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Impact of Pesticides on Microbial Diversity and Enzymes in Soil

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Authors' contributions

This work was carried out in collaboration among all authors. Author GSCO designed the study and wrote the protocol. Author TLA performed the laboratory analyses, statistical analysis and wrote the first draft of the manuscript. Author POO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The presence of pesticides in soils could result in alterations in microbial activities (enzyme activities, microbial populations), soil physical and chemical properties.
Research gap/Challenges: Insufficient literatures on extensive monitoring of soil quality through enzyme activity, during pesticides application. Existing literatures concerning analysis of effect of pesticide application on soil enzyme activity are not comprehensive with respect to number of soil enzymes analysed.
Aim/Objective: The study was conducted to investigate the effect of carbofuran and paraquat on soil biochemical characteristics on certain soils in the Niger Delta region of Nigeria.
Methodology: These pesticides were applied at recommended doses, their effects on soil organic carbon, enzymes activity and microbial populations were assessed using standard methods. The enzymes monitored were amylase, invertase, protease, urease, phosphatase and dehydrogenase. Microbial counts were carried out for total heterotrophic bacteria, fungi, actinomycetes, nitrifying bacteria and phosphate solubilizers using the spread plate method.

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Results: There were variations in the different enzyme activities in carbofuran – and paraquat - treated soil during this research. Dehydrogenase activity increased in treated soils. Also, urease activity was lower compared to other enzyme activities. As the study progressed, variations in values of soil organic carbon were observed. There was a gradual increase in microbial counts and can be traceable to their ability to temporarily mineralize and use the pesticides as carbon and energy source. The soil organic carbon, enzymes and microbial counts values were significantly different at *P=0.05*.
Conclusion: This research revealed that the pesticides cause temporal impact on microbial populations and enzyme activities, associated with the pesticide type at recommended field application rates. A change in numbers, activity and diversity of soil microorganisms may act as indicators of soil fertility and reflect the soil quality.

Keywords: Microbial diversity; soil enzymes; phosphate solubilizers; nitrifying bacteria.

1. INTRODUCTION

A soil microbial diversity index is important in understanding biogeochemical and nutrient cycling influenced by a diverse group of microorganisms. Biological and biochemical processes in soils are important to terrestrial ecosystem function because all members of trophic levels depend on soil for nutrients and energy and for transformation of complex organic compounds. To fully understand microbial functions, processes are studied at multiple levels: molecular (plant fibre structure and enzymatic characteristics of degradation), organismal (functional gene analysis, regulation of enzyme expression and growth kinetics) and community (metabolism, microbial succession and competition between microbial faunal communities) resolution [1].

Soil enzyme activities are sensitive to all kinds of environmental perturbations and can be used to monitor such shifts in the ecosystem. They are useful in identifying positive or negative effects of residue management, soil compaction, tillage, crop rotation and soil contamination during reasonable time period as well as response to different agricultural soil conservation practices such as non-tillage, organic amendments, crop rotation, and organic cultivation. Interestingly, dehydrogenase, urease and phosphatase have been studied mostly due to their roles in organic matter transformation processes and phosphorus cycle in agricultural practices. Soil biochemical assays have been used to analyse soil fertility or productivity, microbial biomass [2] and effects of toxicants. Also, soil enzymes have been used to monitor biogeochemical cycling [3,4], total microbial activity, predict bioremediation alongside its potential success, to understand rhizosphere effect and a potential indicator of soil quality [5].

Urease is the hydrolytic enzyme in conversion of urea into carbon dioxide and ammonia. It plays a major role in the nitrogen-economy of soil. Hence, it has been studied elaborately because of its role and to better our understanding of the mineralization process of nitrogen element and its response to the application of inorganic fertilizers and soil management systems particularly its relationship to the agricultural practices. Furthermore, the survival of ammonium fertilizer oxidizers in forest and agricultural soils depends on it [6]. Studies also showed that fertilization and cropping practices have significant effects on its activity in soils [6].

The release of inorganic phosphorous from organically bound phosphorus to soil as litter and other organic debris is catalyzed by phosphatase. Phosphatase activity is essential for conversion of organic substrates containing phosphorus into inorganic form through hydrolysis in the soil. The amount of phosphatase in any ecosystem is important as it affects the rate of phosphorous cycling. This enzyme action can be inhibited by inorganic phosphate, which produces a feedback inhibition of this enzyme. Over the years, studies on acid and alkaline phosphatase activities have been used to understand the phosphorus cycling as it relates to organic matter and its turnover in soil [7,8,9].

Soil dehydrogenase is an extra-cellular enzyme used to assess microbial oxidative activity. It is an excellent tool used to measure any disruption caused by perturbations such as pesticide application, trace element discharge and soil management practices, as a measure of microbial biomass and measure of soil respiration. Investigations have shown that its plays a major role during the initial stages of the oxidation of soil organic matter as electron or

hydrogen donors from substrates to acceptors. Hence, it has been studied extensively on various fields of agriculture and science due to its importance in the organic matter transformation processes and its potential to indicate the available microbiological activity in the soil [10].

Invertase and amylase are both involved in the carbon cycle. Amylase and invertase catalyze the hydrolysis of polysaccharides and release monosaccharides to provide labile carbon and energy sources for supporting living microbes in soil [11,12].

Protease is an enzyme involved in the initial hydrolysis of protein components of organic nitrogen to simple amino acids which is an important step in the nitrogen cycle. They are widely distributed in soils and perform a range of activity. Protease hydrolyze proteins to polypeptides and oligopeptides to amino acids involved in the nitrogen cycle [13].

Numerous reports, however, have indicated that soil enzyme activities were significantly affected when pesticides were applied to soil at higher than recommended rates over long periods. The studies on alterations in microbial activities and numbers brought about by pesticides have been undertaken by several authors [14,15]. While most of the reports suggest that the application of these chemicals decrease the microbial population [16,17] others increase their population when these products are applied to soil [18].

With this background, the present investigation was undertaken with the aim to evaluate the eco-toxicity of two commonly used pesticides in agricultural systems, representing the carbamates and bipyridium families on soil organic carbon, microbial flora and different soil enzymes under microcosm conditions.

2. MATERIALS AND METHODS

2.1 Place and Duration of Study

The study was conducted in the Department of Environmental Management and Toxicology, Effurun, and Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan, Nigeria/ three months.

