

Effect of Some Commonly Used Herbicides on Soil Microbial Population

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Abstract

Herbicide application has become an integral part of vibrant agricultural productivity in the whole world since its benefit has been overwhelming over the years. However, its 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 in Ghana; 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 rate. Bacterial and Fungal populations were then determined at a five-day interval up to the 15th day after treatment. The data gathered from bacterial enumeration was logarithmically transformed before graphs of mean bacterial were plotted against the exposure period for each selected herbicide. Bacterial population and percentage organic matter did not show any significant differences relative to the exposure period in this study ($p < 0.05$). However, the deleterious impact of the herbicides was seen as Paraquat treatment resulted in reduction in the bacterial population for five, ten and fifteen Days after treatment (DAT) in the treatment with half the recommended field rate. Glyphosate followed with 69.3%, 12.7%, and 18.0%; 2,4-D amine had 44.8%, 33.5%, and 21.6%; and lastly Atrazine had 41.8%, 44.5% and 13.6% bacterial population 5DAT, 10DAT and 15DAT respectively. The inhibition effect on the fungal population was very specific as some fungi (such as *Aspergillus niger*, *Trichoderma viride*, *Collectotrichum gloeosporioides*, *Aspergillus flavus*, *Mucor*, *Penicillium*, *Curvularia lunata*) which were present in the baseline determination (control) did not appear in the treatment. Percentage organic matter for the treatment did not vary much with the baseline determination (control) but the impact was observed in the various levels of treatments for all the herbicides. A similar study should be conducted on a normal field condition where herbicide treatments would be carried out on a normal field condition since most of the previous studies had the herbicide treatment carried out under laboratory condition. It will also be very appropriate if further research work is carried out to identify the specific components of these herbicides which favour the growth and development of certain beneficial microorganisms such as fungi and bacterial.

Keywords: herbicides, microbial population, biomass, baseline, organic matter, treatments.

1.0 INTRODUCTION

The use of herbicides in agriculture has over the years contributed tremendously to both food and cash crop production all over the world of which Ghana is not an exception. But one of the challenges undermining the farming business (Ntow *et al.*, 2006), 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 Ghana. As a result, manufactures have adopted flooding the agrochemical market with all kinds of herbicides that are meant for the elimination of different kinds of weeds at different stages of their growth (Sebiomo *et al.*, 2011). 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 soil bacteria, fungi and actinomycetes whose activities influences the soil fertility (Zain *et al.*, 2013), through organic material degradation, organic matter decomposition and nutrient cycling (De-Lorenzo *et al.*, 2001 and Hutsch, 2001). Nonetheless, over application of these chemicals inhibit some of these natural processes, and decreases the performance of the non-target organisms (Subhani *et al.*, 2000). 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 chemical. However, most of these herbicides are designed to persist longer enough to have the desired effect on the weeds (Greer *et al.*, 1990). The fate of herbicide applied onto the soil environment is governed by two major processes; transfer and degradation. The transfer process involves percolation, runoff, flora and fauna uptake, sorption and desorption, for which the applied chemicals remain physically intact in the soil environment. The degradation processes includes microbial decomposition, plant detoxification, chemical breakdown and photodecomposition which are chemically engineered. These two processes determine the

persistence of herbicides, its efficacy for weeds, as well as its potential for soil and ground water contamination. (Subhani *et al.*, 2000). 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 Ghana 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 (Baran *et al.*, 2007). 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 Ghana in order to assess their inhibitory effects on some of the beneficial microorganisms in the soil.

1.1 MATERIALS AND METHODS

1.1.1 Soil sampling

The top soil (up to 5cm depth) sample was collected from oil palm plantation in Abadwum (Adansi-North district in Ghana) with no prior herbicides treatment. The soil was collected from different points within the plantation and bulked together. It was shaken to mix it thoroughly and portion taken for laboratory analysis. The samples were sieved using a 2.0 mm mesh size to remove stones and plant debris.

