

## Effect of Atrazine, 2,4-D Amine, Glyphosate and Paraquat Herbicides on Soil Microbial Population

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### ABSTRACT

Herbicides' toxic impact on the non-target soil microorganisms which play roles in degrading organic matter, nitrogen and nutrient recycling and decomposition needs to be considered. In the present study, the effect of four (4) most commonly used herbicides, viz; atrazine, 2, 4- D amine, glyphosate and paraquat on soil microorganisms was assessed over a period of fifteen continuous days (exposure period). The herbicide treatments were the normal recommended field rate, (6.67 mg active ingredient per gram of soil for atrazine, 6.17 mg for 2, 4-D amine, 5.56 mg for glyphosate, and 2.46 mg for paraquat), half and double of the recommended field rates. Tables 4.1 through 4.5 showed the various heterotrophic bacterial colony counts obtained from the various treatments, ranging from the control (Day 1; no herbicide applied) to days 3, 6, 9 and 15 after the application of the various herbicides, respectively. Two general trends are readily observed: the first is that, upon application of the herbicide, the microbial growth steadily diminishes, up to the 6<sup>th</sup> day. However, from there it continues to increase till the end of the experiments (day 15). This applies to each herbicide. Firstly, the effect of the addition of the various herbicides was evaluated using two samples, a two-tailed Z-Test for means, at a 95% confidence interval, and the results showed that there is a difference in the bacterial counts before the application of the herbicides (Day 0) and immediately after (day 3), and the difference is statistically significant ( $Z = 3.32$ ,  $Z$  critical = 1.96,  $P = 0.00090$ ). This indicates that the application of the herbicides affects the bacterial population.

### INTRODUCTION

Herbicides are defined as any substances, individually or in a mixture whose function is to control, destroy, repel or mitigate the growth of weed in a crop. Herbicides in their natural state may be solid, liquid, volatile, non-volatile, soluble, or insoluble, hence these have to be made informed suitable and safe for their field use. A herbicide formulation is prepared by the manufacturer by blending the active ingredient with substances like a solvent, surfactants, substance like a solvent, surfactants, sticker and stabilizer to allow mixing dilution application and stability. In Nigeria, herbicides have since effectively been used to control weeds in agricultural systems. As farmers continue to realize the usefulness of herbicides larger quantities are applied to the soil. But the safety of those compounds in the soil becoming increase singly important since they could be leached, in which case groundwater is contaminated or immobile, and persist on the topsoil [1,2]. These herbicides could then accumulate to a toxic

level in the soil and become harmful to microorganisms, plants, wildlife and man. There is an increasing concern that herbicides not only affect the target organisms (weeds) but also the microbial communities present in soils, and this non-target effect may reduce the performance of important soil functions. These critical soil functions include organic matter degradation, the nitrogen cycle and methane oxidation.

The presence of herbicide residues in soil could have direct impacts on soil microorganisms and is a matter of great concern. At normal field recommended rates herbicides are considered to have no major or long-term effect on the microbial population. It has been reported that some microorganisms were able to degrade the herbicide, while some others were adversely affected depending on the application rates and type of herbicide used [3]. Therefore, the effect of herbicide on microbial growth, either stimulating or depressive, depends on the chemicals (type and concentration), microbial species and environmental conditions

[4]. The soil serves as a repository for all agricultural contaminants, and functions as a major habitat for most microbial communities such as soil bacteria, fungi and actinomycetes whose activities influenced soil fertility [5] through organic materials degradation, organic matter decomposition and nutrient cycling [6]. Nonetheless, the over-application of these chemicals inhibits some of these natural processes and decreases the performance of the non-target organisms [7]. However, some soil organisms use these herbicides in the process of degradation as a carbon energy source for their metabolic activities [8–14].

Agriculture remains the backbone of the economy, to ensure agricultural productivity weeds must be dealt with accurately and adequately as it disturbs the peace and interrupts the desired performance of the cultivated crops. Weed growth must be inhibited to give room for the cultivated crops to survive uninterrupted without much competition. Competition of crops with weeds hinders the growth of the crops cultivated which will eventually lead to stunted growth or even total death of the crops. Crops require so many things to thrive these include space, nutrients, sunlight etc. when crops were denied these requirements by weeds definitely the growth, development and performance of crops will be interfered thereby causing the crops to be very disturbed.

Several studies on the wide use of herbicides have already shown that herbicides application leads to changes in soil nutrient levels and alteration to soil microbial activity, diversity and genetic structure and this consequently leads to disturbances of microbial communities ensuring several key ecological processes in the soil such as organic matter degradation and nutrient cycling, could harmfully alter soil fertility and sustainable agriculture productivity (Girvan *et al.*, 2004; Ros *et al.*, 2006) [15–19].

