. The test started with a range finding test to decide the definitive test concentrations. Homogenized and air dried soil were sieved through 2 mm mesh. Adult earthworms were exposed to soil mixed with different concentration of pesticides. The definitive test concentrations for Paraquat were 625, 1250, 2500, 5000, 10000mg/kg and 0.3125, 0.625, 1.25, 2.5, 5.0mg/kg for Carbofuran, respectively. Immediately before the test, the earthworms were washed and weighed individually. Only those with 250 – 300mg weight were selected for the assay. The different test media were polluted with 80ml (test solution) of the various concentrations. The worms were washed with deionized water, placed on absorbent paper for a short period to allow excess water to drain. The test organisms were placed on filter paper for one hour to void the contents of their gut. Ten earthworms were placed on 1000g of natural soil (with different concentrations of pesticides) in a glass bottle/container of 1.2L capacity and allowed to borrow. The earthworms were fed with 5 grams urine-free dried and ground cattle manure (cow dung's) once a week during the experiment. Five grams of fresh pawpaw leaves were placed in each test container as food for the snails to prevent them from starving during study. The snails were kept on moist filter paper to void contents of their stomach before placing them in the test containers. Each container contained ten snails. These set up in three replicates per treatment were prepared for five exposure concentrations and control were also prepared using distilled water. The trial was done at 28±1^{0}C.

2.5 Assessment of mortality

Mortality of earthworms and snails were evaluated at day 7 and 14 to determine the lethal concentration of each pesticide. To check the mortality, the test containers were emptied onto a clean tray and earthworms were sorted from soil. Earthworms were considered dead when they failed to show a reaction to gentle mechanical stimulations with a blunt probe. Live organisms were counted at completion of trial. Direct contact was avoided to prevent induced stress on the test animals. Live organisms were counted and physical alterations were noted. Some snails hibernated secreting a mucus calcareous substance. Test organisms were considered dead under conditions of no movement when the foot region was prodded by a blunt probe or no movement when placed on filter paper and water sprinkled on them for five minutes. Probit analysis was employed to ascertain median lethal concentration.

2.6 Microbial assay

This experiment was performed in pots set-up as randomized block design arranged out in four different blocks. Three kilograms (3kg) of unpolluted soil were weighed in 5L pots. The pots were sprinkled with the pesticides (Carbofuran and Paraquat) individually for a duration of eight weeks at company specified amounts [37]. The control pots received no pesticides but sterile deionised water. Top soil samples (0-5 cm) were obtained from each pot asceptically [38]. Ten soil samples were obtained at random from each pot and thoroughly mixed together to form a composite sample. The influence of various pesticides on soil with respect to microbial growth were evaluated and compared with control soil (without treatment) in replicates every week after the treatment period of eight weeks. The population count of microorganisms was carried out by traditional viable cell counts. One (1) gram of each soil sample was suspended in 9 ml of sterile distilled water. Serial dilution was done aseptically under laminar flow. 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 [39]. Plate count agar (PCA) was used for the enumeration of heterotrophic bacteria [40]. Actinomycetes were enumerated using starch-casein agar [41] and Pikovskaya's medium for phosphate solubilizing microbes [39]. Ashby agar was used to enumerate nitrogen fixers [38] and individual colonies were recorded as colony forming units (CFU).

2.7 Statistical analysis

The susceptibility of the test species (earthworms and snails) to the toxicants were determined using the probit method of analysis for the lethal concentration LC_{50} at day 14. The mean statistical difference between the controls and treatment groups at significance levels of $p < .05$ were evaluated for microbial counts using the analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Acute toxicity assay using earthworms

The Probit results obtained from exposing the earthworms to Carbofuran and Paraquat contaminated soil are presented in Fig.1 and Fig.2. The median lethal concentration LC_{50} of Carbofuran was 10.7mg/kg while that of Paraquat was 159571mg/kg suggesting that the former was more toxic than the latter.