2.2 Soil Sampling

The soil used for experiment was collected from the Federal University of Petroleum Resources, Effurun, Delta State. Soil was collected from 0-15cm layer of a field that had received no pesticides. The soil was sandy loam in texture, neutral in pH, high in organic carbon, medium in N and K and low in P. It was thoroughly homogenised and passed through 2 mm sieve. Microcosms were prepared with 3kg soil samples in sterile containers of 5L capacity. Moisture content was adjusted to field capacity using sterile deionised water. Soil samples were kept in dark for one week before exposing them to the treatments. Soil samples were treated with pesticides at recommended rates [for paraquat (5 ml/kg soil) and for carbofuran (0.1 g/kg soil)] [19] every week for eight weeks while the control received sterile water only. All the containers were incubated at 25 ± 2ºC. The samples were collected for analysis weekly after the eighth week of pesticides application.

2.3 Soil Organic Carbon

Soil organic carbon (OC) in different pesticides treated and control soil samples were determined by partial oxidation method (20) through titration against 1N $(NH4)_2$ $Fe(SO4)_2.6H2O$ using diphenylamine indicator [20]. To 1 g of soil sample in a 250 ml Erlenmeyer flask, 10.0 ml of 1N potassium dichromate was added and the mixture was gently shaken to disperse soil. Thereafter, 20 ml concentrated tetraoxosulphate (iv) acid was added, swirled and allowed to stand for 30 minutes. A 100 ml distilled water was added, followed by the addition of 10 ml O-phosphoric acid. Three to four drops of the indicator was added and titrated with 0.5N ferrous ammonium sulphate till colour changed from green to blue and finally to red (end point).

2.4 Analysis of Soil Enzymes

The enzymatic activities chosen for assay were dehydrogenase, amylase, invertase, urease, protease and phosphatase. Enzymatic activities were determined using Hitachi (220) spectrophotometer according to standard described methods.

2.4.1 Determination of amylase activity

The amylase activity of soil was determined in adaptation to the procedures described by Somogyi [21] and Roberge [22]. To 5 g of sample in a 50 ml Erlenmeyer flask, 1.5 ml toluene was added and the mixture was gently shaken and allowed to stand for 15 minutes. Thereafter, 10 ml distilled water and 5 ml of 2% soluble starch

solution were added to the soil mixture. The flask was stoppered and incubated at 37°C for 5 hours. After the incubation, 15 ml distilled water was added to the soil mixture, the contents were mixed and 10 ml of the suspension was centrifuged at 3000rpm and the clear supernatant was filtered read at 600nm using spectrophotometer. To perform control, the starch solution was added to the reaction mixture after incubation.

Calculation:

Amylase activity (µg glucose released/gram oven dry soil/ hour at 37°C) = CxV/dwt x sw x t

Where;

C is the measured concentration of glucose (pg ml'1) in the sample minus measured concentration of glucose (pg ml'1) in the blank. V is the volume of the suspension in ml, sw is the weight of soil taken (1 g), dwt is the weight of lg oven dry soil and t is the incubation time in hours.

2.4.2 Invertase

The method of Ross [23] was adopted in the estimation of invertase. To estimate invertase activity, 10 ml of 0.1 M acetate buffer (pH 5.8) and 5 ml of 10 % (w/v) sucrose solution (in 0.1 M acetate buffer, pH 5.8) were added to 5 g of toluene treated soil (0.5 ml for 15 min) and incubated at 37°C for 24h. After incubation, 25ml of distilled water was added to the flask and thoroughly mixed. The rest of the procedure was the same as described for the estimation of the amylase activity. Soil amylase and invertase activities were expressed as microgram glucose equivalents (reducing sugars) per gram of dry soil (µg glucose/gram of soil).

2.4.3 Protease

Soil sample (0.5 g) was mixed with 0.3 ml of phosphate buffer, 1.2 ml of tyrosine, 1.2 ml of water and incubated at 30ºC for 1 hour. The reaction was stopped by the addition of 2.0 ml of 0.3 mol/L NaOH. After 30 minutes, 1 ml of acetic acid and 1.0 ml ninhydrin reagent were added to the mixture. The mixture was left in boiling water for 15minutes and immediately cooled in an ice water bath for 30minutes. The absorbance was measured at 570 nm in spectrophotometer and the amount of protease was estimated from the standard tyrosyl leucine solution [24].

2.4.4 Urease

The activity of urease was measured by the method of Tabatabai and Bremmer [25]. One gram of fresh soil was kept in 100 ml volumetric flask and to it 1 ml of toluene was added. It was then allowed to stand for 15 minutes to permit the complete penetration of toluene in to the soil. Thereafter, 10 ml of buffer (pH 7) solution and 5 ml of 10% Urea solution were added. The flask was shaken and incubated at 37°C for 3 hours in an incubator. Where as in control, 10 ml of distilled water was added instead of urea solution. After incubation, the volume was made up to 100 ml by adding distilled water. The content in the flask was mixed thoroughly and was filtered through Whatman filter paper No. 5. Indophenol blue method was adopted for the measurement of ammonia released as a result of urease activity. 0.5 ml of filtrate was taken in a 25 ml volumetric flask and to it 5 ml of distilled water was added. The mixture in the flask was treated with 2 ml of phenolate solution and 1.5 ml of sodium hypochloride solution containing 5% of active chlorine. The final volume was made up to 25 ml by adding distilled water. The optical density was read at 630 nm. The amount of NH_4 + -N released was calculated by a reference-calibrated curve and was expressed as NH_4 + - N mg per gram dry soil per three hours.

2.4.5 Phosphatase

The soil phosphatase activity was estimated employing the procedure of Kelly and Kabana [26]. To 5 g of air dried soil, taken in a 100 ml conical flask, 1.5 ml of toluene was added, mixed well by shaking and then allowed to stand for 15 min. To this 10 ml of 0.1 M Tris -HC1 buffer (pH 7.0) and 5.0 ml of 0.013 M disodium phenylphosphate in Tris - HC1 buffer (pH 7.0) were added. The flask was stoppered and then incubated at 37°C for 3 h. After incubation, 10 ml of the suspension was centrifuged at 3000 rpm for 20 minutes. To estimate the phenol released to 1 ml of the clear supernatant, 1 ml of Folins phenol reagent (1:1 dilution) and 2 ml of 20% (w/v) Na2CC>3 solutions were added. The mixture was kept in boiling water bath for 2minutes and then kept in room temperature for 20 min. After 20 min the solution was brought to a volume (10 ml) and the optical density was measured at 650 nm. Phosphatase activity was expressed as micrograms of phenol released per gram of soil (µg phenol/gram of soil).

