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Effects of Chronic Use of Herbicides on Soil Physicochemical and Microbiological Characteristics

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Authors' contributions

This work was carried out in collaboration between both authors. Author LETA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author TLA managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Herbicide application is a vital strategy of weed control. The effects of these chemicals on the non-target soil microorganisms are very intense; have adverse impact on physicochemical parameters of the soil, which in turn affect soil fertility and plant growth.
Research Gap: There are insufficient literatures on extensive monitoring of the effects of prolonged herbicides use. Existing literatures concerning analysis of effect of prolonged herbicides application on soil are not comprehensive with respect to number of soil characteristics analyzed.
Aim: This study assessed the effects of Atrazine and Glyphosate on physicochemical properties and microbial population of carrot and maize farm soils, exposed to prolonged use at Songhai Delta.
Place and Duration of Study: The study was conducted in Songhai Delta farms and the Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, from April to July, 2019.
Methodology: These pesticides were applied according to the manufacturers' instructions at sublethal concentrations. Their effects on soil pH, cation exchange capacity, total organic carbon,

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nitrates, phosphates, sulphates and microbial populations at two depths (0-15 and 15-30 cm) were assessed using standard methods. Microbial counts were carried out for total heterotrophic bacteria and fungi using the pour plate method.

Results: There were variations in the different properties of the carrot and maize farm soils. Soil pH was higher in maize farm (5.91±0.10) than in carrot farm (5.88±0.06) at the depth of 15-30 cm. The pH, total organic carbon, nitrate content had no significant difference while phosphate and microbial counts were significantly different at *P=.05.*

Conclusion: This assessment has shown that the herbicides had no influence on pH, total organic carbon, nitrate but a negative one on bacterial and fungal populations with prolonged use. A modification in physicochemical and microbiological characteristics of soil could be used to predict the fertility and health status of soils.

Keywords: Herbicides; Atrazine; Glyphosate; Physico-chemical Parameters; Total Heterotrophic Bacteria; Fungi.

1. INTRODUCTION

The use of herbicides in agriculture have contributed tremendously to both food and cash crop production all over the world. One of the challenges undermining the farming business [1], has been the invasion of many common weed species due to favorable environmental conditions such as abundance of rainfall, adequate sunlight, fertile soil etc. in Nigeria. Hence, manufacturers have adopted flooding the market with all kinds of herbicides that are meant for the elimination of different kinds of weeds at different stages of their growth [2]. Perhaps, the efficacy of these herbicides in controlling the target weeds has resulted in the application of these chemicals by most farmers.

The soil serves as the repository for all agricultural contaminants, function as a major habitat for most microbial communities such as bacteria, fungi and actinomycetes whose activities influences the soil fertility [3], through organic material degradation, organic matter decomposition and nutrient cycling [4,5]. Nonetheless, over application of these chemicals inhibit some of these natural processes, and decreases the performance of the non-target organisms [6].

Atrazine (6 – chloro - N^2 – ethyl - N^4 – isopropyl - 1, 3, 5 – triazine - 2, 4 - diamine) belongs to the group of triazines. Triazines interfere with photosynthesis in plants. Atrazine is a selective systemic herbicide used for pre - and post - emergence control of annual grasses and broad leaf weeds in a variety of cultivated crops [7].

Glyphosate (N-phosphonomethylglycine) is a broad spectrum non-selective systemic herbicide and crop dessicant. It is an organophosphorus compound precisely a phosphonate which acts by inhibiting the plant enzyme 5 – enolpyruvylshikimate - 3 -phosphate synthase *via* the Shikimic acid pathway which is ubiquitous in microorganisms that link primary and secondary metabolism [8].

However, some soil organisms use these herbicides in the process of degradation as carbon energy source for their metabolic activities. Numerous studies have shown that the level of contamination of soil with these chemicals depends on the persistency of the herbicides in the soils environment, the quantity, frequency of application and the toxicity of the chemicals. However, most of these herbicides are designed to persist longer enough to have the desired effect on the weeds [9,10,11].

The fate of herbicides applied onto the soil environment is governed by two major processes; transfer and degradation. The transfer process involves percolation, runoff, flora and fauna uptake, and sorption and desorption, for which the applied chemicals remain physically intact in the soil environment. The degradation processes include microbial decomposition, plant detoxification, rhizosphere chemical breakdown and photodecomposition which are chemically engineered. These two processes determine the persistency of herbicides, its efficacy for weeds, as well as its potential for soil and ground water contamination [6]. Therefore, there is the need to understand the factors affecting the degradation processes of herbicide in order to adopt effective strategies to reduce its persistent period within the soil environment.