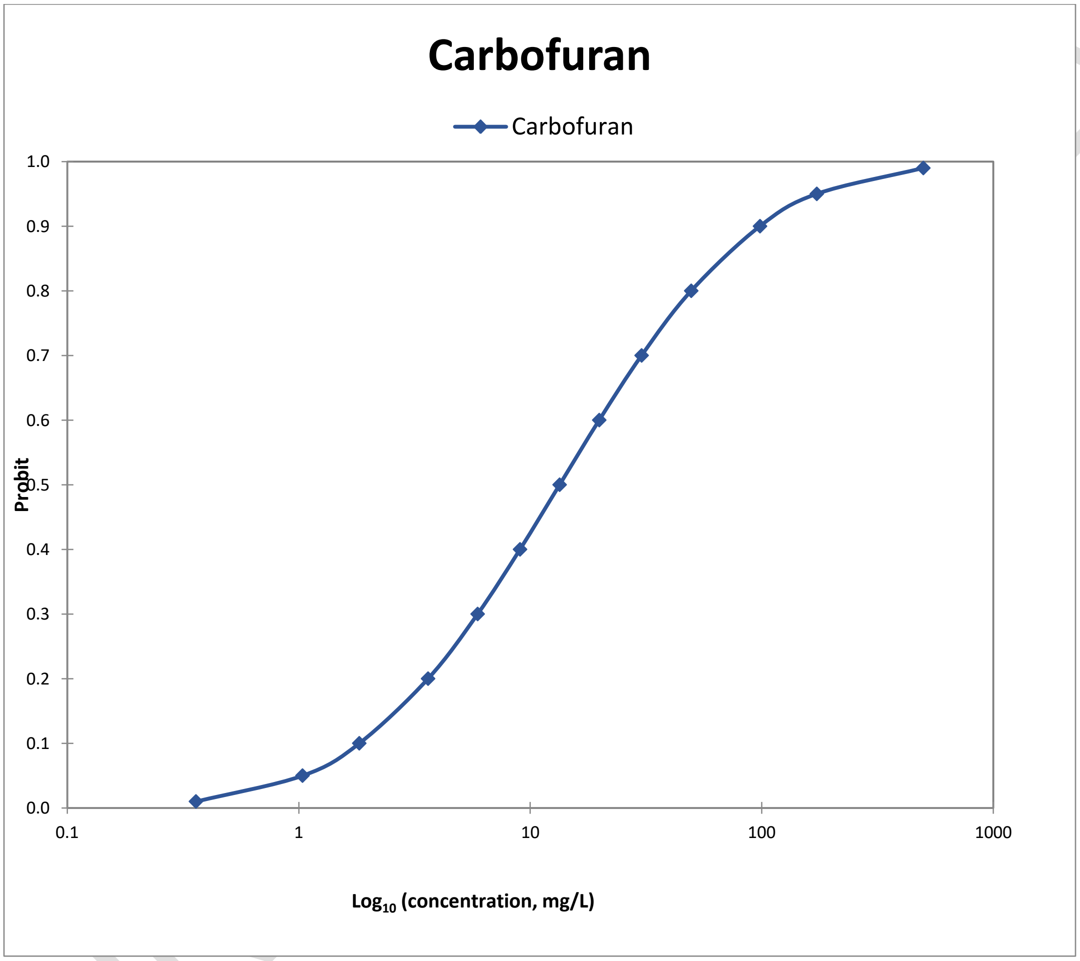

Fig.1 Median lethal concentration (LC_{50}) of Carbofuran for earthworms = 10.7mg/kg

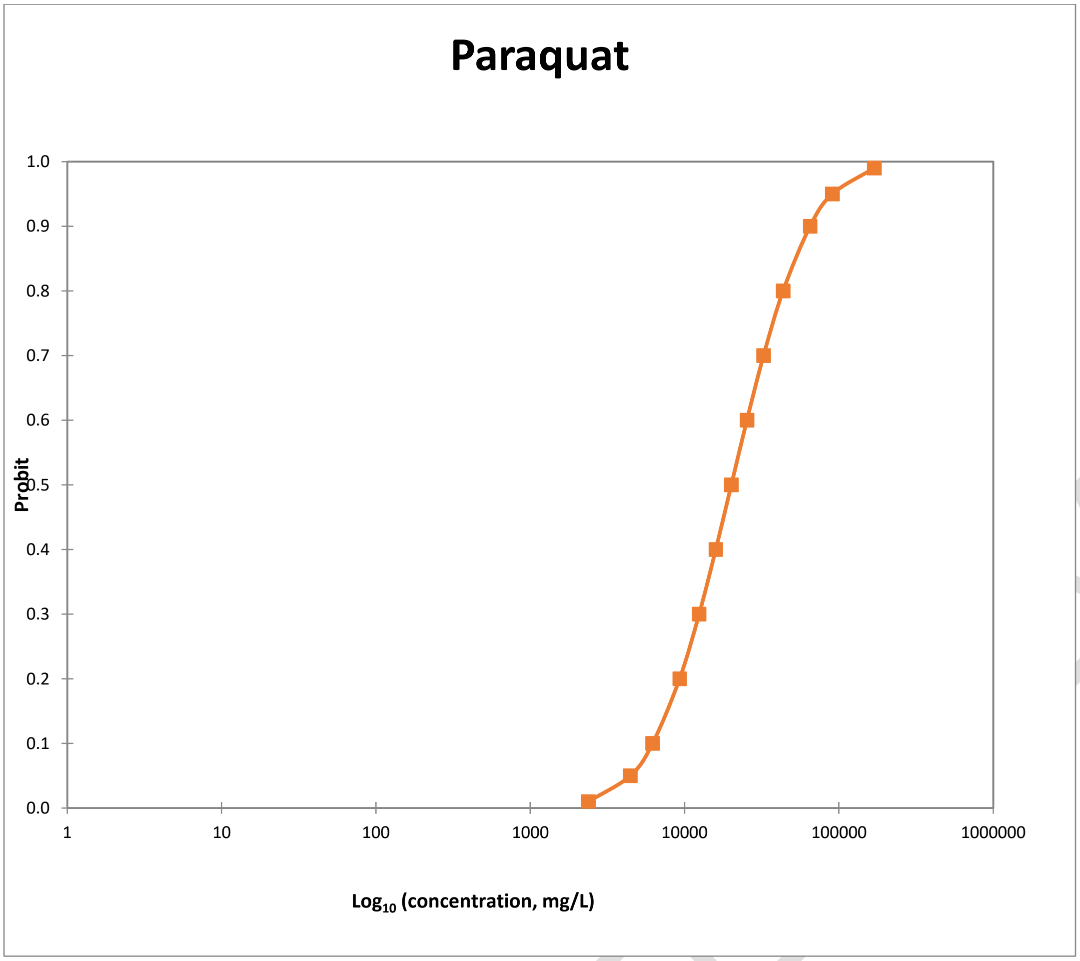

Fig.2. Median lethal concentration (LC_{50}) of Paraquat for earthworms = 159571mg/kg