2.4.6 Dehydrogenase

The 2-3-5-Triphenyl tetrazolium chloride (TTC) reduction technique [27,28] was used for the estimation of dehydrogenase activity in soil. One gram of fresh soil was taken in a test tube. The soil was then mixed with 0.1 g of calcium carbonate ($CaC0_3$) and 1 ml of 1 % TTC solution. The mixture was then shaken and plugged with a rubber stopper and incubated at 30° C for 24 hours in an incubator. Three replicates were maintained in each case. The resulting slurry was transferred on Whatman filter paper No.1 and extracted with successive aliquots of concentrated methanol. The volume of the filtrate was made to 50 ml by adding methanol. The optical density of the filtrate was read at 485 nm, using methanol extract as a blank. The activity was represented in terms of concentration of formazan, which was calculated by a standard curve of triphenyl formazan in methanol. Dehydrogenase activity per gram dry soil was expressed in terms of milligram formazan per gram dry soil per hour.

2.5 Assessing the Microbial Population

The population count of microbes; bacteria, actinomycetes, fungi and two functional groups viz., nitrifying bacteria and phosphorus solubilising microbes (PSM) were taken to examine the effect of pesticides on their respective populations. Microbial counts were carried out using the plate count agar (PCA) for bacteria [29], Rose Bengal chloramphenicol agar for fungi [30], starch casein agar for actinomycetes [31], Pikovskaya's medium for phosphate solubilizing bacteria [32] and Ashby medium for nitrifying bacteria [33]. Serial dilution plate count method was used for enumeration of colony forming units (cfu). Microbial population counts were taken after an incubation period of 48 hours for bacteria, 48–72 hours for fungi, 96 hours for PSM and seven days for nitrifying bacteria.

2.6 Statistics and Statistical Analysis

Data was subjected to two way analysis of variance (ANOVA) for the significance of treatment effects and the mean values were compared using least significant difference (LSD) test.

3. RESULTS AND DISCUSSION

3.1 Soil Physico-chemical Analysis

The results of the physico-chemical analysis on the unpolluted soil sample are shown on Table 1 below:

Table 1. Physico - chemical properties of the soil sample

Parameter	Values
Total organic carbon (%)	8.6
pH	6.71
Nitrates (mg/kg)	36.28
Phosphates (mg/kg)	2.33
Sodium (meq/100 g)	11.52
Potassium (meq/100 g)	4.71
Calcium (meq/100 g)	6.11
Magnessium (meq/100 g)	0.01
Soil particle size distribution (%)	
Loam	2.65
Sand	97.192
Clay	0.158

3.2 Soil Organic Carbon

There were variations in the total organic carbon present in the different pesticide treated soil as seen in Fig. 1. In carbofuran treated soil, there were increases in organic carbon (OC) till day 21 and then a decrease at day 28. The organic carbon in paraquat increased from day 7(1.62%) to 14(2.45%), followed by a decrease at day 21(1.94%) but later increased at day 28(2.48%). This was similar to the findings of Baboo et al. (30). There was a gradual increase in the TOC from day 7 to 28 in the unpolluted soil (control). Organic carbon values were statistically significant with respect to the different pesticide treatments at *P=0.05* value. Some researchers [34,14,30] have established that the degradation of some pesticides is faster in soils with high concentration of organic carbon probably as a result of high microbial activities. More so, the fate of these pesticides is highly influenced by the presence of organic carbon which supports their removal from the soil.

3.3 Enzyme Activities

The protease and urease enzymes were chosen for their importance in the nitrogen cycle while amylase and invertase in the soil carbon cycle. Dehydrogenase was chosen for the estimation of

the overall microbial activity and phosphatase for its importance in the phosphorus cycle. Results of the enzyme analyses for the pesticides treated soil are shown below.

Amylase activity increased from day 7 (22.50 µg glucose/g soil) to 14 (23.01 µg glucose/g soil) and decreased from day 21 (22.10 µg glucose/g soil) to 28 (21.21 µg glucose/g soil) for carbofuran (Fig. 2). However, the amylase activity increased continuously for paraquat from 29.50 µg glucose/g soil (day 7) to 33.32 µg glucose/g soil (day 28) throughout the study. The variation in amylase activity with respect to different pesticides and days was significant at *P=0.05* value. The differences in the values of amylase activity could be due to the pesticide induced changes in starch degrading enzyme [30,35]. Consequently, stress is induced by nutrients unavailability. These pesticides are degraded by soil microbial enzymes and in so doing incorporate the intermediates or products into their biomass. Furthermore, some microbial groups (pesticide degrading bacteria and fungi) could start the breakdown of these pesticides almost immediately after their introduction into the environment [36]. Different researchers reported that buprofezin and acephate at higher concentrations were toxic to the amylase activity [11,37,38]. In some other pesticide studies, activity was stimulated at lower concentrations, but was negatively affected when higher rates of monocrotophos, quinalphos, cypermethrin, fenvalerate, malathion and permethrin were applied to soil [11].

Fig. 3 shows the results of invertase activity during this study. The invertase in paraquat exhibited a consistent decrease from the 7th day (35.09 µg sucrose/g soil) to the 28th day (31.87 µg sucrose/g soil). There were variations in invertase activity in the carbofuran treated soil. The activity ranged between 26.82 µg sucrose/g soil – 28.52 µg sucrose/g soil. The activity increased in control from day 7 (36.75 µg sucrose/g soil) to 14 (37.68 µg sucrose/g soil), a decrease was observed at day 21 (31.05 µg sucrose/g soil) and an increase at day 28 (33.83 µg sucrose/g soil). The variation in invertase activity with respect to different pesticides and days was significant at *P=0.05* value. Studies have shown that the invertase enzyme is a very stable, persistent and associated with different soil components [30]. However, there were variations in the invertase enzyme activity in carbofuran treated soil during this research. The decrease in the activity could be attributed to the cellular destruction by toxic compounds present in the pesticides in addition to the decreased nutrient mobilization and glucose concentration [39].