In Nigeria, and many other countries the world over, over the past five decades, herbicides have been increasingly added to the environment under intensively managed cultivation practice leading to contamination of natural bodies. Of late, there has been increasing concern about the non-target effect of herbicides [20–26]. Soil microorganisms are among the important non-target organisms most affected by herbicide [27]. The intensive use of the herbicide has caused environmental concern especially unforeseen consequences on soil microbes. Since herbicides can be potentially toxic to the non-target organism. Determining the impact of herbicide on non-target organisms such as microbes in the soil has been of considerable interest [28–30].

Studies on herbicide residual effects on soil microorganisms are often done in soil microcosm small-scale experiments which can be interpreted accurately at larger scales [31]. Microcosms containing soil microfauna of field communities offer a higher resolution of ecotoxicological effects of chemicals in soil environments [32]. As the precise assessment of the potential non-target effects of herbicides on soil microorganisms is of growing interest, therefore, soil microcosm can provide a better understanding of the possible response of soil microbes to herbicides. Also, an ideal herbicide should have the quick ability to be degraded into non-toxic substances that ultimately exert fewer toxic effects on soil microbes (especially non-target organisms) [1,4,33,34]. The research aims to determine the effect of herbicide on soil bacteria

## METHODOLOGY

### Soil sampling

The topsoil (up to 5 cm depth) sample was collected from Dutsin-Ma water board (Water board area) with no prior herbicide treatment in the last 5 years. The soil samples were collected in quadruplicate at each of the four sampling sites. The four samples were mixed thoroughly, and portions were taken for laboratory analyses. The samples were sieved using a 2.0 mm mesh size to remove stones and plant debris.

### Herbicides Selection.

The herbicides were obtained from local agricultural input dealers in Dutsin-Ma. The selected herbicides were the most commonly used ones which contain the following active ingredients: Paraquat (Sun-Paraquat 200 SL), Glyphosate (Sunphosate 360 SL), 2, 4-D amine (720 SL) and Atrazine (Atrazine 500).

### Soil Treatments

The soil treatment was carried out in four (4) different concentrations double the recommended field rate (RFR), half the RFR and normal the RFR over three days for fifteen (15) days. The rate of treatment is according to the manufacturer's recommended rate of 2.4 mg of the active ingredient per a gram of soil for Paraquat, 5.56 mg per gram of soil for Glyphosate, 6.17 mg per gram of soil for 2,4-D Amine and 6.67 mg per gram of soil for Atrazine. Each of the treatments was in two replicates.

### Baseline determinations (Control)

This was the point where the bacteria population in the soil was determined without any chemical treatment to serve as the baseline to compare with the soils that were treated with various herbicides. The soil organic matter was determined before the chemical treatment and after treatment.

### Determination of Bacterial Load

The enumeration of the bacteria population was done using Pour Plate Counter. The plate count agar was prepared by suspending 8.96g of dehydrated medium (powder) in 320 mL of distilled water. The content was heated and boiled for one minute with constant agitation until the powder was completely dissolved. The agar was poured into a conical flask and sterilised in an autoclave at 121 °C. One gram of each treated soil sample was weighed and serially diluted. One (1) mL aliquot was taken from an inch below the surface with a sterilized 1 mL pipette and placed on an empty sterile plate.

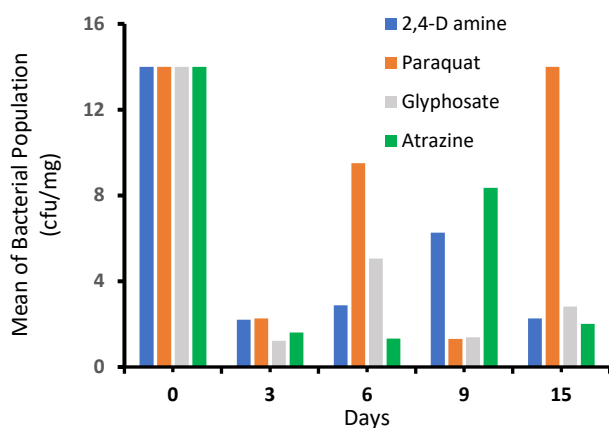
Twenty (20) mL of the melted plate count agar which has been cooled to 45 °C was poured into the diluted sample. This was swirled to ensure that the mixture was thoroughly mixed and cooled to solidify on a flat laboratory bench before incubation was done under a lamina flow. These labelled specimens were inverted to prevent them from being soaked through condensation. Incubation was done at room temperature of 37 °C for 24. The total viable colony on each plate was counted using the colony counter and the data was recorded. Statistical Analyses of the data were performed using Microsoft Office Excel Data Analysis ToolPak (2016 version).

