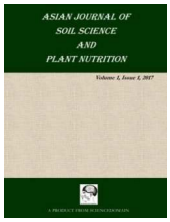

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Assessment of the Microbial Populations and Chemical Characteristics of Paraquat Treated Soil

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Authors' contributions

This work was carried out in collaboration between all authors. Author JKD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BCI and TTD managed the analyses of the study. Author TTD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at assessing the microbial population and chemical components of paraquat treated soils. The soil samples were treated with the low (0.3 ml/L), recommended (0.6 ml/L) and high (0.9 ml/L) doses of paraquat respectively. Microbial and chemical assessments were carried out using standard procedures. The results of the study showed that, microbes such as *Staphylococcus* sp., *Micrococcus* sp., *Pseudomonas* sp., *Escherichia coli, Bacillus* sp., *Actinomycetes bovis, Actinomycetes israeli, Streptomycetes* sp., *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Absidia corymbifera* and *Rhizopus stolonifer* were found present in the paraquat treated soils of different doses with the control soil samples having most of the fungal species isolated. The application of the paraquat to the soils at different weeks after application (WAA) affected the microbes as most were found absent in some of the treated soil samples and

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this was proved by the result of the control soil samples. The organic matter and carbon contents of the paraquat treated soils were at the 4th WAA had the significantly (*P=.05*) highest contents with 1.93%, 1.93% and 1.84% and 1.34%, 1.12% and 1.12% respectively whereas the pH of the soils was at 2nd WAA the highest compared to those of the other soils at other WAA as well as the control soils. Different doses of paraquat at different WAA affect soil microbial populations as well as the chemical components of the soil. So, the effects of paraquat on soil microbial population and chemical components depended on the concentrations used and the duration of application. Since the fungi, bacteria and actinomycetes species identified in this study were sensitive to herbicide application, they may serve as a reliable indicator of the biological value of soils.

Keywords: Herbicide; soil; effect; determination; microbial population.

1. INTRODUCTION

Herbicides are chemical compounds that are used to kill unwanted plants. These are used to clear waste grounds, industrial sites, railways and farm lands [1]. Prior to the wide spread use of chemical herbicides, cultural controls such as altering soil pH and fertility levels were used to control weeds. Mechanical control (including tillage) was also (and still is) used to control weeds [1]. Today, chemical herbicides are used by farmers in order to control weeds, thus increasing their plant production. Without the use of these herbicides, control of weeds would not have been easy and the effects, one of which is hunger and food shortage as a result of the weeds affecting plant production, would have been more serious than the Ecotoxicological effect which they are intended [2]. Numerous experiments have been conducted that demonstrated the potential positive impacts of the use of herbicides by farmers; for instance; maize yields double and production costs fell by 61% in Nigeria [3]. [4] Also reported in Nigeria that the cost of weed control in rice with herbicides was 50% lower than hoe weeding.

In achieving the optimization of agricultural resources thus satisfying human needs and at the same time maintaining the quality of the environment and sustaining natural resources, the soil microbial community composition is of great importance, because they play a crucial role in carbon flow, nutrient cycling and litter decomposition, which in turn affect soil fertility and plant growth [5,6,7].