A large number of the populace in Nigeria can't read and understand herbicide label. This has resulted in the contamination of streams, rivers and ground water which is an important natural resource [12]. These contaminations do not pose danger to only the non-target organisms and the environment but exposes human beings to many

health implications. Hence, the need to study the effects of some of these herbicides which are commonly used in Nigeria in order to assess their inhibitory effects on some of the beneficial microorganisms in the soil.

In conclusion, herbicides are unique in that they are designed to kill plants. Sufficiently high doses will kill both crop and weed, while small doses have no effect on crops and weed. The action of an herbicide is usually determined by its chemical and physical properties, its effect on plant metabolism, the plant and the environment. The present study evaluated the effects of herbicides on the fertility and microbial population density of farm soils exposed to prolong herbicides use in the farms at Songhai Delta, Amukpe.

2. MATERIALS AND METHODS

2.1 Place and Duration of Study

The study was conducted in Songhai Delta farms, Amukpe - Sapele and the Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria, from April to July, 2019.

2.2 Sample Collection

Soil samples were collected at three different locations; Carrot farm, Old maize farm and the control. The farms had been exposed to the herbicides (Glyphosate and Atrazine) for over three years. Soil samples were collected at depths of (0-15 cm) and (15-30 cm). The carrot and the old maize farm soil samples were collected from Songhai Delta farms, Amukpe-Sapele, Delta State. where the herbicides have been applied for over three years. The control was collected from a farm without the history of herbicide use in Federal University of Petroleum Resources, Effurun. A total of 6 samples were collected at each location, mixed to form a composite sample, placed in clean polyethylene bags and transported to the laboratory where it was preserved at a room temperature before it was required for used. Carrot and maize farm as well as the control farm had sandy loam soils.

2.3 Determination of the Physico-Chemical Parameters

2.3.1 Determination of pH

Ten (10) gram of each of the soil sample was weighed into 50 ml beakers and 25 ml distilled-deionized water was added to form 1:2.5 soil/water mixtures. The mixture were stirred for 30 minutes and allowed to stand for about 5 minutes. Two point calibrations were done with buffer solution having pH of 7 and 4 (buffer 7 and buffer 4). Finally, the pH meter electrodes (JENWA 3510) were immersed into the soil/water mixture and the pH was measured on the upper part of the suspension [13].

2.3.2 Determination of organic carbon

The oxidation method of Walkley and Black [14] with potassium dichromate ($K_2Cr_2O_7$) using O-phenanthroline indicator was used to assess the organic carbon. The acidified dichromate oxidizes the organic carbon as shown in the following reaction: $2Cr_2O_7^{2-} + 3C + 16H^+ \rightarrow 3CO_2 + 8H_2O$. Weigh 1g soil sample and transfer to 250ml Erlenmeyer flask. Pipette 10ml of 1N $K_2Cr_2O_7$ solution into flask and swirl gently to disperse the soil. Add rapidly 20ml concentrated H_2SO_4 (tetraoxosulphate (vi) acid) using an automatic pipette. Immediately, swirl more vigorously for one minute and allow to stand on a sheet of asbestos for about 30minutes. Then, add 100ml of distilled water allow to stand for 30minutes. Finally, add 3-4 drops of indicator and titrate with 0.5N ferrous sulphate solution. At endpoint, add the ferrous sulphate drop by drop until the colour changes sharply from blue to red (maroon color in reflected light against a white background). Make the blank titration in the same manner but without soil to standardize the dichromate. The result is calculated as follows:

$$\%\ \text{Organic carbon} = \frac{\text{N (V1} - \text{V2)} \times 0.3\text{F}}{\text{W}}$$

N = Normality of ferrous sulphate solution
V_1 = ml ferrous ammonium sulphate required for the blank
V_2 = ml ferrous ammonium sulphate required for the sample
W = mass of sample in gram
F = correction factor = 1.33

2.3.3 Determination of nitrate

The NO_3^-, - nitrogen forms in the soil were determined by shaking 1 g of the representative soil sample with 50 ml of 2.5% acetic acid and extracted. The solution was filtered into a clean beaker and labelled. The NO_3^-N forms were analysed using the Brucine method of APHA [13]. Twenty five milliliter of the Brucine reagent and 2 ml concentrated sulphuric acid were added. A

yellow coloured solution was formed and read at 470 nm in the spectrophotometer (Hitachi 220 spectrophotometer) using water as blank. The standard concentration of nitrate in the sample was extrapolated from a standard nitrate graph.