1.1.2 Herbicides selection

The herbicides were obtained from a local agricultural input dealer in Akumadan in the Ashanti Region in Ghana. 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 (Agrazine 500).

1.1.3 Soil treatments

The soil treatment was carried out in three (3) different concentrations double the recommended field rate (RFR), half the RFR and normal the RFR over an interval of five days for fifteen (15) days exposure period in addition to the control sample. The rate of treatment was by the manufacturer of the herbicides 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 three replicates.

Formula for calculating the treatments (Zain *et al.*, 2013):

$$Y \text{ (mg/g)} = \frac{\text{RFR (g a.i / ha)}}{\text{Am. AiF (g a.i / L) x 450 L/ha}} \times \frac{1000 \text{ mg}}{1 \text{ g}}$$

Where;

Y - milligrams of chemical per gram of soil

RFR- recommended field rate

Am. AiF - amount of active ingredient in formulation

1.2 Enumeration of microbial population

1.2.1 Baseline determinations (Control)

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

1.2.2 Bacteria

The enumeration of the bacteria population was done using Pour Plate Counter. The plate count agar was prepared by suspending 20.5 g of dehydrated medium (powder) in one litre 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 flask and sterilized in an autoclave at 121 °C. One gram of each treated soil sample was weighed and serially diluted. 1 ml aliquot was taken from an inch below the surface with sterilized 1ml pipette and placed in an empty sterile plate. 15 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 it from being soaked through condensation. Incubation was done at room temperature of 25 °C for 24 – 48 hours. Total viable colony on each plate was counted using the colony counter and the data recorded.

1.2.3 Fungi

The enumeration of the fungi was done by using Potato Dextrose Agar (PDA) supplemented with each of tetracycline and streptomycin to inhibit bacterial growth. The PDA was prepared by weighing 200 g of freshly peeled and washed potato in the laboratory. It was then boiled, mashed and the pulp squeezed through a fine sieve. 20 g agar was added and boiled to dissolve and again 20 g dextrose was added and boiled to dissolve and

make up to one litre with water. The content was then sterilized at 15 psi for 20 minutes in an autoclave. 1 ml of the test samples was added to a sterile Petri dish and then a required amount of sterile, molten agar was added to the test sample. The content was cooled to 45 °C and swirled gently to mix well before it was allowed to solidify. Incubation of the fungi was done under a lamina flow at room temperature of 25 °C for 48 hours and identified with reference to Bergey's manual of systematic bacteriology. The total number of a particular organisms on each plate was identified and scored based on a maximum count of four (4) on a particular plate (Barnett and Hunter, 1972; Alexopoulos and Beneke, 1968; sebiomo *et al.*, 11).

1.3 Determination of Organic matter

The organic matter content was determined by the wet combustion (Walkley and Black, 1934). One gram of the sample soil was weighed out into a 500 ml Erlenmeyer flask and 10 ml of 1.0 N Potassium dichromate (K₂Cr₂O₇) solution added using a burette (Potassium dichromate oxidizes Carbon in the organic matter, itself being reduced in the process). This was followed by the addition of 20 ml conc. H₂SO₄ to generate heat to facilitate the reaction between carbon and Cr₂O. The mixture was swirled for one minute to ensure that the solution was in contact with all the particles of the soil. The flask and the content were allowed to cool on an asbestos sheet for 30 minutes. Two hundred milliliters of distilled water was added, followed by 10 ml orthophosphoric acid (to sharpen the colour change at the end point of titration). One milliliter of diphenylamine indicator was added and the solution titrated with 1.0 M normal ferrous sulphate solution until the colour changed to blue, and then finally to a green end-point. The titre value was recorded and the blank solution corrected. Organic carbon was calculated using the formula below by (Sebiomo *et al.*, (2011) :

$$\{m. e. K_2Cr_2O_7 - m. e. FeSO_4\} \times 0.003 \times f \times 100$$

$$\% \text{ Organic C in Soil} = \frac{\text{Weight of Soil}}{\text{Weight of Soil}}$$