This study tested the effect of Carbofuran and Paraquat on survival of *Lumbricus terrestris* using standardized ecotoxicological tests (after OECD guidelines with few adaptations for tropical conditions). Commercial formulations of Carbofuran and Paraquat were tested individually in tropical soil. The pesticides tested were lethal to *L. terrestris*, but noxious level obtained hinged on concentrations used. The probit outcome of hither research revealed LC_{50} of Carbofuran as 10.7mg/kg while that of Paraquat was 159571mg/kg hence, Carbofuran was more lethal than Paraquat. From our findings, Carbofuran was very lethal and Paraquat practically not toxic conferring to soil toxicity rating [43]. Similar reports have been published for pesticides in diverse investigations. Yuguda et al. [20], in their studies reported variations in toxicity of a number of pesticides (insecticides, fungicides and herbicides) to *L. terrestris* and established that insecticides were more poisonous than herbicides. This was in corroboration with our findings. Carbofuran induced high toxic effect by revealing high magnitude of mortality while paraquat demonstrated rather lesser fatal effect. In addition, worms displayed other physical behavoiurs such as coiling and swelling due to toxicity of pesticides (Carbofuran and Paraquat). Different researchers have reported that animals' progressive change in signs and symptoms due to toxicity levels range from visibly undetectable marks to coiling, extrusion of coelomic fluid, segmental constriction and swelling. In several animals the swollen portion burst causing bloody lesions, limp and eventually death [19,21].

When interpreting ecotoxicological test in predicting environmental risk, death is a major factor measured for drop in numbers of topsoil organisms. So many researchers have reported reduction in worms' abundance, in which some have attributed the reduction to limitations in putrefaction of organic matter. Furthermore, population reductions are rarely interconnected with lethal effects.

San-Miguel et al. [43], recounted some pesticides may possibly present a low lethal risk to earthworms in the wild (natural conditions), and that lower abundance maybe as a consequence of organisms' ability to avoid areas that are seriously contaminated with these toxicants. Declines in their numbers may well be traceable to impacts on reproduction; including the malformation of gametes and embryos, inability to hatch and teratogenic effects [16]. Patel & Prajapati [22] reported a hyper toxicity (LC_{50} = 0.9ppm) of cypermethrin (that is used in farms to control mites) to earthworms, *Eisenia fetida*. Furthermore, [24] in their findings stated that acetamiprid exhibited an LC_{50} of 6.69mg/kg on *Lumbricus terrestris*. In our study, the tested pesticides caused mortality of test animals (earthworms).

3.2 Acute toxicity assay using snails

The Probit results obtained from exposing the snails to Carbofuran and Paraquat contaminated soil are presented in Fig.3 and Fig.4. The median lethal concentration LC_{50} of Carbofuran was 23.22 mg/kg while that of Paraquat was 759000mg/kg for the snail.