2.3.4 Determination of phosphate

The available phosphate in the soil was extracted with Olsen's extracting solution and analyzed using the ascorbic acid- molybdenum on a spectrophotometer [13]. A 5 ml soil sample extract and 0.8 ml of the combined agent was measured into a clean beaker, and then left for 10 minutes. The bluish solution formed was read at 888 nm using a spectrophotometer (Hitachi 220 spectrophotometer) and distilled water as a blank. Potassium hydrogen orthophosphate and the combined reagent were used in preparing the standard phosphate concentration. This was absorbed at same wavelength. The concentration of phosphate in the sample was extrapolated from a standard graph of phosphate.

2.3.5 Determination of sulphate

The sulphate in the soil was extracted with a 500ml solution of potassium orthophosphate and the sulphate determined by barium chloride ($BaCl_2$. $2H_2O$) – Gelatine turbidimetric method [13]. Pipette 10ml of the sample aliquot into a 25ml volumetric flask, add distilled water to bring the volume to approximately 20 ml and add 1ml of the gelatin – $BaCl_2$ reagent. Make up to volume with distilled water, mix the content thoroughly and allow the mix to stand for 30 minutes. Determine the sulphate at 420 nm within 30 to 60mins using a spectrophotometer. Prepare a set of standard solution containing 1ml of gelatin-$BaCl_2$ reagent and 10ml of the blank digest or extracting solution.

2.4 Microbial Counts

2.4.1 Determination of total heterotrophic bacteria and fungi

The method of Chikere and Ekwuabu [15] was adopted. Nutrient agar (NA) was used for isolation of bacteria while potato dextrose agar (PDA) was used for fungi isolation. Both media were prepared according to manufactures specification. Ten-fold serial dilution was carried out using 1 g of soil sample and 0.85% (w/v) sodium chloride as diluent. The standard pour plate method was used by inoculating 0.1 ml aliquot of the different dilutions into sterile Petri dishes and 15 ml- 20 ml of cooled media was poured into each of the plates. Swirl plates for homogenization, allow the plates to solidify and incubate at 28 ± 2°C for 18-24hours (bacteria) and 48-72hours (fungi). After incubation, individual colonies were recorded as colony forming units (CFU).

2.5 Statistical Analysis

Data generated from the physiochemical parameters, nutrient contents, soil texture and the microbial enumeration were subjected to logarithm transformation and subsequently expressed in graphs. Statistical package for social sciences (SPSS) was used to compare the means of the effect of herbicides on the different farms (old maize and carrot) against the control and analysis of variance (one way-ANOVA) to test the significant differences between the farm soils.

3. RESULTS AND DISCUSSION

3.1 Soil pH

The pH values estimated from the different farms are shown in Fig. 1. The soils' pH in carrot and maize farms were higher than the control at the both depths (0-15 cm and 15-30 cm). However, the pH was higher in maize soil (5.91±0.10) than in carrot soil (5.88±0.06) at the depth of 15-30 cm, while the rerverse was the trend at 0-15cm depth. Furthermore, pH at the depth of 15-30 cm was higher (5.91 ± 0.10) than at 0 to 15 cm (5.87 ± 0.06) in the maize soil. Again, the pH at the depth of 0-15 cm (5.50±0.10) was higher than at 15 to 30cm (5.40±0.04) in control soils. At both depths, the pH values were acidic for all soil samples, this indicated that the herbicides had no effects on the tested soils relative to the control and this maybe due to the nature of the soil in Delta state. According to [16] most farmyard soils have pH between 5.5 and 8.0 but under different agricultural practices, soils' pH values may increase or reduce. The solubility of soil macronutrients, micronutrients or essential trace elements are influenced by soil pH [17]. Also, Ahn et al. [18] reported that in higher pH soils lesser amount of these herbicides is bound to soil particles, making them more available for plant uptake. The one-way ANOVA showed that there was no significant difference in pH values at both depths relative to control soil at $P=.05$ value.