Where;

m.e. = milli equivalent = normality of solution × ml of solution used, 0.003 = m.e. weight of C, f = correction factor = 1.33, % Organic matter was calculated using the formula:

$$\text{Percentage (\%) organic matter} = \text{Percentage organic carbon} \times 1.724$$

1.4 Statistical Analysis

Data generated from bacterial enumeration was subjected to logarithm transformation and subsequently expressed in graphs whilst data obtained from fungi enumeration was expressed in tables. Analysis of Variance (ANOVA) was run to compare the means of the different exposure periods of the herbicide. The data was again subjected to Duncan Multiple Range Test (DMRT) to compare the mean values between the baseline determinations and chemical treatments and to bring out the differences that exist between the treated soils.

1.5 Results

1.5.1 Effect of the exposure period of herbicide in relation to the baseline determination

The bacterial population after the herbicide (Atrazine) treatment was higher in the baseline (Control) in all the exposure period followed by the treatment with doubled recommended field rate (RFR). Even though, there was a steady declining rate of the bacterial population in relation to the exposure period, somehow the Atrazine application above the recommended field rate supported the growth of soil bacteria.

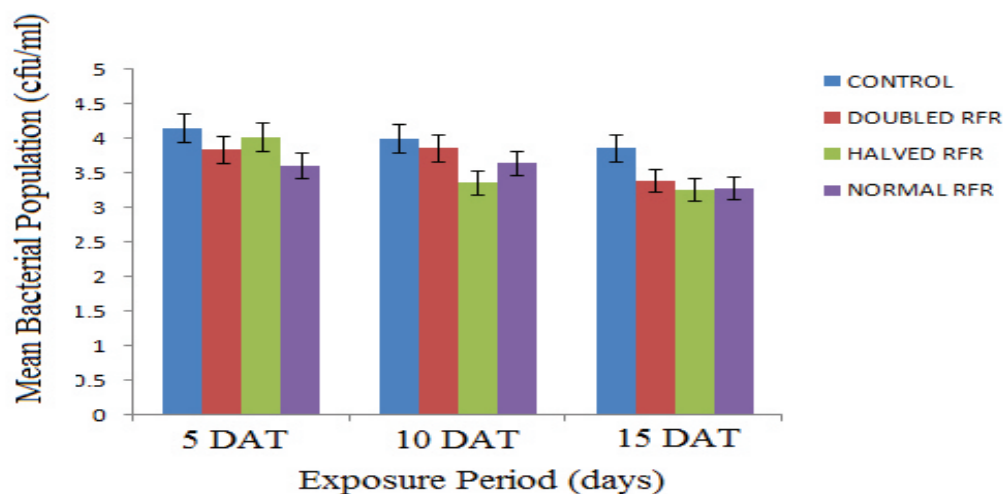


Figure 1. Mean Bacterial Population with Atrazine treatment in relation to the exposure period DAT, is the days after treatment, RFR, is the recommended field rate.

Description of the above figure

Fig.1. above is a graph showing the mean bacterial population of soil treated with Atrazine herbicide against fifteen days exposure period. The baseline determination recorded the highest bacterial population followed by herbicide treatment double of the normal recommended field rate. The only exception was found in the first five days after treatment. Here, half of the manufacturing company's recommended field rate was used and it recorded the second highest bacterial population, but recorded the least bacterial population in both the 10 and 15 DAT exposure periods.