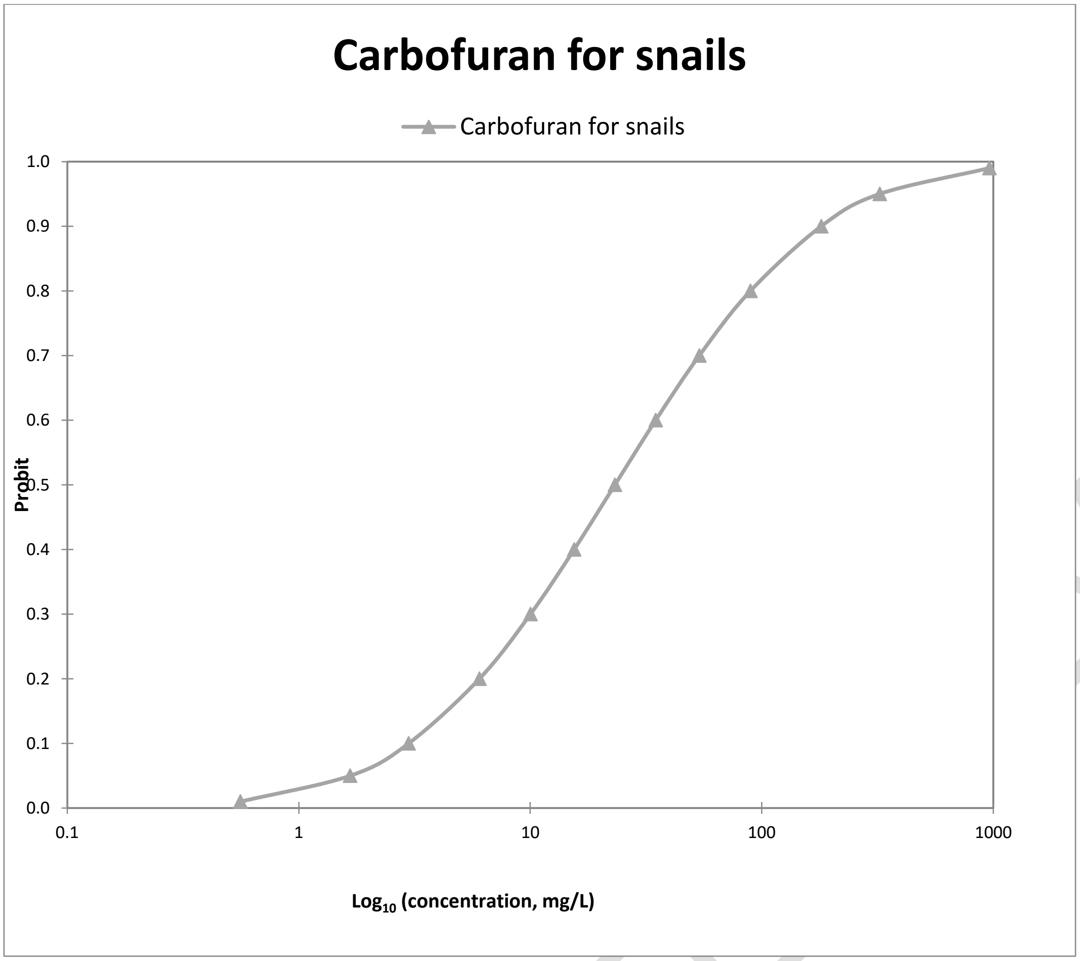

Fig.3. Median lethal concentration (LC_{50}) for snails in Carbofuran = 23.22 mg/kg