3.2 Cation Exchange Capacity (CEC)

The cation exchange capacity values of soils from different farms are as presented in Fig. 2. The cation exchange capacity in the soil of carrot

and maize farms were higher relative to the control at 15-30cm depth, while at 0-15 cm depth, the cation exchange capacity was higher in the carrot farm, but lower in the soil of maize farm (78.65±0.08 meq/g) at 0-15 cm. The CEC values for control soil were 82.86±0.16 meq/g and 68.79±0.10 meq/g at 0-15 cm and 15-30 cm, respectively. However, the cation exchange capacity was higher in carrot soil than in maize soil at both depths. Also, the cation exchange capacity at the depth of 15-30 cm (86.61±0.12meq/g) was higher than at 0-15 cm (85.19±0.13meq/g) in the carrot soil, while CEC at the depth of 0-15 cm (78.65±0.08meq/g) was higher than at 15-30 cm (74.42±0.06meq/g) in maize soil. Again, there is an indication that the herbicides had no effect on the cation exchange capicity when compared to the control which may be attributed to the long exposure of herbicide on the tested soils. In addition, it could be traceable to high organic matter on the soil surface leading to high cation exchange capacity [19]. At *P=.05* there was no significant difference in CEC values using the one-way ANOVA.

3.3 Total Organic Carbon (TOC)

Fig. 3 shows the total organic carbon (TOC) content of soils from the different farms. The total organic carbon content in the soil of carrot and maize farm were lower at both 0-15 cm and 15-30 cm depth when compared to the control, except at 0-15 cm depth where TOC value equalled that of the control. However, the total organic carbon content was higher in carrot soil than in maize soil at both depths. Furthermore, TOC contents in control (4.59±0.14%), carrot (4.59±0.14%), and maize (4.59±0.08%) at the depth of 0-15 cm were higher than at 15-30 cm in the control (4.00±0.10%), carrot (3.83±0.08%) and maize (3.07±0.17%) in all the studied farms. The herbicides used in this experiment had no effect on the total organic carbon of the tested soils and may be linked to the long use of herbicides. There are reports that the concentration of herbicides present in the soil depends on the carbon content of such environments. According to [20], the higher the soil carbon content the lower the concentration of herbicide in the soil. This is presumably due to vigorous microbial activity. Furthermore, the fate of herbicides is greatly affected by the presence of soil organic matter by aiding their disappearance [21,22]. The one-way ANOVA showed that there was no significant difference in TOC values at both depths (*P=.05*).

3.4 Nitrate

The nitrate content of soils from different farms are shown in Fig. 4. The nitrate content in the carrot farm (26.68±0.07 mg/kg) and maize farm (26.58±0.15 mg/kg) were higher than the control (25.23±0.14 mg/kg) at 0-15cm depth, but the reverse was observed at 15-30 cm depth. However, the nitrate content were higher in carrot farm soil (26.68±0.07 mg/kg and 26.58±0.20 mg/kg) than in maize farm soil (26.68±0.15 mg/kg and 22.76±0.06 mg/kg) at both depths, respectively. Furthermore, nitrate