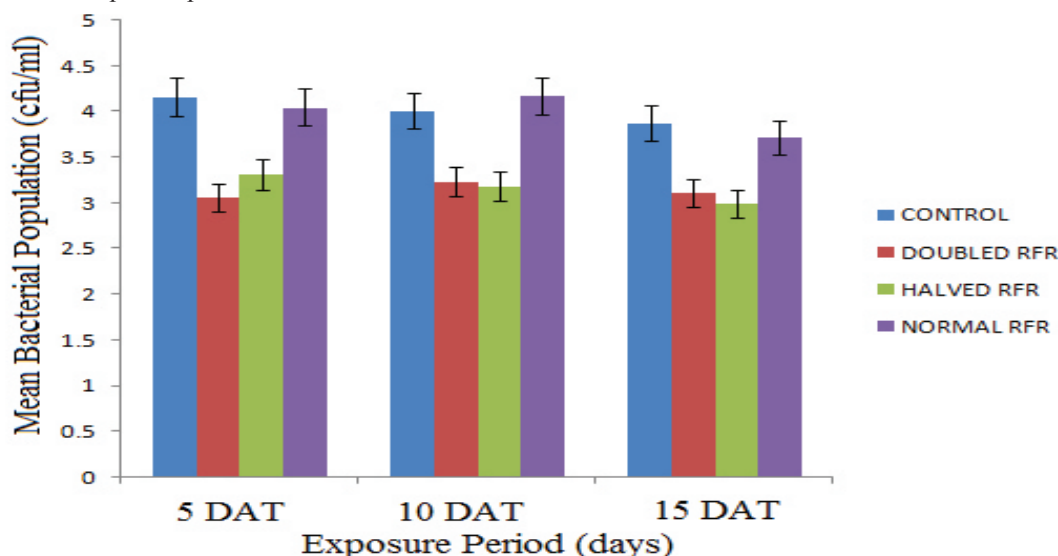


Figure 2. Mean values of Bacterial Population after 2, 4-D amine treatment in relation to the Exposure periods

DAT, is the day after treatment, RFR, is the recommended field rate.

Description of the above figure

The graph above shows (Fig. 2) the mean bacterial population of sample soil treated with 2, 4-D amine herbicide against fifteen days exposure period. The bacterial population in the sample treatment based on the normal recommended field application rate did not differ much throughout. However, bacterial population decreased in the sample with treatment double of the normal RFR in relation to the controlled sample in all the exposure period. But a close look at the halved sample treatment reveals gradual decline in the bacterial population from day five (5) up to day fifteen (15) after treatment.

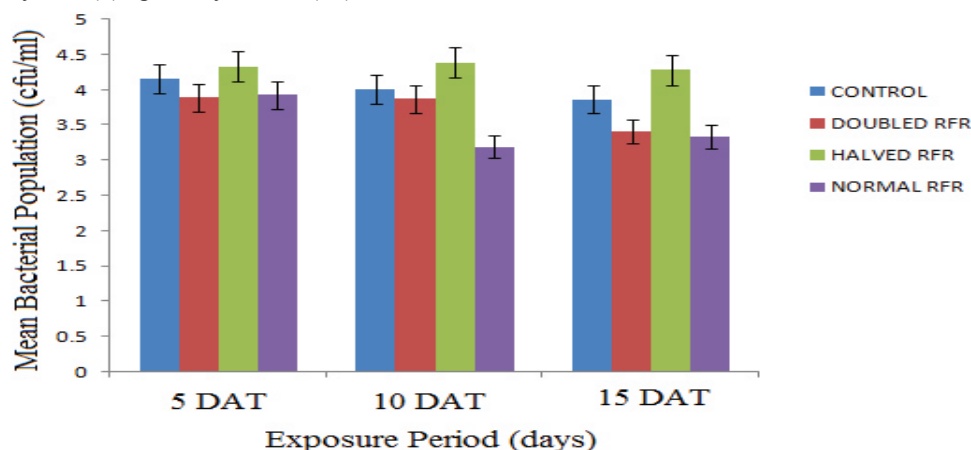


Figure 3. Mean Bacterial Population after Glyphosate treatment over the exposure periods.

DAT, is the day after treatment, RFR, is the recommended field rate.