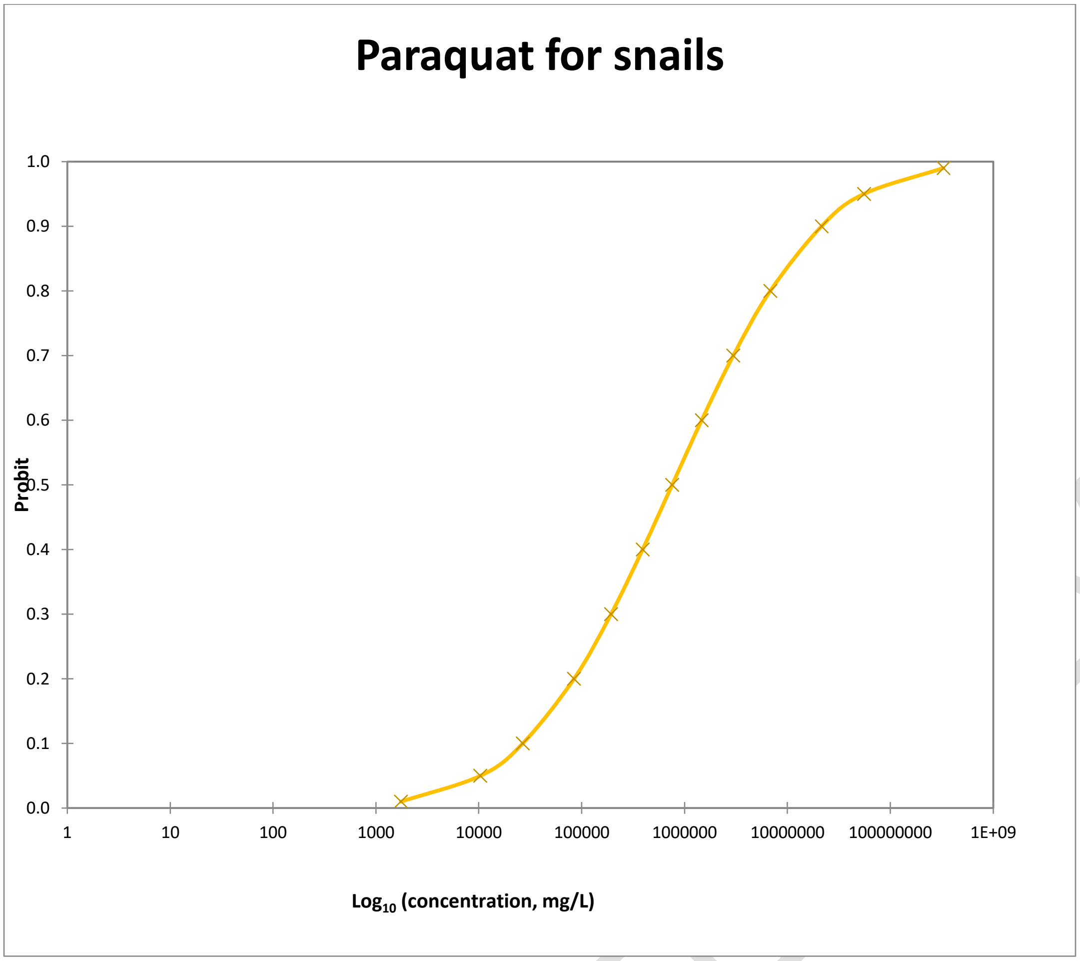

Fig.4. Lethal concentration (LC_{50}) for snails in Paraquat = 759000mg/kg

This study showed that increases in pesticide concentration resulted in mortality of test snails presenting a correlation between concentration and mortality or death. Carbofuran had an LC_{50} value of 23.22 mg/kg whereas Paraquat had 759000 mg/kg. From the results, Carbofuran was highly poisonous to snails while Paraquat was not underpinning soil toxicity rating prescribed by the Organization for Economic Co-operation and Development [42]. The LC_{50} showed that Carbofuran was highly harmful to snails than Paraquat. This report is analogous with previous reports on species where the toxicant concentrations interrelated with lethality of test animals [44]. Ogeleka et al. [16], reported LC_{50} value of Grassate as 1.731 ± 0.05mg/kg which connotes lethality. Also, [23] recounted that Renovan (chlorpyrifos) had an LC50 value of 9.6mg/L and a molluscidal effect on *Biomphalaria alexandrina* snails. Our study established that Carbofuran, a carbamate had a noteworthy impact on snails during exposure and may perhaps cause a drastic drop in population of these viable animals.

3.3 Microbial counts

Figure 5 shows the bacterial counts during the experiment. The total heterotrophic bacterial counts increased in all the pesticides treated soil after the initial decline in numbers.

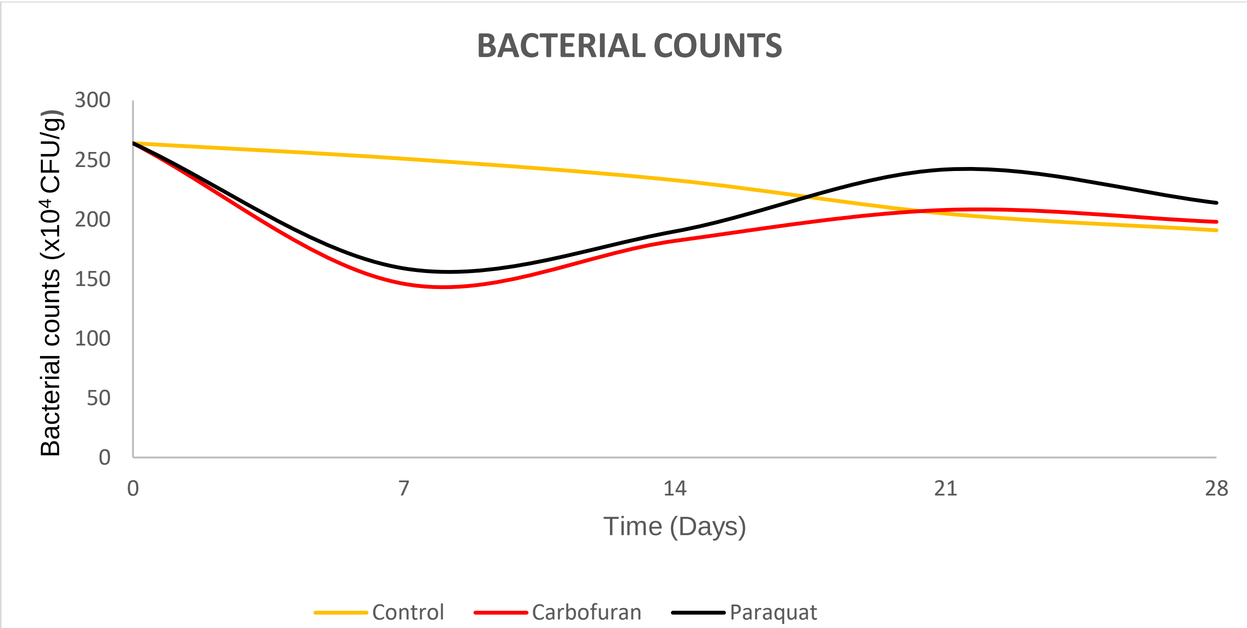

Fig.5. Total heterotrophic bacterial counts.

Total heterotrophic bacterial (THB) counts in Carbofuran and Paraquat treated soils increased from 1.59 × 10^6 CFU/g to 2.41 × 10^6 CFU/g and 1.46 × 10^6 CFU/g to 2.08 × 10^6 CFU/g, respectively for day 14 to 21. A reverse trend was observed in counts from control soil. Diez et al. [45], reported an initial drop in numbers of heterotrophic bacteria and fungi, but counts amplified as the days progressed after the application of pesticides in soil. This was partly observed during our study.

Fungal counts obtained from the study is shown in Fig.6 below. There was a general decrease in fungal counts in the different treatments initially except the control soil.