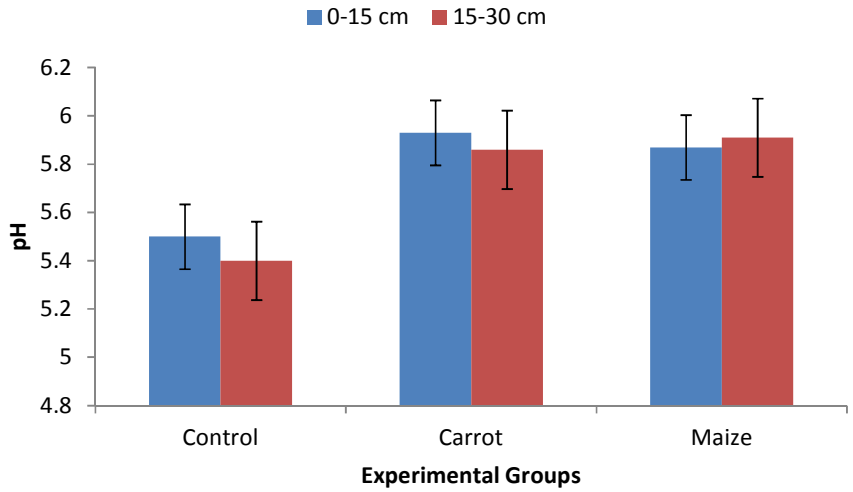

Fig. 1. Effect of herbicides on soil pH in different farms

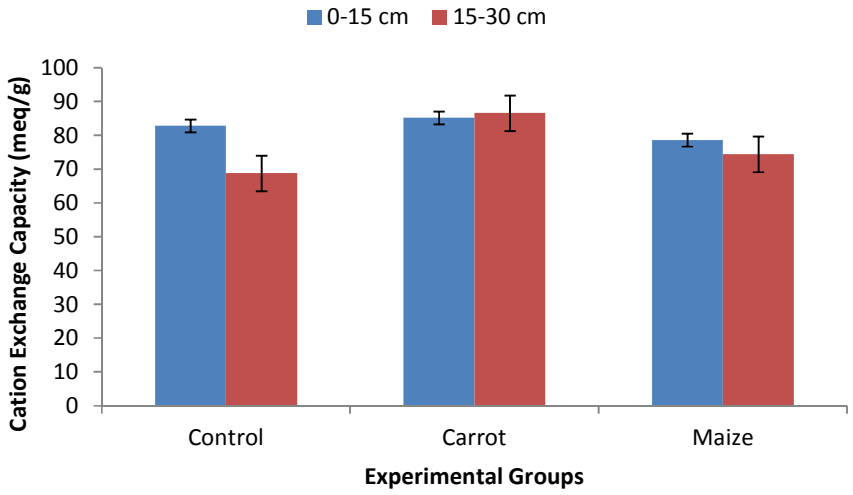

Fig. 2. Effect of herbicides on soil cation exchange capacity in different farms

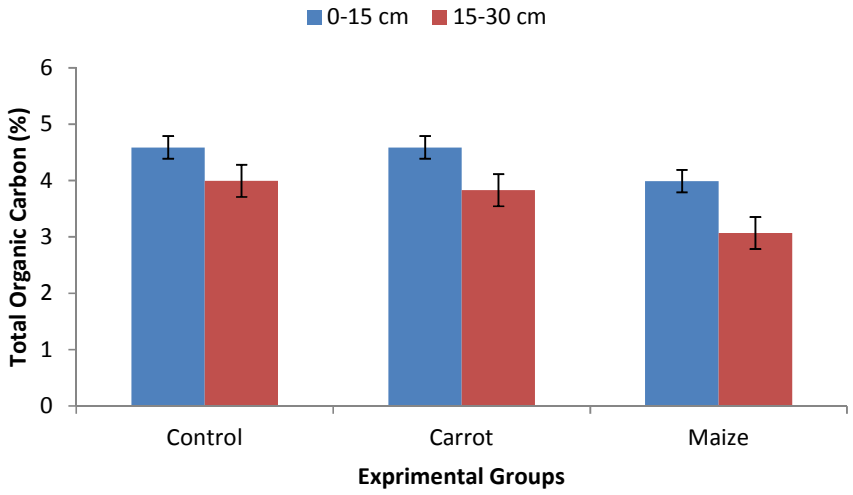

Fig. 3. Effect of herbicides on soil total organic carbon in different farms

content at the depth of 15-30 cm (26.68±0.17 mg/kg) was higher than at 0-15 cm (25.23±0.14 mg/kg) in the control, while nitrate content at the depth of 0-15 cm (26.68±0.07 to 26.58±0.15 mg/kg) was higher than at 15-30cm (26.58±0.15 mg/kg to 22.76± 0.06 mg/kg in carrot and maize soil. This indicate that the herbicide has no effect on nitrate content of the soil and there is increase in nitrate content of the tested soils when compared to the control, this might be due to the used fertilizers that led to much increase in the tested soils. Nutrient management practices like application of organic manures and mineral fertilization can cause an increase in the abundance of nitrate content in the soil. Balezentiene and Kilimas [23] have reported that soil nutrient are influenced by the agricultural management practices, input of fertilizers and pesticides as well as increases with depth. The one-way ANOVA shows that there was no significant difference in nitrate values in different farm soils at both depths at *P=.05*.