Description of the above figure

Fig. 3 shows the mean bacterial population of soil treated with Glyphosate herbicide against fifteen days exposure periods. Bacterial population half of the recommended field rate increased constantly from the first five day after treatment to the fifteen days after treatment suggesting an improvement in the bacterial growth and development in relation to the controlled experiment. Again, a decrease in bacterial population was recorded in the fifteen days after treatment with double and normal the company's recommended rate of application.

However, bacterial population steady declined throughout the exposure period might be due to a shortage of carbon source which was provided in the treated samples.

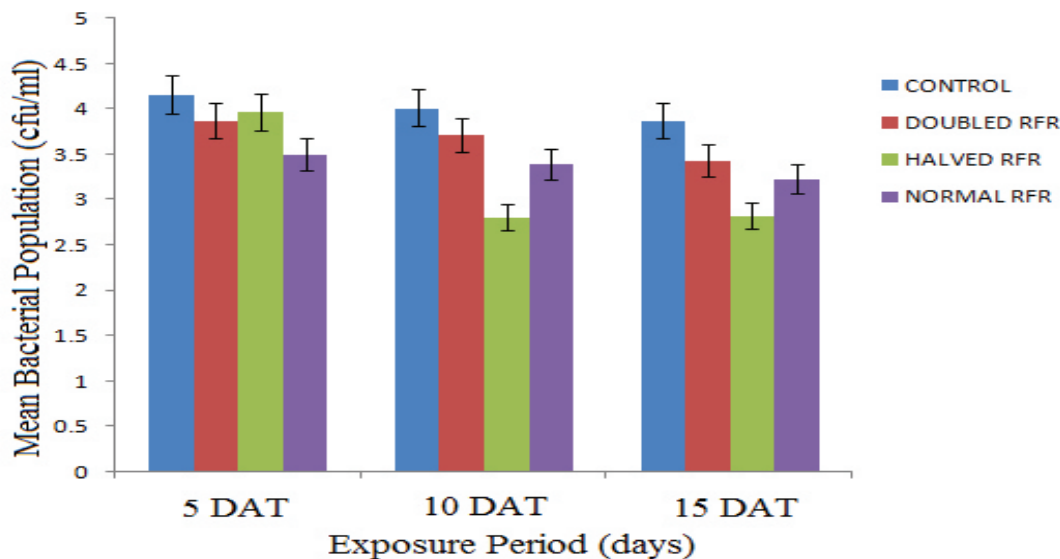


Fig. 4: Mean bacterial population after Paraquat treatment against exposure periods

DAT, is the day after treatment, RFR, is the recommended field rate

Description of the above figure

Fig. 4 shows the mean bacterial population against fifteen exposure periods for sample soil treated with Paraquat. From the fig. 4, the baseline determination recorded the highest bacterial population and subsequently declined gently from the first five days (5 DAT) to day fifteen (15 DAT). The first five days after Paraquat application revealed an increased bacterial population beyond the entire treated sample halved the recommended field rate, but it sharply declined after day five to day fifteen. This decreasing trend in all the treatment towards the fifteen days suggests a decline in carbon source to support the initial population of the bacteria.

1.6 Effect of different exposure period and concentration of the selected herbicides on fungal population

Enumeration of fungi was scored on the basis of four (4) counts or scored on a plate under the different concentration and three different exposure periods for Atrazine, 2, 4-D amine, Glyphosate and Paraquat was recorded. The cumulative fungal population of the four herbicides under different concentration in relation to the recommended field rate (RFR) was calculated and seven specific fungi were recorded from the plates.

The seven fungi recorded were represented with letters (a-g) as: **a** - *Aspergillus flavus*;

b – *Colletotrichum gloeosporioides*; **c** - *Aspergillus niger*; **d** – *Tichoderma viride* **e** – *Mucor*; **f** – *penicillium*; **g** – *Curvularia lunata* indicated in a table below for three different exposure periods. From the table 1 a cumulative score of 13 fungi was recorded as against a zero cumulative record for the baseline.