However, despite the benefits of using these herbicides by farmers as noted by [2] and [4], the increased use of herbicides in agricultural soils causes the contamination of the soil with toxic chemicals, thus may exert certain effects on non-target organisms including soil microorganisms [8]. Paraquat is the most highly acutely toxic herbicide to be marketed over the last 60 years. Yet it is one of the most widely used herbicides in the world and in most countries where it is registered, it can be used without restriction. It is used on more than 100 crops in about 100 countries. Gramoxone, manufactured by Syngenta, is the most common trade name for paraquat, but the herbicide is also sold under many different names by many different manufacturers. Paraquat is used as an herbicide, desiccant, defoliant and plant growth regulator [9]. It is also used for controlling broadleaf weeds and grasses in more than 100 different crops, including plantations [10]. Again, it is used as a pre-harvest defoliant or desiccant on crops such as cereals, cotton, beans, hops, sugar cane, pineapple, soy, potatoes, and sunflowers; and as a post-harvest desiccant to speed up removal of spent plants such as tomato plants. Furthermore, it is used for weed control in non-agricultural areas such as roadsides, airports, around commercial buildings, drains, irrigation ditches, and waterways [11]. In Nigeria, especially Mubi local government area of Adamawa State as well as most of the northern part of Nigeria where farming is the major occupation, strict observance of manufacturer's instructions on how to make use of these herbicides are not being adhered to as they are ignorant of its danger to the environment. Natural ways of controlling pests and pathogens of crops which are friendly to the environment have been forfeited by farmers for synthetic herbicides as the synthetic herbicides provide quick solutions to pests or pathogens problems as noted by [2]. The use of paraquat as herbicide of choice in this study was as a result of it being the most widely used herbicide to control broad-leaf weeds and grasses especially in the study area. Therefore, there is need to occasionally assess the effects of this herbicide on micro flora and other soil organisms as reported by [12] so as to know the measures to be taken.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at Modibbo Adama University of Technology Yola, in the open field of the Department of Plant Science Botanical garden, Girei Local Government Area of Adamawa state, Nigeria. It is located at the North Eastern part of Nigeria and lies between Latitude 7° and 11℃N of the equator and between longitudes 10° and 14℃E of the Greenwich meridian. The botanical garden where the study was carried out has sandy-loamy type of soil. The study area has a tropical climate characterized by dry and wet seasons. It has an average annual rainfall of 759 mm. The choice of the study area was informed because of the fact that, the area was never applied any herbicide prior to this study.

2.2 Experimental Design

Completely randomized block design (CRBD) was used for the experiment. For each treatment, it was one block which was replicated three times.

2.3 Preparation of Herbicides

The paraquat used was purchased from Jimeta Modern Market Yola, Adamawa State of Nigeria from the recommended dealer of herbicides. The preparation/concentration of the paraquat for application was prepared according to the manufacturer's recommendations. The concentrations prepared were: 0.3 ml/L of water (low concentration), 0.6 ml/L of water (manufacturer's recommended dose) and 0.9 ml/L of water (high dose). Each of the concentrations has three replications.

2.4 Soil Treatment

The soil was treated with concentrations of the herbicide prepared above by using a knapsack sprayer. Polythene bag was used to cover the soil so as to avoid the chemical drifting to the next neighboring blocks/plots thus, avoiding contamination during the treatment. The control blocks were not treated or applied herbicide.

2.5 Sample Collection

The top soil samples were collected at the depth of 0-5 cm from each block of the field during each collection. It was then put into a sterilized polythene bags and were taken to the laboratory for isolation. The soil samples were then made free of large stones and plant debris using 2.0 mm mesh sieve and stored at 4℃ before processing. The soil collection was done at two weeks interval for a period of eight weeks.

2.6 Isolation of Microorganisms

2.6.1 Isolation of bacteria

Isolation of bacteria was done through serial dilution. 1 g of the soil sample collected from the field was put in a test tube containing 10 ml of distilled water. One (1) ml of the sample solution was then taken from the first test tube and put in the second test tube containing 10 ml of distilled water and this continue until the test tube. For this study, dilution 10^{-2} and 10^{-4} were used. For pouring the sample solution on a media, spread method was used. Micro peptide was used in taken 0.5 ml of the sample solution from the desired test tube and dropped on prepared nutrient agar medium plates under sterilized conditions. The inoculated plates were then after sometimes incubated at 37℃ for 24-48 hours. After growth was observed, sub-culturing was done for the isolation of pure culture. The pure cultures were then subjected to Gram's staining so as to identify it and characterized based on their morphological and microscopic features using Bergey's manual as described by [13].

2.6.2 Isolation of fungi

The isolation of fungi was performed using serial dilution. The soil sample solution (dilution 10^{-4}) was then pour on a Potato Dextrose Agar (PDA) medium plates prepared under sterilized conditions and was spread using a sterilized glass rod. The inoculated plates were then incubated at room temperature for 5 days. After incubation period, on the presence of visible growth, the pure cultures were then obtained through sub-culturing. Identification and characterization of the fungal species were done based on their morphological and microscopic characters analysis by using taxanomic guides and standard procedures as described and done by [14].