3.5 Phosphate

The phosphate content of the soil from different farms are shown in Fig. 5. The phosphate content in the carrot and maize farm soils were

higher relative to the control during the study. The phosphate content was higher in maize soil (34.05±0.06 mg/kg and 36.16±0.08 mg/kg) than in carrot soil (28.9±0.21 mg/kg and 34.40±0.07 mg/kg) at both depth. In addition, phosphate contents at the depth of 15-30 cm were higher than at 0-15 cm in all the studied farms. Phosphate content in control at both depts were 21.46±0.14 mg/kg (0-15 cm) and 25.72±0.07 mg/kg (15-30 cm). The study has revealed that herbicides had no effect on the phosphate content of the soil when linked to the control. This could be traceable to the prolonged used of herbicides and application of fertilizers on the tested soil. Lalfakzuala et al. [24] reported that fertilizer application increases nutrient content. Likewise, an appreciable number of fungi present in this environment have been known for immobilizing or retaining, nutrients in the soil. Dickie et al. [25] has reported that in exchange for carbon from the plant, fungi help solubilize phosphorus and bring soil nutrients (phosphorus, nitrogen, micronutrients and perhaps water) to the plant. At *P=.05* there was a significant difference in phosphate values at both depths using the one-way ANOVA.

3.6 Sulphate

Fig. 6 shows the sulphate content of soils from the different farms. The sulphate content in the carrot and maize farm soils were higher than the control. The sulphate in the control soil was 3.40±0.16 mg/kg and 7.88±0.11 mg/kg at 0-15 cm and 15-30 cm depth. Sulphate contents in carrot farm soil were 7.01±0.23 mg/kg and 11.94±0.11 mg/kg while maize farm soil were 9.43±0.11 mg/kg and 8.11±0.11 mg/kg at 0-15 cm and 15-30 cm, respectively. The sulphate content was higher in maize soil than in carrot soil at 0-15 cm depth, while the sulphate content was higher in carrot soil than in maize soil at 15-30 cm depth. Additionally, sulphate content at the depth of 15-30 cm was higher than at 0-15 cm in the farms, except in maize farm where its depth at 0-15cm was higher than that of 15-30 cm. The present day study has shown that Atrazine and force up had no effect on the sulphate content of the soil relative to the control probably, as a result of thier sustained use on the soil. Lalfakzuala et al. [24] reported that fertilizer application increase nutrient content. The one-way ANOVA showed no significant difference in sulphate values among the different farms at both depths (*P=.05*).

3.7 Total Heterotrophic Bacterial Count (THBC)

The total heterotrophic bacterial count (THBC) of soils from different farms are shown in Fig. 7. The THBC in the soil of maize (3.86±0.09 CFU/g and 3.86±0.09 CFU/g) and carrot (4.12±0.08 CFU/g and 4.38±0.09 CFU/g) farms were very low when compared to the control (6.40±0.10 CFU/g and 7.24±0.05 CFU/g) at both 0-15 cm and 15-30 cm depth. The counts were higher in carrot soil than maize soil at both depths. Also, THBC at the depth of 15-30 cm was higher than at 0-15 cm in both carrot and farms. Nevertheless, there was a sharp decrease in bacterial population at 0-15 cm in the carrot and