Table 1. Cumulative fungal Population score of herbicide treatment under three different concentrations of Atrazine

Herbicide Exposure Period	Specific fungal population and their cumulative score						
	a	b	c	d	e	f	g
5 DAT	3	2	2	3	1	0	0
10 DAT	2	6	0	2	2	0	0
15 DAT	2	5	4	0	1	0	0
Cumulative Score	7	13	6	5	4	0	0
Baseline (Cumulative)	4	0	5	0	0	0	3

Description of the above table

Letters: (a – g) represent the seven specific fungi identified, Cumulative score is the sum total of all the fungi identified under the three different herbicide concentration in relation to the recommended field rate (RFR), which is the recommended rate of application on the product label.

The above table (table 1) shows the cumulative fungal population score of herbicide treatment under tree

different concentration. From the table it is clearly seen that some of the fungi identified in the herbicide treated samples were not in the baseline determination. Fungi labeled (b) recorded the highest cumulative score of 13 with the highest enumeration occurring in the first ten days after treatment followed by fungi labeled (a) with the least cumulative fungi recorded for fungi (e) as compared to fungi labeled (g) in the baseline determination though it is absent in the herbicide treatments.

Table 2. Cumulative fungal Population score of herbicide treatment under three different concentration of 2, 4-D amine

Herbicide Exposure Period	Specific fungal population and their cumulative score						
	a	b	c	d	e	f	g
5 DAT	4	0	4	0	1	2	0
10 DAT	7	0	0	3	2	0	0
15 DAT	7	0	4	0	1	0	0
Cumulative Score	18	0	8	3	4	2	0
Baseline (Cumulative)	4	0	5	0	0	0	3

Description of the above table

Letters: (a – g) represent the seven specific fungi identified, Cumulative score is the sum total of all the fungi identified under the three different herbicide concentration in relation to the recommended field rate (RFR), which is the recommended rate of application on the product label.

The table 2 above depicts the cumulative fungal population score of 2, 4-D amine herbicide treatment under three different concentrations for fifteen days continuous exposure period. Fungi labeled (a) had an impressive cumulative score of 18 with 7 fungi enumerated in each of 10 DAT and 15 DAT compared to cumulative score of 4 recorded in the baseline determination. According to the table some fungi were enumerated in the herbicide treatment (b, d, e, and f) but were absent in the baseline determination which might be as a result of a particular component of the herbicide favoring their growth and multiplication. The fungi labelled (f) recorded the least cumulative score of 2 though it was absent in the baseline determination.

Table 3. Cumulative fungal Population score of herbicide treatment under three different concentration of Glyphosate

Herbicide Exposure Period	Specific fungal population and their cumulative score						
	a	b	c	d	e	f	g
5 DAT	2	6	2	3	1	0	3
10 DAT	1	11	0	0	0	0	4
15 DAT	0	1	1	0	0	0	3
Cumulative Score	3	18	3	3	1	0	10
Baseline (Cumulative)	4	0	5	0	0	0	3

Description of the above table

Letters: (a – g) represent the seven specific fungi identified, Cumulative score is the sum total of all the fungi identified under the three different herbicide concentration in relation to the recommended field rate (RFR), which is the recommended rate of application on the product label.