2.6.3 Isolation of actinomycetes

Isolation and characterization of the actinomycetes were carried out using [15] method.

2.7 Determination of Soil pH

The pH of soil sample was determined using pH meter 3150 Jenway model according to the method described by [16].

2.8 Determination of Organic Matter and Organic Carbon

Organic carbon and organic matter in the soil was determined using [17] method.

2.9 Statistical Analysis

All data obtained were subjected to one-way analysis of variance (ANOVA) and where there was significance difference, Ducan Multiple Range Test (DMRT) was used to separate the means.

3. RESULTS

3.1 Fungi, Bacteria and Actinomycetes species identified from Paraquat Treated and Control (untreated) Soil Samples at Two (2) Weeks after Application (WAA)

The results of the fungi, bacteria and actinomycetes identification in paraquat treated and untreated (control) soil samples at two weeks after application (WAA) showed that in paraquat low dose (PLD), paraquat recommended dose (PRD), paraquat high dose (PHD) and the control (CON), fungi, bacteria and actinomycetes such as *A. fumigatus*, *Pseudomonas* sp., and *A. israelis*; *A. flavus, E.coli* and *Streptomycetes* sp.; *A. corymbifera, Pseudomonas* sp. and *A. bovis; A. fumigatus*, *A. flavus, A. corymbifera, A. niger, R. stolonifer* and *Micrococcus* sp. and *Streptomycetes* sp. respectively were identified (Table 1).

3.2 Fungi, Bacteria and Actinomycetes Identified from Paraquat Treated and Untreated (control) Soil Samples at Four (4) Weeks after Application (WAA)

At four (4) weeks after application, the fungi, bacteria and actinomycetes identification in paraquat treated and control soil samples showed that, in PLD, PRD, paraquat high PHD and the CON, fungi, bacteria and actinomycetes such as *A. flavus, Bacillus* sp. and *A. bovis*; *A. niger, E. coli* and *A. Israeli; R. stolonifer, Pseudomonas* sp. and *Streptomycetes* sp.; *R. stolonifer, A. flavus, A. niger, A. corymbifera, A. fumigatus* and *E. coli* and *A. bovis* respectively were identified (Table 1).

3.3 Fungi, Bacteria and Actinomycetes Identified from Paraquat Treated and Control Soil Samples at Six (6) Weeks after Application (WAA)

The result of the identification revealed the presence of fungi, bacteria and actinomycetes such as *A. niger, Staphylococcus* sp. and *A. bovis; A. flavus, Pseudomonas* sp. and *Streptomycetes* sp.*; R. stolonifer, E. coli* and *A. israeli; A. niger, A. flavus, R. stolonifer, A. coymbifera, A. fumigatus* and *Bacillus* sp. and *Streptomycetes* sp. in PLD, PRD, PHD and CON soil samples respectively (Table 1).

3.4 Fungi, Bacteria and Actinomycetes Identified from Paraquat Treated and Control Soil Samples at Eight (8) Weeks after Application (WAA)

Identified from the PLD, PRD, PHD and CON soil samples were fungi such as *R. stolonifer, A. flavus, A. niger,* and *R. stolonifer; A. flavus, A. niger, A. corymbifera* and *A. fumigatus* respectively. *Bacillus sp, Pseudomonas* sp., *Bacillus* sp. and *E. coli* and *A. bovis, Streptomycetes* sp., *A. Israeli* and *A. bovis* respectively were the bacterial and actinomycetes species identified from the four soil samples (Table 1).

3.5 Effect of Paraquat Treatment on Fungal Count at 2 – 8 Weeks after Application (WAA)

At the 2^{nd} WAA of PLD, PRD and PHD, the result showed that the fungal count were significantly higher (*P=.05*) compared to those of the other WAA with 4.00, 3.00 and 4.00 x 10^4 cfu/mg respectively. However, that of PRD was not significantly different (*P=.05*) from those of 4^{th} – 8^{th} WAA. The CON on the other hand, showed the highest fungal count at 6^{th} and 8^{th} WAA with 5.00 and 5.00 x 10^4 cfu/mg respectively which were significantly different (*P=.05*) from those of 2^{nd} and 4^{th} WAA (Table 2).