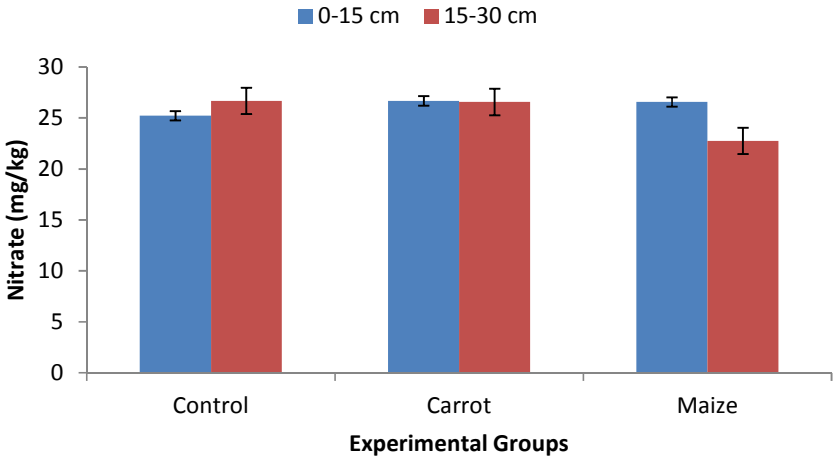

Fig. 4. Effect of herbicides on soil nitrate in different farms

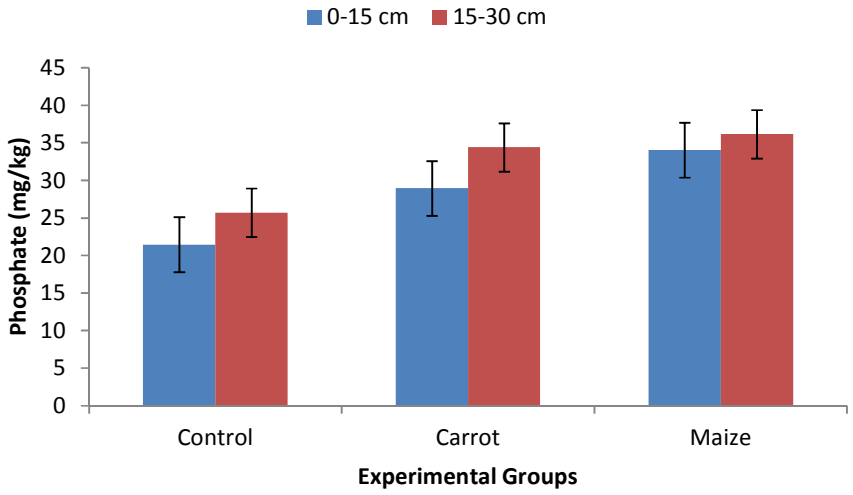

Fig. 5. Effect of herbicides on soil phosphate in different farms

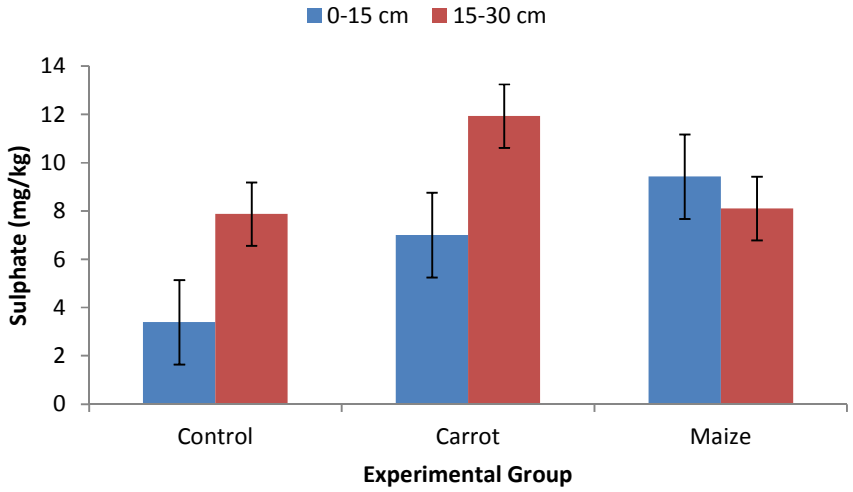

Fig. 6. Effect of herbicides on soil sulphate in different farms

maize farms. Similarly, there was an overall steady decrease in bacterial population from maize and carrot in comparison with the control in the study. This could be ascribed to the fact that the bacterial populations were adversely affected with the subsequent increase in exposure to Atrazine and force up. These results were in line with the findings of Anderson et al. [26] and Xia et al. [27]. Also, Rosli et al. [3] recorded such free-fall decrease in microbial population under similar soil treatment in Malaysia. A one-way ANOVA showed that the variation in total heterotrophic bacterial count with respect to different farms was significant at *P=.05*.

3.8 Total Heterotrophic Fungal Count (THFC)

Fig. 8 shows the total heterotrophic fungi count (THFC) of soil from the carrot, maize and control farms. The THFC in the carrot soil and maize farm soils were lower than in the control. Counts in the carrot and maize farms were 2.12±0.02 CFU/g and 3.24±0.02 CFU/g); (3.86±0.03 CFU/g and 4.20±0.05 CFU/g at 0-15 and 15-30 cm, respectively. In control soil, THFC was 5.36±0.10 CFU/g (0-15 cm) and 6.20±0.02 CFU/g (15-30 cm). Again, the THFC was much lower in the soil of carrot and maize farm when compared to the

control at the depth of 15-30 cm than at the depth of 0-15 cm. Although, THFC was higher in maize soil than in carrot soil at both depth, counts at 15-30 cm depth were higher than at 0-15 cm in all the studied farms. Our findings were in corroboration with the reports of other researchers [28,29]. The fungal count were lower compared to the control at 0-15 cm (surface) due the prolonged used of herbicide at the top surface. Again, the herbicides concentration are higher at the top surface of the soil and a high proportion reaches and accumulates in the microbiologically active top layer of 0 to 15 cm of soil. A one-way ANOVA showed that the variation in total heterotrophic fungal count with respect to different farms at 0-15 cm was significant at *P=.05* and the reverse trend was observed at 15-30 cm.