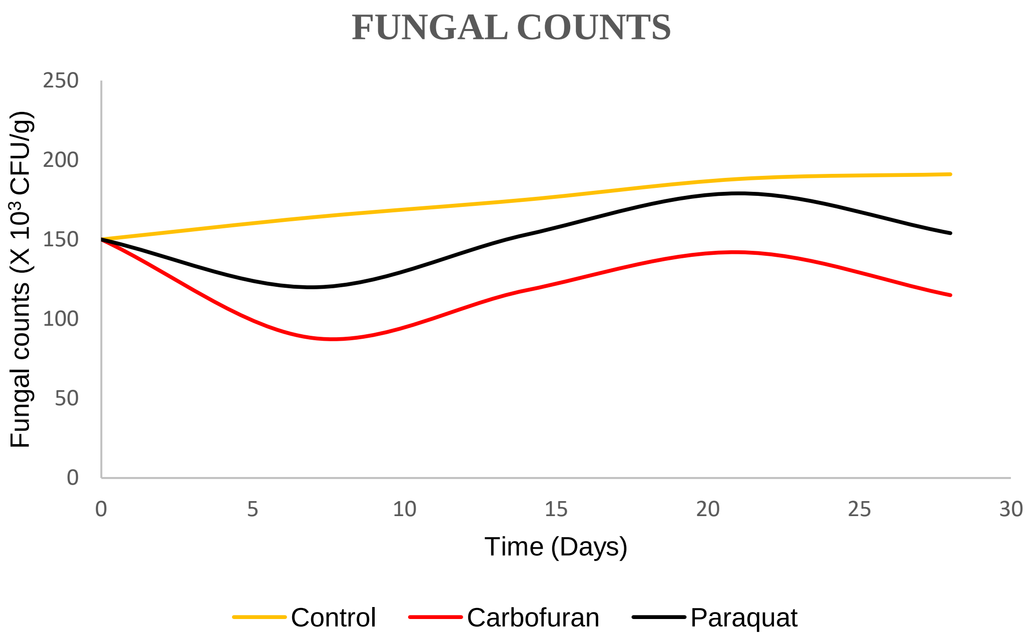

Fig.6. Fungal counts in the different treatments

Fungal counts amplified in Carbofuran (1.18×10^5 CFU/g to 1.42×10^5 CFU/g); Paraquat (1.53×10^5 CFU/g to 1.79×10^5 CFU/g) from day 14 to day 21 and were in the magnitude of 10^4 to 10^5 CFU/g for both pesticides. There were increases in fungal population throughout the study from 1.50×10^5 CFU/g to 1.91×10^5 CFU/g for the control soil.

Actinomycetes counts in response to the different treatments are represented on Fig.7 below.

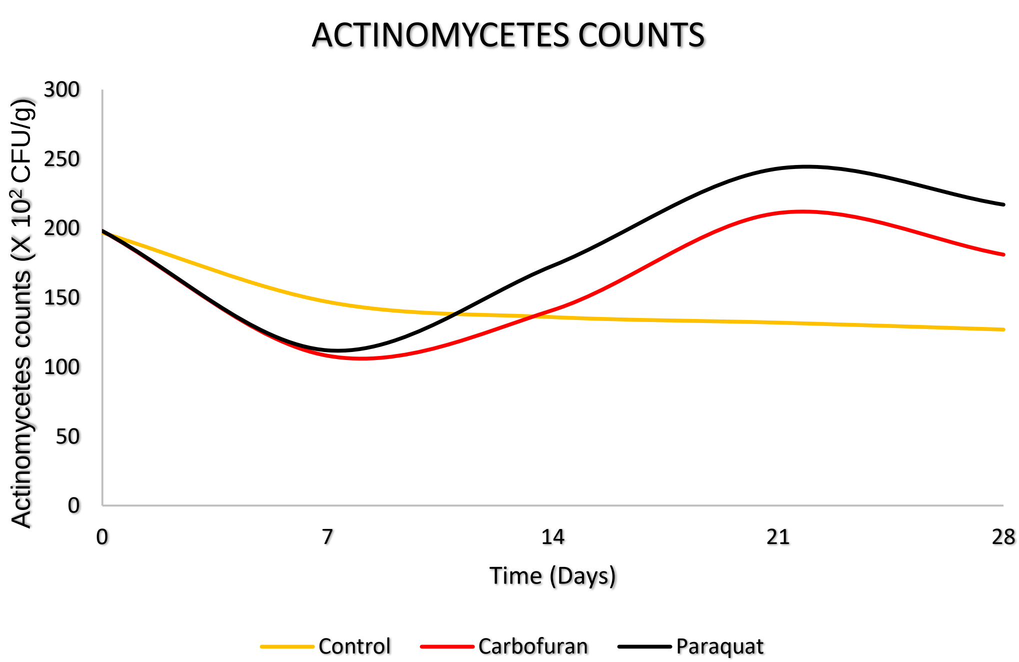

Fig.7. Actinomycetes counts in the different treatments

The actinomycetes counts increased for both treatments from day 14 to day 21, followed by a decline (day 28). Counts increased from 1.41×10^4 CFU/g to 2.11×10^4 CFU/g for Carbofuran and 1.73×10^4 CFU/g to 2.43×10^4 CFU/g for Paraquat. The reverse was the trend for the control soil from 1.97×10^4 CFU/g to 1.27×10^4 CFU/g.

Figure 8 shows the phosphate solubilizers counts during the experiment. Phosphate solubilizers' counts dropped throughout study.