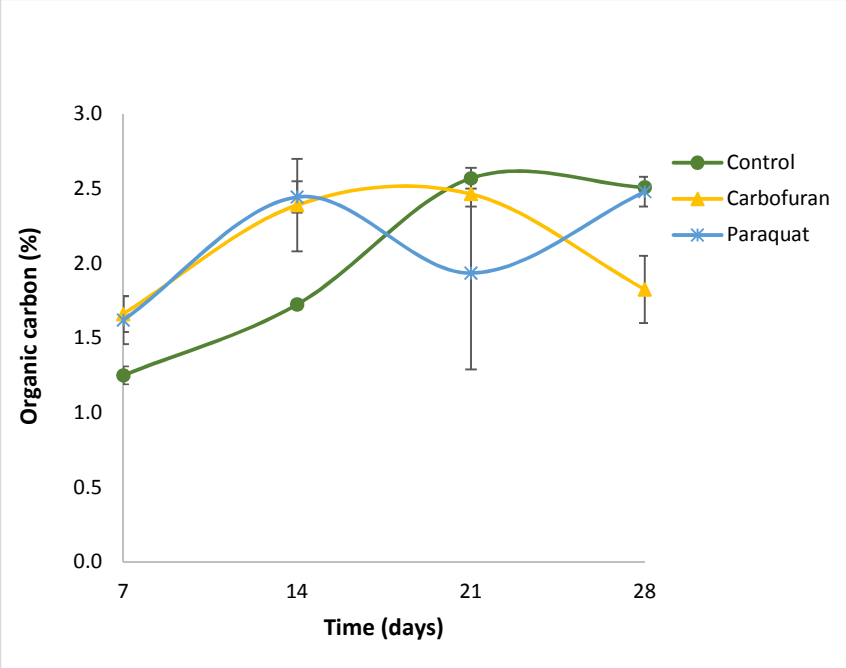

Fig. 1. Effect of herbicides on organic carbon on different days after treatment

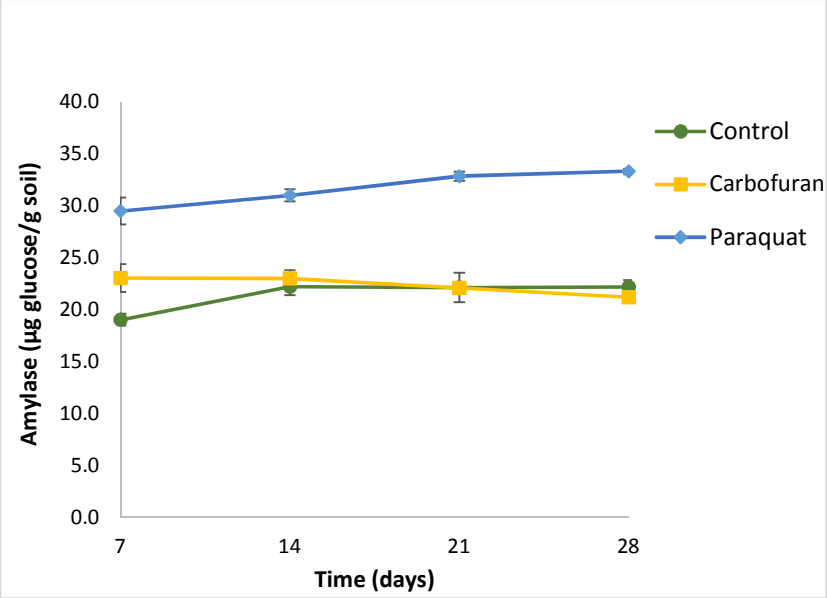

Fig. 2. Effect of pesticides on amylase on different days after treatment

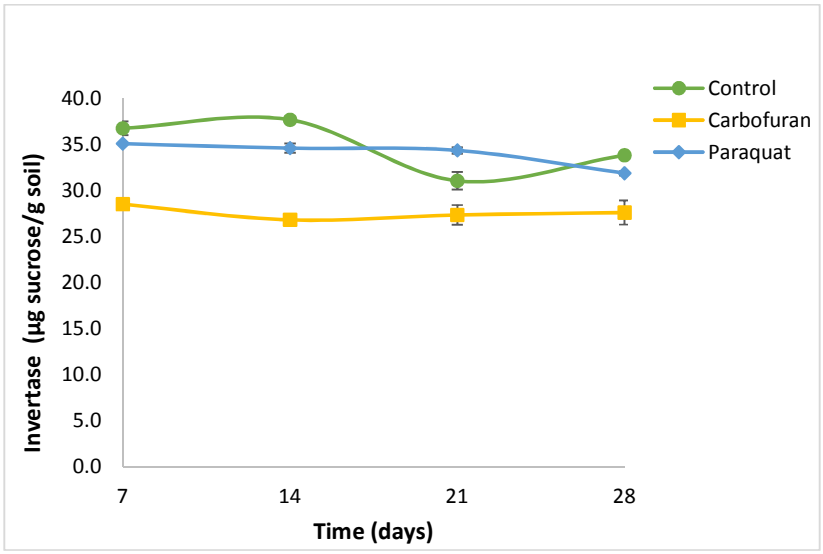

Fig. 3. Effect of pesticides on invertase on different days after treatment

Moreso, invertase is one of the extracellular enzymes present in the soil such that the death of some microorganisms would lead to the reduction in the production and release of these enzymes' activity. However, these enzymes can be harnessed following the adaptation and growth of microorganisms as well as the effective degradation of these pesticides releasing adequate nutrients to support microbial growth. Madella and Kadiyala [38] investigated the effects of buprofezin and acephate on invertase activity and reported that they were detrimental to the enzyme by the repeated applications and at higher rates. Invertase activity increased by 110.9% at 1.6 µg baythroid g^{-1} soil and was decreased by 40.3% at the highest level studied [37]. Sreenivasulu and Rangaswamy [40] reported that invertase activity in soil increased with increasing concentrations of fungicides but application of the pesticides at higher rates had negative effects on the enzyme activities. On the other hand, carbaryl insecticide applied at a

normal agricultural dose had no inhibitory effect on invertase activity. According to Madalla and Kadiyala [38], long-term atrazine applications significantly reduced the activity of invertase in soil. From our study, the selected pesticides had impacts on the selected enzymes in vitro but remains unclear how these are actually influenced at field level. In conclusion, the strict use of pesticides at recommended doses only should be encouraged.