The above table also shows the cumulative fungal population scores of Glyphosate herbicide treatment under three different concentrations at fifteen days continuous exposure period. According to the table 4.3 some fungi like (b, d, e and f) were absent in the baseline determination but were present in the Glyphosate treated sample. Fungi labeled (b) recorded the highest cumulative score of 18 with the highest enumeration occurring at the 10 DAT. It is followed by fungi labeled (g) which recorded cumulative score of 10 compared to a score of 3 in the baseline determination

Table 4. Cumulative fungal Population score of herbicide treatment under three different concentration of Paraquat

Herbicide Exposure Period	Specific fungal population and their cumulative score						
	a	b	c	d	e	f	g
5 DAT	4	2	4	2	0	0	0
10 DAT	3	6	1	0	2	0	0
15 DAT	2	3	1	2	1	0	3
Cumulative Score	9	11	6	4	3	0	3
Baseline (Cumulative)	4	0	5	0	0	0	3

Letters: (a – g) represent the seven specific fungi identified, Cumulative score is the sum total of all the fungi identified under the three different herbicide concentration in relation to the recommended field rate (RFR), which is the recommended rate of application on the product label.

The table reveals the cumulative fungal population scores of herbicide treatment under three different concentrations of Paraquat for 15 days continuous exposure period. It clearly seen from the table that some fungi such as (b, d, e, and f) were absent in the baseline determination but were present in the Paraquat treatment samples. Fungi labelled (b) which was absent in the baseline determination recorded the highest cumulative score of 11 with the highest population enumerated at 10 DAT. Least cumulative score of 3 each was recorded from fungi labelled (e) and (g).

1.7 Mean percentage organic matter under different concentration of four herbicides

The mean values of the % organic matter calculated for each of the four herbicides treatment under three different concentrations did not differ significantly ($p < 0.05$) with the baseline determination as can be seen in the table 11. The 2, 4-D treatment above the normal field application rate recorded a higher mean value of 3.88 ± 0.01 for the percentage organic matter as compared to 3.2 ± 0.01 in the baseline determination

Table 5. Mean \pm SE of % Organic matter of soil treated with the selected herbicides under three different concentrations

HERBICIDE TREATMENT CONCENTRATION (mg a.i / g of soil)	MEAN \pm SE		
	NORMAL RFR (X)	DOUBLED RFR (2X)	HALVED RFR (0.5X)
ATRAZINE	3.07 ± 0.07	3.13 ± 0.08	3.03 ± 0.06
2, 4-D AMINE	3.59 ± 0.02	3.88 ± 0.01	3.06 ± 0.02
GLYPHOSATE	3.11 ± 0.02	3.15 ± 0.01	3.07 ± 0.03
PARAQUAT	2.95 ± 0.01	2.94 ± 0.01	3.13 ± 0.03
BASELINE DETERMINATION	3.20 ± 0.01		

Means and SE is the standard Error; RFR, is the recommended field rate of application.

Description of the above table

The above table shows the mean plus the standard error of percentage organic matter of soil treated with the four selected herbicides under three different concentrations these chemicals. All the mean obtain followed similar trend which is not different from the mean value recorded for the baseline determination

1.8 Discussion

1.8.1 The effect of some selected herbicides on bacterial population

Generally, the effect of the four selected herbicides was not significant to the bacterial population at DMRT ($p < 0.05$), but there was an appreciable change in microbial population in both bacteria and fungi. The three levels of herbicide concentration either impacted a detrimental effect on the microbial population or supported population growth structure of some of the organisms. In figure 4.1, the bacterial population gradually increased from (6.945×10^3 cfu/ml) of the first 5DAT to (7.400×10^3 cfu/ml) of the 10DAT exposure period with Atrazine treatment when the normal field application rate was doubled relative to the baseline which decreased from

(14.462×10^3 cfu/ml) of the 5DAT to (10.245×10^3 cfu/ml). This increase suggests the capacity of the organisms to degrade some aspect of the herbicide and utilize it as a carbon source to support their growth and multiplication (Wardle and Rahman, 1992).

The increase in bacterial population after the herbicide treatment is in support of the research conducted by Sebiomo *et al.*, (2011), who also recorded an increased bacterial population in the first and second week of the same herbicide treatment. However, there was a sharp decrease in bacterial population which might be due to the fact that the rise in bacterial population became lethal with the subsequent increase in exposure period as reported by (Anderson *et al.*, 2000). Similarly, there was a steady decrease in bacterial population from 5DAT, 10DAT and 15DAT respectively, compared to the baseline determination in the current study. A study conducted by (Zain *et al.*, (2013) also recorded such free-fall decrease in microbial population under similar soil treatment in Malaysia.