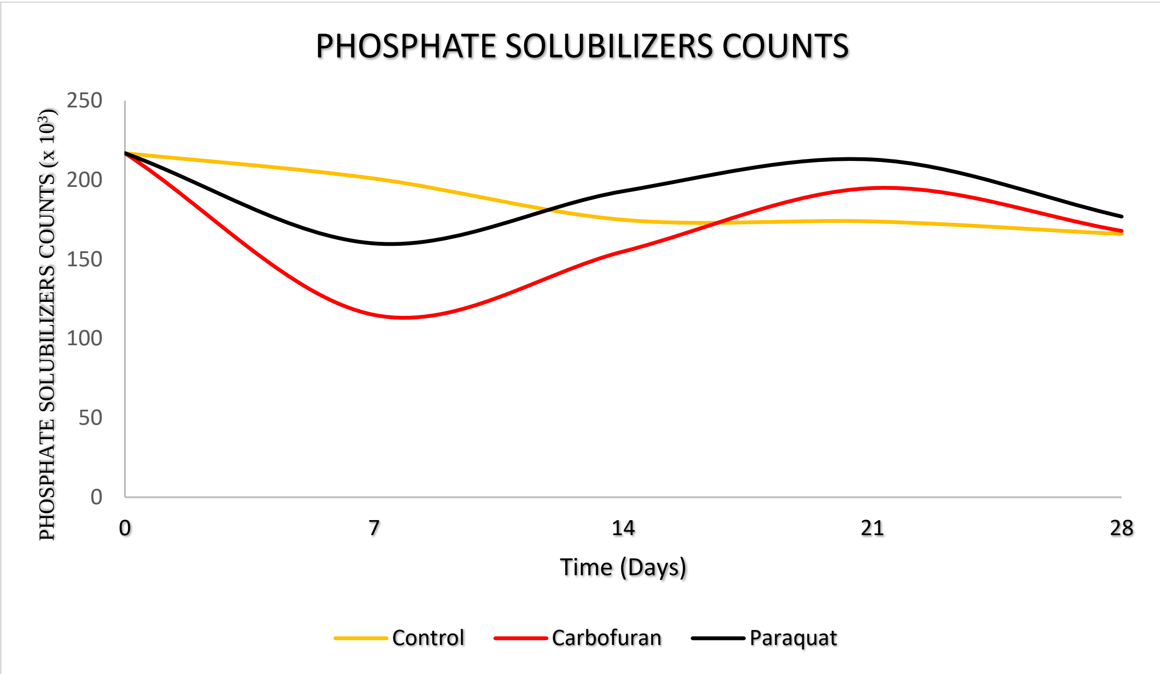

Fig.8. Phosphate solubilizers counts in the different treatments

In Carbofuran treated soil, the phosphate solubilizers increased from 1.55×10^5 CFU/g to 1.95×10^5 CFU/g and 1.93×10^5 CFU/g to 2.13×10^5 CFU/g for Paraquat at days 14 and 21, respectively.

The nitrifying bacterial counts obtained during this study is shown in Fig.9. Furthermore, there was an initial drop in the nitrifiers counts (day 7) and thereafter increases at day 14 and day 21.