The protease activity in paraquat decreased at day 14 (30.405 µg tyrosine/g soil) and increased from 30.71 µg tyrosine/g soil (day 21) to 32.33 µg tyrosine/g soil at the 28th day. There were variations in the activity values in the control as well as the carbofuran treated soils (Fig.4). The variation in protease activity with respect to different pesticides and days was significant at *P=0.05* value. There are reports that protease activity relies on the concentration of available proteinaceous compounds, ammonium nitrate deposits present in the soil as well as the presence of proteolytic microorganisms [41].

The urease activity in paraquat peaked at day 14 (4.505 µg urea/g soil) and reduced till day 28 (1.365 µg urea/g soil). Again, there were variations in the soil treated with carbofuran throughout the study (Fig. 5). The variation in urease activity with respect to different pesticides and days was significant at *P=0.05* value. It's been established that soils exhibit urease activity; that the enzyme are extracellular and bound to inorganic and organic soil colloids making them to persist in the environment. This study revealed that urease activity was the least amongst other enzyme activities evaluated. This was also the reports of Baboo et al., [30] in their work. It can be inferred that the presence of these enzyme activity in soil could be due to a proportion of these enzymes locked up in the proliferating microbes, non-proliferating microbes, in association with/or in cell debris and these fractions are not assessed during studies. Hence, one can conclude that such urease proportion predominate in soils.

From Fig. 6, the phosphatase activity showed a decrease in paraquat from day 7 (13.00 µg nitrophenol/g soil) to day 21 (10.855 µg nitrophenol/g soil) and then increased at day 28 (12.07 µg nitrophenol/g soil). There were variations in the values for carbofuran and the control during the investigation. The variation in phosphatase activity with respect to different pesticides and days was significant at *P=0.05* value. Filimon et al. [42] reported a significant decrease in phosphatase activity when cypermethrine and thiamethoxam were applied to soil at field recommended rates.

The pesticides and days of incubation had significant effect (*P=0.05*) on soil dehydrogenase activities. The results in Fig.7 shows that as the days of incubation increased, the soil dehydrogenase activities increased concomitantly to the 28th day in the treated soils. The results of the control samples showed decreases than those of the pesticide treated soils which dropped upon treatment with pesticides initially. During the investigation, paraquat treated soil exhibited the highest dehydrogenase activities of 10.245 µg formazan/g soil/hr and 16.605 µg formazan/g soil/hr at the 21st and 28th day, respectively. The activity in carbofuran treated soil increased throughout the study period from 5.615 µg formazan/g soil/hr (day 7) to 13.74 µg formazan/g soil/hr (day 28). The variation in dehydrogenase activity with respect to different pesticides and days was significant at *P=0.05* value. In this study, dehydrogenase activity decreased at day 7 (first week of sampling). This might be due to shock or toxic effect of the treatment (pesticide) leading to decreases in microbial populations with the capability of utilizing the pesticides as carbon source. However, dehydrogenase activity increased after the 7th to the 28th day of incubation in the present study for all treatments. This can be attributed to the ability of the microbial populations to overcome the stressors; developing the capability of utilizing them as carbon source and proliferating in such environment.

There are reports that there was an increase in metabolic activity with atrazine concentration and with incubation time [43,14]. Results from this study further confirms these reports. The intracellular dehydrogenase enzyme is a measure of the overall microbial activity because it is linked with the microbial mediated oxidation-reduction processes. Dehydrogenase plays an important role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors. Thus, soil dehydrogenase activity is seen as a valuable tool to examine the impacts of peaticides treatments on the soil microbial biomass.

This study has shown that the effects of the different chemicals on both enzymes activities and microbial communities reduced with time. Increases in both microbial counts and enzymes activities after the 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 higher amount of utilizable nutrients released by the dead fraction of the microbial populations [16,44]. 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 [34,14].