3.6 Effect of Paraquat Treatment on Soil Bacterial Population at 2–8 WAA

The bacterial population was significantly the highest (*P=.05*) at the 4^{th} – 8^{th} WAA of PLD, PRD and PHD with 33.20, 32.00 and 32.30 x 10^4 cfu/mg respectively. The control, however, was at 2^{nd} and 6^{th} WAA recorded the highest

population of bacterial with 35.00 and 35.00 x 10^4 cfu/mg respectively (Table 2).

3.7 The Effect of Paraquat Treatment on Actinomycetes Population at 2 – 8 WAA

The population of actinomycetes at the 2nd WAA of PLD and PRD and even the CON were significantly the highest (*P=.05*) with 2.50, 1.40 and 3.48 x 10^8 cfu/mg respectively. The same thing was observed for PRD at 6th WAA with 6.80 x 10^8 cfu/mg (Table 2).

3.8 The Effect of Paraquat Treatment on Soil Organic Carbon at 2 – 8 WAA

In Table 3, the application of paraquat doses recorded the most effective action on the soil organic carbon only at the 4th WAA with 1.34%, 1.12% and 1.12% respectively except for its high dose at the 6th WAA which also had the highest effect (1.10) that was not significantly different (*P=.05*) with that at the 4th WAA. The control, on the other hand, recorded the most effective action at the 6th and 8th WAA with 1.42% and 1.43% respectively (Table 3).

3.9 The Effect of Paraquat Treatments on Soil Organic Matter at 2 – 8 WAA

In Table 3, the application of the different doses of paraquat significantly (*P=.05*) showed the most effective action at the 4th WAA with 1.93%, 1.93% and 1.84% respectively. The control, however, was only effective on the soil organic matter only at the 8th WAA with 2.46%.

3.10 The Effect of Paraquat Treatment on Soil pH at 2nd - 8th WAA

The pH of the soils was at the 2nd WAA significantly (*P*=.05) the highest with 7.39%, 7.49% and 7.49% respectively. Similar result was also observed at 6th WAA for PHD with 7.49% which was significantly the same (*P=.05*) with that at the 2nd WAA. The control also was at the 2nd WAA had the highest pH with 7.52% (Table 3).

4. DISCUSSION

In this study, assessment of fungal species in paraquat untreated (control) soil samples from the 2 – 8 WAA presents fungal species such as *A. fumigatus, A. flavus, A. niger, A. corymbifera* and *R. stolonifer* which were found common in all the control soil samples. Similar observation was made by [18] in their control soil samples when they determined the effect of atrazine on soil microbes. This occurrence could be attributed to the fungi being well adapted to the soil conditions or probably the health status of the soil as similarly reported by [19] that associated soil microbial activity and content to soil health. Also, the similarity in fungal species isolated in the control soil samples of paraquat (of this study) and atrazine [18] treated soils could be as a result of these studies being carried out in the same study area with probably the same environmental conditions as such factor according to [20] affect soil microbes. On the other hand, fungal species such as *A. fumigatus, A. flavus, A. corymbifera, A. niger* and *R. stolonifer* were found present in the paraquat treated soils with *A. niger, A. flavus* and *R. stolonifer* being the commonest especially from the 4-8th WAA. This finding was supported by the findings of [21] who reported the presence of *A. niger, A. flavus* and *Penicillium* sp. in their herbicides treated soil. However, their findings differ in one of the fungal species identified probably as a result of the differences in the type of agrochemicals, concentrations, mode of applications, environmental conditions and herbicides combination used as these according to [22] and [19] affect soil's microbes content. Apparent from the assessment of the type of fungal species present in the untreated (control) soils of this study was the fact that fungal species, *A. corymbifera,* was not present in the paraquat treated soils especially from 4th – 8th WAA. This could be that the paraquat had a fungicidal effect on the fungi species.