According to Latha and Gopal [30] the detracting effect of herbicides towards all bacteria, fungi and enzyme activities decrease with time and depth. This increase suggests the capacity of the organisms to degrade some aspect of the herbicides and utilize it as a carbon source to support their growth and multiplication [18,22,31]. However, the initial decreases in microbial counts in treated soils may be attributed to the fact that microbial populations were susceptible to the products of soil-pesticide interactions that could possibly be deleterious [32].

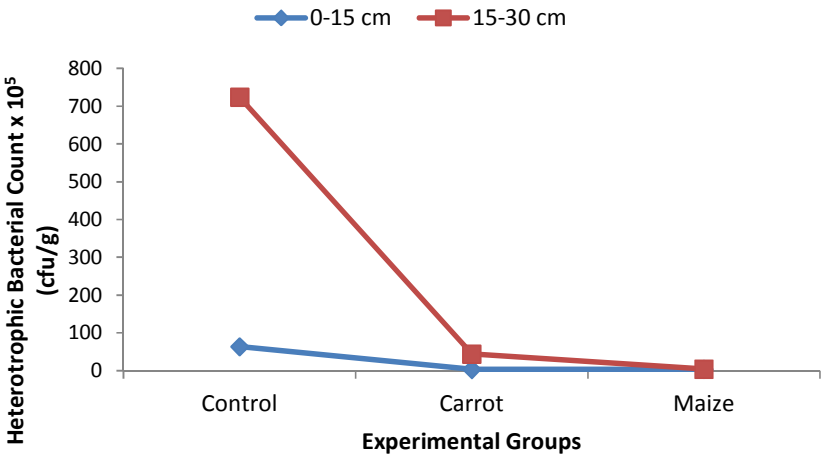

Fig. 7. Total heterotrophic bacterial count from different farms

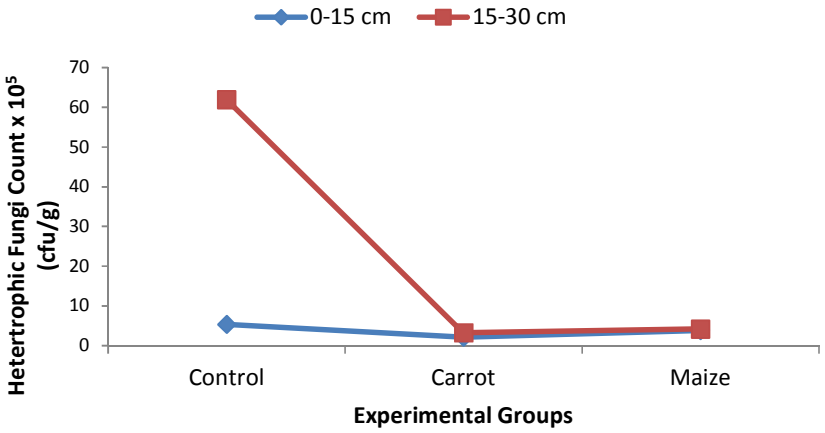

Fig. 8. Heterotrophic fungi count from different farms

4. CONCLUSION

The undiscriminating use of herbicides has progressively turned out to be a matter of environmental disquiet modifying soil fertility status due to their adverse effects on soil microbial communities and physico-chemical properties of soil. Their residual impact must be well thought-out for environmental safety despite the vital usefulness in managing weeds. The herbicides are used either as pre-emergence or as post-emergence; a high proportion of herbicides reaches the soil and accumulates in the microbiologically active top soil altering microbial populations, enzyme activities and biodiversity, which are good indicators of the balance in the agro-ecological system. The study confirmed that the herbicides (Atrazine and Glyphosate) may alter the microbial populations with respect to prolonged treatment thus, disturbing various soil microbial activities such as functions of microbes in biogeochemical cycles, which may lead to soil infertily.

In the application/use of herbicides in Nigeria for control of weeds, the national agricultural policy should consider the toxicity of these herbicides to soil health, degradation, and mitigation/ amelioration of the effects of the herbicides before approval for use in the Nigerian environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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