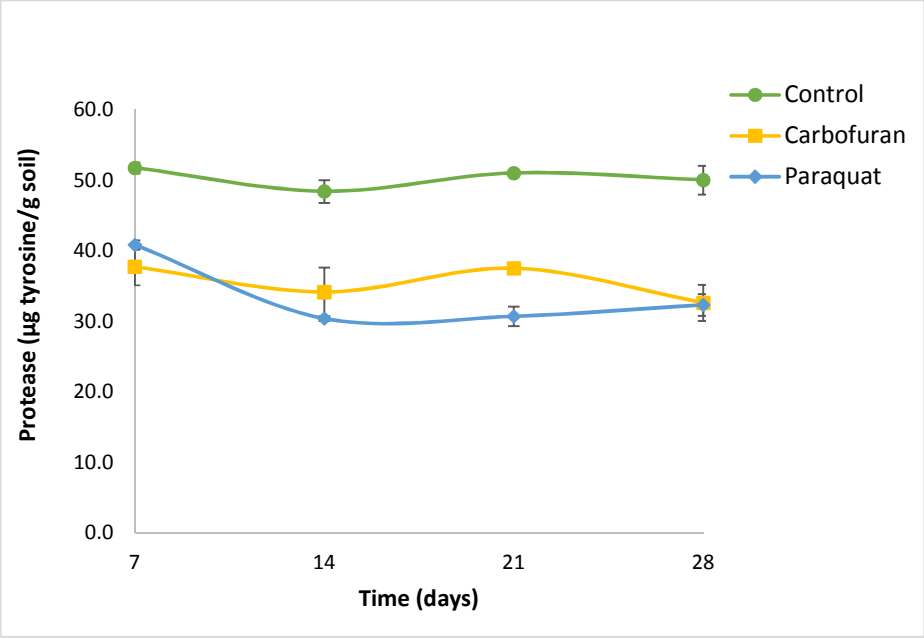

Fig. 4. Effect of pesticides on protease on different days after treatment

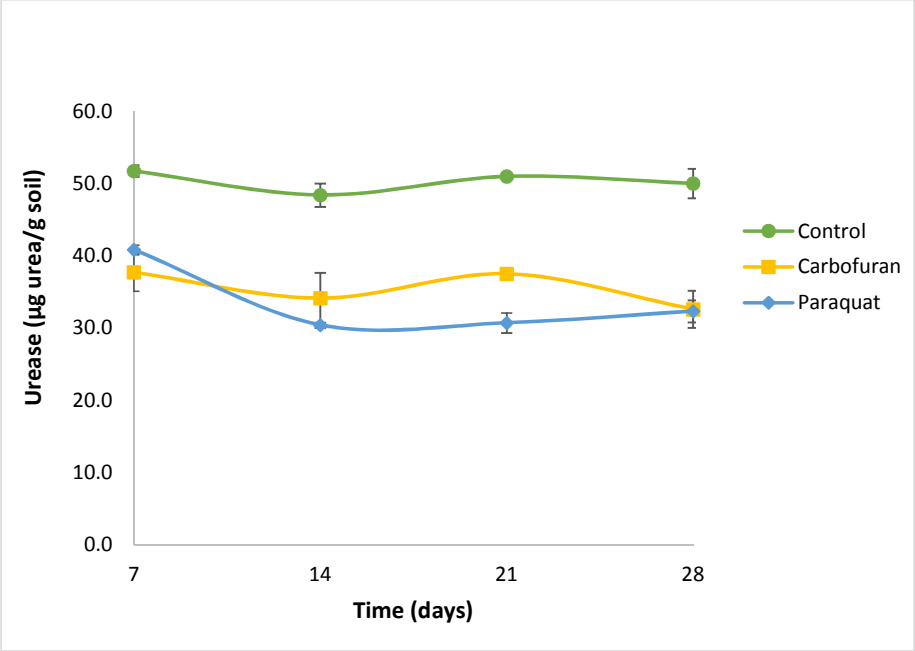

Fig. 5. Effect of pesticides on urease on different days after treatment

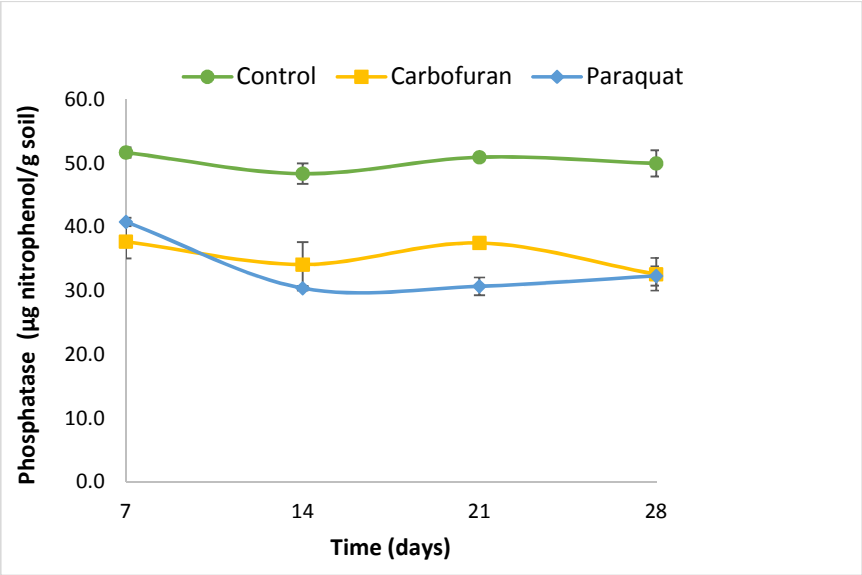

Fig. 6. Effect of pesticides on phosphatase on different days after treatment

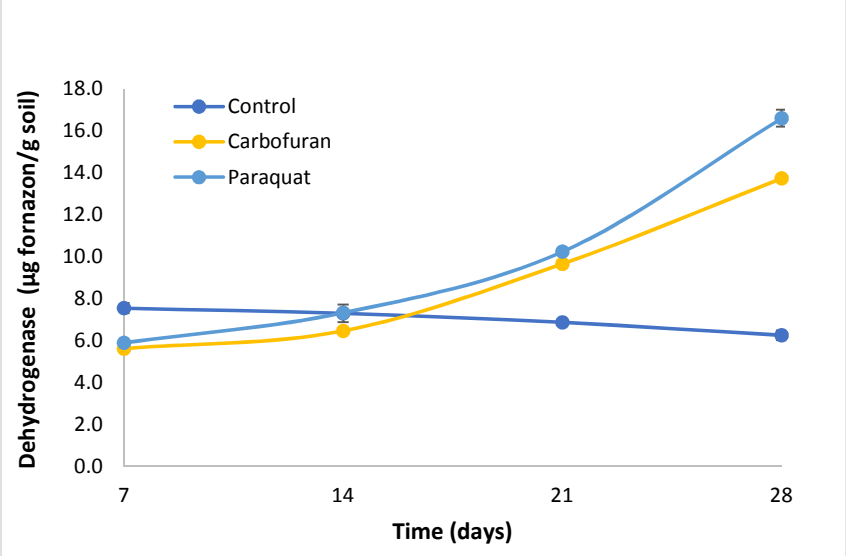

Fig. 7. Effect of pesticides on dehydrogenase on different days after treatment

Hence, pesticides either stimulate or suppress soil microbial population depending on the agrochemicals (type/formulation and concentration), mode of application, groups of microorganisms and environmental conditions [45,46]. It has been established that the degradation of herbicides is faster in soils with high organic matter, probably due to vigorous microbial activity. Use of herbicides can reduce total microbial populations in soil, where some studies have traced it to reduced input of organic residues [30]. The more organic matter present in a soil, the longer an insecticide persists in it. According to several researchers [16,34,47, 48,49,50], pesticides can cause qualitative and quantitative change in soil microbial populations.

The microbial abundance and distribution of different groups of soil microorganisms were enumerated and expressed in terms of colony forming units per gram soil (cfu/g). During this study, there were observed differences in the microbial population present in the different

pesticide treated soils. The variations in microbial population are presented in cfu/g soil.

The total heterotrophic bacterial (THB) counts increased in all the pesticides treated soil after the initial decline in numbers (Fig. 8). THB counts in carbofuran and paraquat treated soils increased, ranging from 2.18 x 10^6 cfu/g soil to 7.7 x 10^6 cfu/g soil and 2.38 x 10^6 cfu/g soil to 1.10 x 10^7cfu/g soil, respectively. A reverse trend was obtained in the counts from the control soil. Cycon and Piotrowska-Seget [51] reported that there was an initial decline in the population of heterotrophic bacteria and fungi, but was stimulated at higher doses of an organophosphate insecticide (diazinon) in soil, which we also observed from this study. The variation in bacterial counts with respect to different pesticides and time were significant at *P=0.05* value.