The results of the identification of bacteria and actinomycetes present in the treated and control (untreated) soil samples showed that, bacteria such as *Staphylococcus* sp., *Bacillus* sp., *Streptococcus* sp., *Pseudomonas* sp., *E. coli* and *Micrococcus* sp. were present in both the treated and untreated (control) soil samples with *Pseudomonas* sp. and *E. coli* being the most common in occurrence in the soil samples from the 2nd – 8th WAA. On the other hand, actinomycetes such as *A. bovis*, *A. israelis, Streptomycetes* sp. were identified and even the commonest in all the WAA.

These bacteria and actinomycetes species identified, differ across the number of WAA of the treatments as well as the doses probably as a result of the effect of the chemicals on them. This

was so because herbicides could adversely affect soil microbes depending upon the application rate/dose and the type of herbicide used [21,23]. The bacterial species identified in the treated and control soil samples of this study were similarly reported by [24] in the control and paraquat treated soils. However, some of the bacterial species identified in this study such as *Staphylococcus* sp., *Streptococcus* sp., and *E. coli* were not reported in their findings probably as a result of the differences in concentration used, period of application and collection of samples for assessment and the depth at which the soil samples were collected.

Table 1. Fungi, Bacteria and Actinomycetes species identified from paraquat treated and untreated soil samples

Week interval	Treated soil	Fungal species	Bacterial species	Actinomyetes species
2nd WAA	Paraquat Low Dose	*Aspergillus fumigatus*	*Pseudomonas* sp.	*A. israeli*
	Paraquat Recommended Dose	*Aspergillus flavus*	*E. coli*	*Streptomycetes* sp.
	Paraquat High Dose	*Absidia corymbifera*	*Pseudomonas* sp.	*A. bovis*
	Control (untreated soil sample)	*A. fumigatus* *A. flavus* *A. corymbifera* *Aspergillus niger* *Rhizopus stolonifer*	*Micrococcus* sp.	*Streptomycetes* sp.
4th WAA	Paraquat Low Dose	*A. flavus*	*Bacillus* sp.	*A. bovis*
	Paraquat Recommended Dose	*A. niger*	*E. coli*	*A. Israeli*
	Paraquat High Dose	*R. Stolonifer*	*Pseudomonas* sp.	*Streptomycetes* sp.
	Control (untreated soil sample)	*R. stolonifer* *A. flavus* *A. niger* *A. corymbifera* *A. fumigatus*	*E. coli*	*A. bovis*
6th WAA	Paraquat Low Dose	*A. niger*	*Staphylococcus* sp.	*A. bovis*
	Paraquat Recommended Dose	*A. flavus*	*Pseudomonas* sp.	*Streptomycetes* sp.
	Paraquat High Dose	*R. stolonifer*	*E. coli*	*A. Israelis*
	Control (untreated soil sample)	*A. niger* *A. flavus* *R. stolonifer* *A. corymbifera* *A. fumigatus*	*Bacillus* sp.	*Streptomycetes* sp.
8th WAA	Paraquat Low Dose	*R. stolonifer*	*Bacillus* sp.	*A. bovis*
	Paraquat Recommended Dose	*A. flavus*	*Pseudomonas* sp.	*Streptomycetes* sp.
	Paraquat High Dose	*A. niger*	*Bacillus* sp.	*A. Israelis*
	Control (untreated soil sample)	*R. stolonifer* *A. flavus* *A. niger* *A. corymbifera* *A. fumigates*	*E. coli*	*A. bovis*

WAA: Week after application

Table 2. Effect of paraquat on soil fungi, bacteria and actinomycetes populations