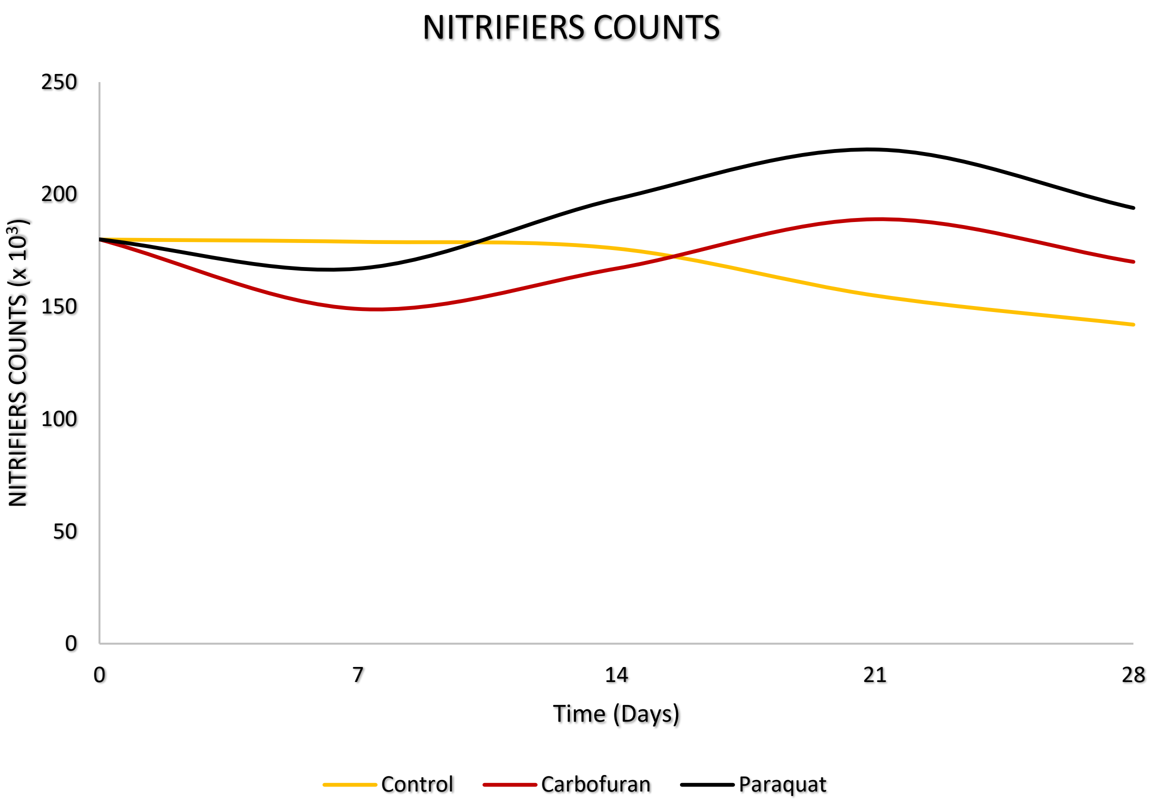

Fig.9. Nitrifiers counts in the different treatments

The counts increased from 1.67 × 10^5 CFU/g to 1.89 × 10^5 CFU/g for Carbofuran treated soil and 1.98 × 10^5 CFU/g to 2.20 × 10^5 CFU/g for Paraquat treated soil. On the other hand, there was a gradual decline in counts from 1.80 × 10^5 CFU/g to 1.42 × 10^5 CFU/g in the control soil.

Microorganisms are used to consider responses to anthropogenic pollution because microbial cells are in direct contact with their environment and are sensitive to these disturbances [46,47,48,49,50]. However, those that are competent and adjust to these ecological changes become the most dominant microbial species. This ability to adjust to anthropogenic disturbances allows microbial analyses in discerning soil health status, shifts in microbial populations and activities; hence, can perform excellently as indices of soil health fluctuations. Sometimes, dynamics in microbial community structure or function provides prompt signal of soil enhancement or caution of soil degeneration prior to demonstrable alterations in chemical and physical properties [51].

The present day study has revealed that the effect of different chemicals/toxicants on microbial populations reduced with time. Increases in counts after initial suppression were pointers to microbial recovery from shock presented by the stressors; their adaptations to these toxicants and their abilities to degrade them. Furthermore, these increases could be traced to the proliferation of microbes on the larger volume of utilizable nutrients released by the dead segment of the populations [52,53,54]. There are reports that several microorganisms possess the ability to breakdown pesticides while others lack such abilities and are highly affected by the type of pesticide and dose applied [46,55].

Hence, pesticides either stimulate or suppress population depending on agrochemicals (type/formulation and concentration), method of use, microbial groups and ecological conditions [55,56]. It has been established that availability of high organic matter accelerates herbicides degeneration in soils, possibly because of dynamic microbial action/exertions. The use of herbicides can reduce total microbial populations in soil, where some studies have traced it to reduced input of organic residues [38]. The more organic matter present in a soil, the longer an insecticide persists in it. According to several researchers [57,58,59] pesticides leads to qualitative and quantitative adjustment in soil microbial communities/groups.

Culture-dependent protocol revealed that there was an increase in total heterotrophic bacteria (THB) population in both pesticide treated soils, after an initial decrease in counts was observed during this study. Bacterial counts were in the magnitude of 10^6 CFU/g soil for Carbofuran and Paraquat treatments. Jiang et al [60] reported that the long term application of acetochlor had no significant impact on the overall soil microbial community composition after years of application.

A significant difference (P= 0.05) was recorded in fungal counts with reference to both pestcides and duration (time). Fungal counts increased from 10^4 CFU/g soil to 10^5 CFU/g soil for Carbofuran and Paraquat-treated soils. Actinomycetes counts were in magnitude of 10^4 CFU/g soil for both Carbofuran and Paraquat treated soils, while population of phosphate solubilizers and nitrifiers were in 10^5 CFU/g soil.

4. CONCLUSION

Research has shown that pesticides have caused serious impact on soil environment. Such soils have negative effects on humans and the natural ecosystem. There's need for the protection of the health of the natural terrestrial ecosystem and in turn protect human health as well as other vertebrates and invertebrates. Growth of earthworms and snails exposed to agro-pesticides are useful bio-indicators of soil pollution. Acute toxicity test indicated test organisms (earthworms and snails) were more endangered by Carbofuran (LC_{50} 10.7 mg/kg; 23.22 mg/kg) relative to Paraquat (LC_{50} 159571 mg/kg; 759000 mg/kg), respectively. Toxicity test estimates the possibility that antagonistic environmental impacts/influence might take place or are taking place due to susceptibility to sole or additional pesticides.

In practice, pesticides are applied multiple times under various environmental conditions, and commercial formulations contain a range of additional compounds that are not disclosed. The soil used in this study was collected from a pesticide free environment. Therefore, it is possible that either pesticide-tolerant organisms were present or these organisms developed the abilities. Despite being largely consistent with previous studies, our results must be inferred with restraint and additional work is crucial to effusively understand the impending influences of pesticides on soil microbial populations. Pesticides caused transient impact on microbial populations at recommended field application rate and were particularly sensitive to small changes in the environment. The gradual increase in microbial counts may be attributed to their ability to temporarily mineralize and use pesticides as energy source. No research is encompassing, hence there's need to assess information on the chemical nature, mode of action and approaches to degradation of these toxicants in the terrestrial ecosystem to reduce the negative effects to fauna and organisms at higher trophic levels.

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