There was a general decrease in fungal counts in the different treatments initially except the control soil as shown in Fig. 9. Fungal counts increased in all the treatments from day 7 to day 21. The fungal counts were 8.53 x 10^4 – 1.40 x 10^5 cfu/g soil for carbofuran and 1.99 x 10^5 – 1.73 x 10^5 cfu/g soil for paraquat, respectively. There were decreases in the fungal population throughout the study from 1.58 x 10^5 to 1.04 x10^5 cfu/g soil for the control. Thus, the application of both pesticides resulted in higher fungal counts as compared to control. This is in conformation with the reports of Baboo et al. [30]. The variation in fungal counts with respect to different pesticides and time were significant at *P=0.05* value.

Actinomycetes counts increased in both pesticides treated soil from day 7 to day 21 and then a reduction was observed for the samples at day 28. Carbofuran increased from 1.05 x 10^4 to 2.08 x 10^4 while paraquat increased from 1.08 x 10^4 to 2.39 x 10^4 cfu/g soil. At day 28, actinomycetes counts reduced to 1.79 x 10^4 and 1.95 x 10^4 in carbofuran - and paraquat - treated soil, respectively as shown in Fig. 10. There was a gradual decrease in actinomycetes counts from 2.01 x 10^4 to 1.25 x 10^4 cfu/g soil in the control samples. The variation in actinomycetes count with respect to different pesticides and time after treatment was significant at *P=0.05*.

Also, there were increases in nitrifying bacterial counts during this study as seen in Fig. 11. The counts decreased from 1.84 x 10^5 to 1.35 x 10^5 cfu/g soil in the control soil, however, increased in carbofuran treated soil from 1.46 x10^5 to 1.83 x10^5 cfu/g soil and from 1.70 x10^5 – 2.16 x 10^5 cfu/g soil in paraquat treated soil. The variation in nitrifying bacterial count with respect to different pesticides and days was significant at *P=0.05* value.