Week	Fungal count (10^4 cfu/mg)				Bacterial population (10^4 cfu/mg)				Actinomycetes (10^8 cfu/mg)			
	PLD	PRD	PHD	CON	PLD	PRD	PHD	CON	PLD	PRD	PHD	CON
2nd WAA	4.00 a	3.00 a	4.00 a	2.00 b	24.00 b	16.00 c	9.80 c	35.00 a	2.50a	2.70d	1.40a	3.48a
4th WAA	3.00 ac	2.00 a	1.00 b	3.00 b	33.20 a	15.20 c	12.30 b	16.40 b	0.55c	4.00c	0.12c	0.15c
6th WAA	3.00 ac	2.00 a	2.00 b	5.00 a	21.40 bc	32.00 a	32.30 a	35.00 a	1.32b	6.80a	0.17c	2.39b
8th WAA	2.00 bc	3.00 a	2.00 b	5.00 a	19.00 c	20.30 b	9.00 c	9.50 c	0.82c	5.30b	1.23b	2.76b

Key: Means with the same superscript(s) along the column are not significantly different at P=.05.
PLD: Paraquat low dose; PRD: Paraquat recommended dose; PHD: Paraquat high dose;
CON: Control; WAA= Week after application

Table 3. Effect of paraquat treatment on soil organic carbon, Organic matter and soil pH

Week	Organic carbon (%)				Organic matter (%)				Soil pH			
	PLD	PRD	PHD	CON	PLD	PRD	PHD	CON	PLD	PRD	PHD	CON
2nd WAA	0.38d	0.55d	0.38c	0.95c	0.65c	0.95d	0.62d	1.63d	7.39 a	7.49 a	7.49 a	7.52 a
4th WAA	1.34a	1.12a	1.12a	1.07b	1.93a	1.93a	1.84a	2.33c	6.49 b	6.81 ac	6.84 ac	6.83 ac
6th WAA	1.01b	0.99c	1.10a	1.42a	1.62b	1.89b	1.57c	2.44b	5.73 cd	6.35 bc	7.49 a	6.55 bc
8th WAA	0.95c	1.06b	0.94b	1.43a	1.63b	1.82c	1.62b	2.46a	6.23 bd	6.94 ac	6.36 bc	6.74 bc

Key: Means with the same superscript(s) along the column are not significantly different at P=.05.
PLD: Paraquat low dose; PRD: Paraquat recommended dose; PHD: Paraquat high dose;
CON: Control; WAA= Week after application

The effect of paraquat treatments on soil fungal count at the 2nd WAA of PLD, PRD and PHD showed a higher fungal count compared to those of other WAA. Similar report was given by [24] who reported a decrease in the fungal count of the soil treated with butachlor, pyrazosulfuron and paraquat with passage of time from 7th to 28th day after treatment. The decrease of the fungal count observed in this study could be due to the adverse effect of the herbicide on the fungal component of the soil. This was proven by the fungal count of the control as the fungal count was higher especially at 6th and 8th WAA.

Bacterial population was low for most of the paraquat doses at the 2nd WAA, but increased at other WAA. [21] Discovered that higher concentrations of herbicide treatments resulted in much lower microbial counts when compared to soils treated with recommended doses. This study somehow agrees with the above statement because the bacterial population of the control soil samples had the highest compared with those of paraquat treated soils.

Actinomycetes population was high at 2nd WAA of PLD, PRD and the control as well as at the 6th WAA of PHD. This could be influenced by the factors such as temperature, pH, organic carbon content, aeration and moisture content of the soils as reported by [15]. Similar result was published by [24] who reported a significant increase in actinomycetes population from 7th to 28th day as a result of herbicide application.

The behavior of most herbicides is generally influenced by the content of organic carbon [25]. The phenomenon of herbicide leaching was primarily related to the adsorption of herbicides in the different soils, mainly due to their contents of fulvic-acids, humus in the organic fraction of the soils, followed by parameters such as the cat-ion exchange capacity, acid dissociation constant [26]. Variation in the effect of paraquat treated soils on organic carbon content was observed with respect to the doses of the herbicides and the weeks after application. Same was observed for the effect of paraquat on the soil organic matter content as well as pH of the soils. This could be due to vigorous microbial activities in the soil [27].

5. CONCLUSION

Different doses of paraquat at different WAA affect soil microbial populations as well the chemical components of the soil. So, the effects of paraquat on soil microbial populations and chemical components depended on the concentrations used and the duration of application. It also determines the type of microbes present in the soil by getting rid of those that cannot withstand its effects, in this way allowing only the resistible species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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