## RESULTS

The various heterotrophic bacterial colony counts obtained from the various treatments (Table 1 and Fig. 1), ranging from the control (Day 1, no herbicide applied), to days 3, 6, 9 and 15 after the applications of the various herbicides, respectively show two general trends are readily observed: the first is that upon the application of the herbicide, the microbial growth steadily diminishes, up to the 6<sup>th</sup> day. However, from there it continues to increase till the end of the experiments (day 15). This is applicable to each herbicide. On the second day, soil treated with paraquat shows the highest number of colonies, followed by 2, 4-D amine, and then atrazine, while glyphosate has the least colony. On day 6 paraquat maintain the highest colonies. On day 9, atrazine has the highest number of colonies and paraquat has the least colonies. On day 15, paraquat exhibited the highest number of colonies followed by glyphosate and then 2,4-D amine and atrazine have the least (Fig. 1).

**Table 1.** Bacterial colony counts were obtained from various herbicide treatment.

Sampling Point	Soil Treatment	Mean of Bacterial Count (CFU/g)				
		Days of incubation				
		0	3	6	9	
A	2,4-D amine	TNTC	2.2x10 <sup>9</sup>	2.87x10 <sup>8</sup>	6.26x10 <sup>8</sup>	2.26x10 <sup>9</sup>
B	paraquat	TNTC	2.27x10 <sup>9</sup>	9.5x10 <sup>8</sup>	1.315x10 <sup>9</sup>	TNTC
C	glyphosate	TNTC	1.222x10 <sup>9</sup>	5.060x10 <sup>8</sup>	1.38x10 <sup>9</sup>	2.81x10 <sup>9</sup>
D	atrazine	TNTC	1.605x10 <sup>9</sup>	1.32x10 <sup>9</sup>	8.36x10 <sup>8</sup>	2.01x10 <sup>9</sup>



**Fig. 1.** Trends of bacterial colony counts obtained from various herbicide treatments.

## DISCUSSION

Firstly, the effect of the addition of the various herbicides was evaluated using a two-sample, two-tailed Z-Test for means, at a 95% confidence interval, and the results showed that there is a difference in the bacterial counts before the application of the herbicides (Day 0) and immediately after (day 3), and the difference is statistically significant ( $Z = 3.32$ ,  $Z_{critical} = 1.96$ ,  $P = 0.00090$ ). This indicates that the application of the herbicides has an effect on the bacterial population, and this is in agreement with previous submissions [34].

Moreover, to determine the overall effect of the herbicides on the bacteria as the experiments runs, over time, Spearman's Product Moment Correlation was determined for the average colony counts obtained, from the first day to the last, and a weak,

negative correlation was obtained ( $R = -0.37$ ), and this shows that the application of the herbicides initially had negative effects on the bacteria, as some of them may not be able to tolerate it, due to its toxicity, therefore the microbial population generally reduces. However, when the same analysis was repeated excluding the control, i.e. day without application of any herbicide, the correlation was found to be weak, but positive ( $R = 0.46$ ). This reflects the positive increase in growth at the latter stages of the experiment, where two factors, namely, the decrease in the amount of herbicide and the acclimatization of the microbes to the various herbicides, make the growth rates increase [4,7,35–37].

Finally, a two-way Analysis of Variance was used to compare whether the individual colony counts obtained from each herbicide differ from each other with regard to the stage of the experiment (i.e. the time after the application of the herbicide) or the type of herbicide applied. The results showed that there is no significant difference in terms of the effects of one herbicide compared to another, amongst the herbicides tested ( $p = 0.17$ ); however, there is a significant difference in terms of the effects of the tested herbicides on the microbial counts, as the time of the experiment progresses ( $p = 0.0001$ ). This shows that all the herbicides had virtually the same toxicity or effects on the microbes, however, this changes over time, which is attributable to acclimatization and the onset of biodegradation, as described earlier [32].

## CONCLUSION

Owing to the apparent devastations that the four different herbicides exert on soil bacterial flora which play significant roles in soil ecology and enhance beneficial plant-microbe interaction, their usage needs to be checked. The results of the study indicate that the presence of Atrazine, 2, 4-D amine, Glyphosate and Paraquat in the soil exert considerable change in the growth and development of soil microorganisms. The effect of the addition of the various herbicides was evaluated using a two-sample, a two-tailed Z-Test for means, at 95% confidence interval, and the results showed that there is a difference in the bacterial counts before the application of the herbicides (Day 0) and immediately after (day 3), and the difference is statistically significant ( $Z = 3.32$ ,  $Z_{critical} = 1.96$ ,  $P = 0.00090$ ). This indicates that the application of the herbicides has an effect on the bacterial population, and this is in agreement with previous submissions (Araujo *et al.*, 2003). Further research should be done in this area, such as those focusing on bacteria with the ability to degrade the selected herbicides in those contaminated soils, and their response to the changing concentration.

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