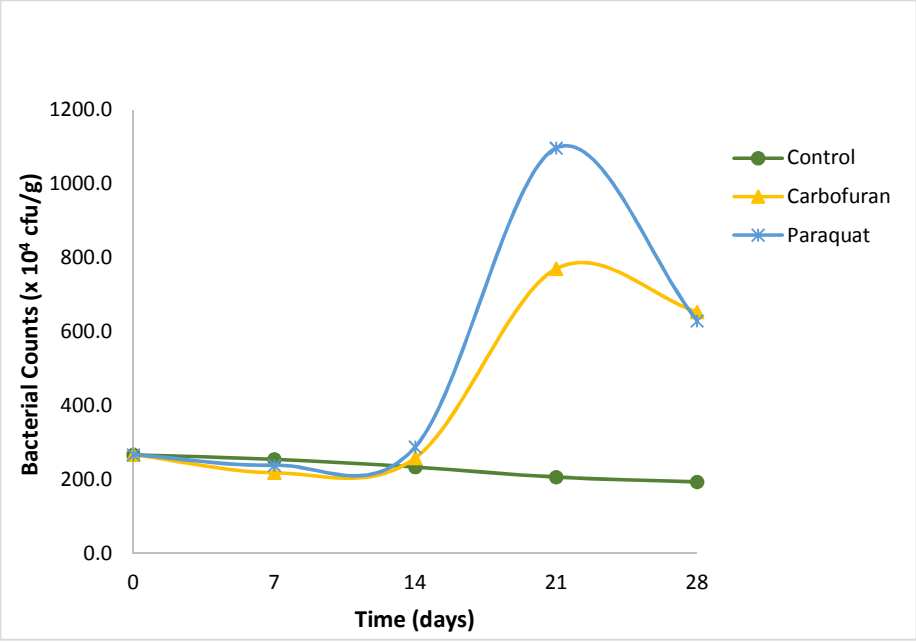

Fig. 8. Enumeration of total heterotrophic bacteria in pesticides treated soil

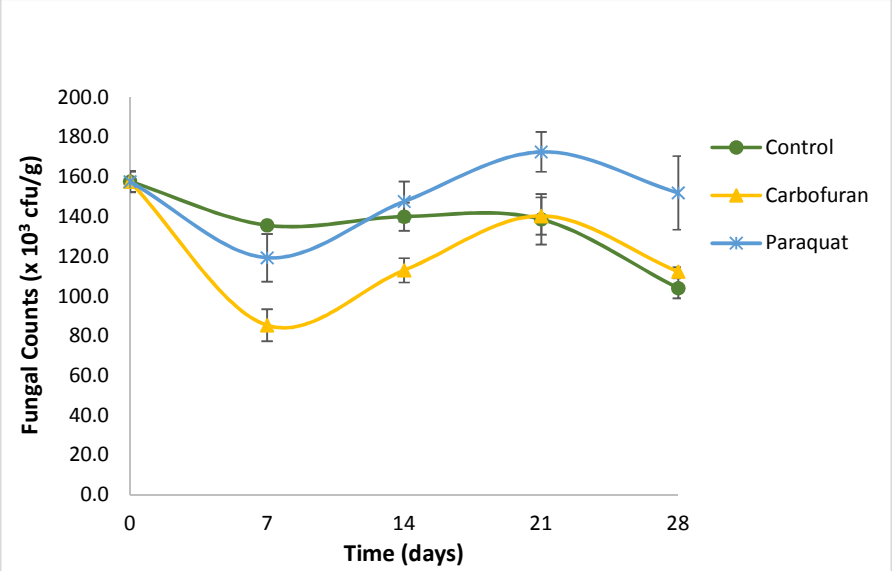

Fig. 9. Enumeration of fungi in pesticides treated soil

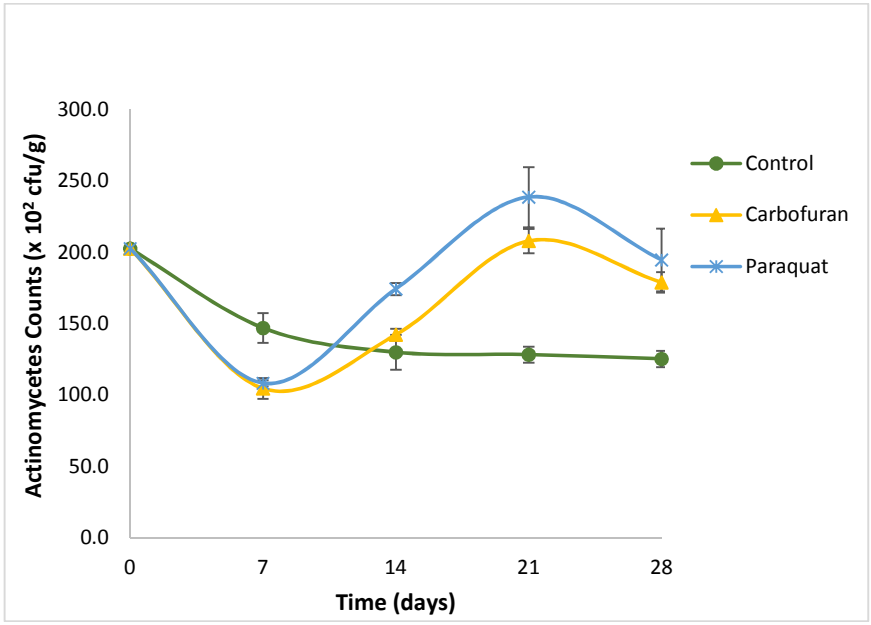

Fig. 10. Enumeration of actinomycetes in pesticides treated soil

Similar trend was observed in phosphate solubilizers counts during this study. There were increases in the pesticides treated soils and a reverse in the control soil. Carbofuran increased from 1.11 x 10^5 - 1.94 x 10^5 cfu/g and paraquat 1.62 x 10^5 to 2.08 x 10^5 cfu/g soil (Fig. 12). The variation in phosphate solubilizers count with respect to different pesticides and time was significant at *P=0.05* value.

In this study, there was a gradual increase in heterotrophic aerobic bacteria, actinomycetes fungi, phosphate solubilizers as well as nitrifiers counts in different pesticides treated soil as the study progressed. The rise in microbial counts in pesticides treated soil may be due to their ability to mineralize or breakdown and use these chemicals as carbon and energy source [52]. However, the initial decreases in microbial counts in treated soils may be attributed to the

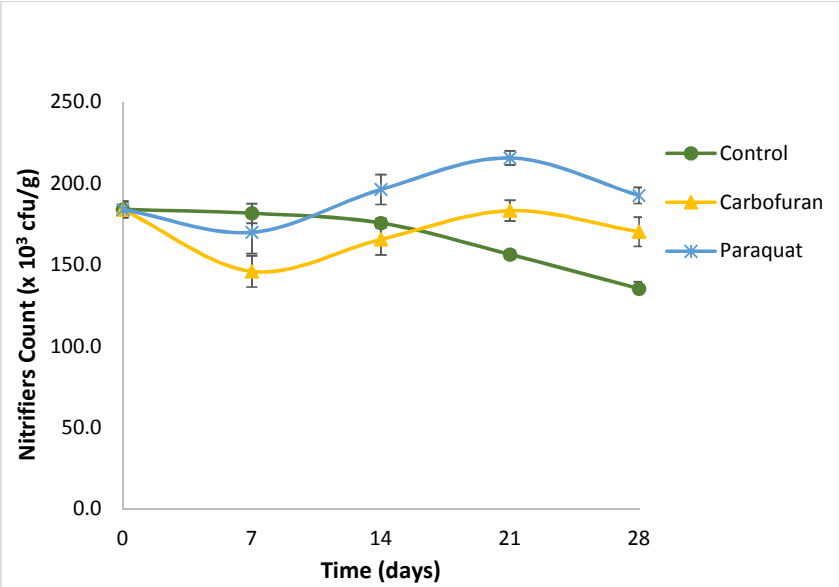

Fig. 11. Enumeration of nitrifiers in pesticides treated soil

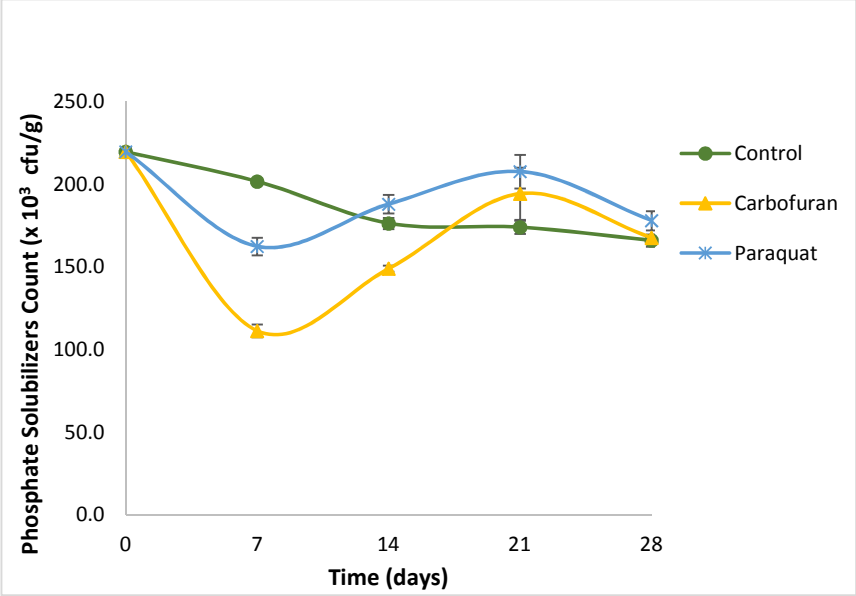

Fig. 12. Enumeration of phosphate solubilizers in pesticides treated soil

fact that microbial populations were susceptible to the products of soil-pesticide interactions that could possibly be deleterious [53].

4. CONCLUSION

This study was borne out of the fact that there exists insufficient literatures on extensive monitoring of soil quality through enzyme activity, during pesticides application. Existing literatures concerning analysis of effect of pesticide application on soil enzyme activity are not comprehensive with respect to number of soil enzymes analyzed. Also, it has been established that pesticides, even when applied at field recommended rates, a good fraction of it gets to the soil, altering its microbial population and distribution as well as the physicochemical characteristics of the ecosystem. This study thus, revealed the inhibitory and non-inhibitory effect of

the pesticides studied, on the different soil enzymes and microbial population evaluated. Hence, soil enzyme activity and microbial population can be used as important indices for studies concerning soil pollution. Therefore, further studies addressing other soil parameters (for instance, soil micro fauna and other enzymes) affecting soil microorganisms, their activities as well as the soils health should be conducted to assess the pesticides impact on the environment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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