Contrary, to the above trends where there is an increased microbial population from 5DAT to 10DAT and a subsequent decrease to 15DAT, bacterial population of 69.3% under normal field application rate declined sharply to 12.7% and rose again to 18.0% after Glyphosate treatment (See fig. 3). Glyphosate is a P-containing amino acid that functions both as a sole P source for in vitro microbial growth and as a readily available C and N source when degraded in soil (Busse *et al.*, 2001), hence, the sharp declination observed in table 4 under normal field rate might be due high mortality rate since high population would lead to fast depletion of the carbon source. Some studies report increased populations of actinomycetes and fungi after treatment with Glyphosate increased soil microbial biomass (Hanley *et al.*, 2002). In figure 4.4, Paraquat treatment recorded an impressive 87.2% bacterial population for the first 5DAT but the effect of its toxicity was felt from 10DAT (6.5%) to the 15DAT (6.4%). Similarly, Zain *et al.*, (2013) reported a drastic inhibition of both bacterial and actinomycetes populations by Paraquat to about 70 to 82% at recommended rate. The inhibitory capacity of Paraquat stems from the fact that it is known to be bounded strongly and coherently to soil component, including clay minerals and organic matter, therefore limits the access of microorganisms to Paraquat in soil water. The result of this study for Paraquat is consistent for all the treatment levels (Doubled, Halved and Normal recommended field rate of application).

1.8.2 The effect of some herbicides on soil fungal biomass and % organic matter

The fungal population was scored on the basis of four (4) maximum counts on a plate. With this as many as seven (7) different fungi were observed and are represented with the letters (a – g). For this reason cumulative score for each herbicide treatment were tabulated taking into consideration the treatment levels as indicated in table (4.1 - 4.4). In this study, a cumulative score of 7 was recorded for a particular fungus by Atrazine treatment compared to 4 score for the baseline determination. Again, cumulative score of 13 was scored as compared to zero (0) for the baseline (control). A laboratory study conducted by Estok *et al.*, (1989) and Busse *et al.*, (2001) confirm this inhibitory effect to some fungi. It can be seen from table 4.1 that at some exposure periods some fungi scored 0 relative to the baseline and even under normal field rate of the herbicide application. Most of these fungi were present in the baseline determination but were absent in the treatment samples. This means that some of the herbicides are toxic whilst others may be moderately toxic to some fungi. Claims have been made that repeated application of Atrazine does not affect the number of viable fungi in any way (Cole, 1976), suggesting that herbicides can elicit different reactions by different fungi. Certain fungal species are benefitted by herbicide addition, while others are inhibited. This trend is consists in all the herbicide treatment with some cumulative score as far as 18cfu/ml.

In this study the percentage organic matter of the control and the various herbicide treatments did not differ ($P=0.05$) much with the Mean \pm SE of 3.2 ± 0.01 for the baseline determination. Only 2, 4-D amine recorded 3.88 ± 0.01 when the concentration was doubled compared to the normal field rate of application. According to table 5 it could be seen that almost all the % organic matter revolved around the baseline determination with no prominent significant differences at ($p < 0.05$). This unaffected pattern of the percentage organic matter might be due to the short term nature of the exposure period for the herbicides treatment, since Sebiomo *et al.*, (2011) recorded from a study conducted in Nigeria that soil organic matter increased after continuous application from the second to the six week of treatment.

1.9 Conclusion

The results of the study indicates 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 microorganism. The toxic effect of some of the herbicide was felt shortly after its application whilst herbicide treatment like Paraquat had lasting effect on most microorganisms. For instance, the population of bacteria sharply increased to about 87.2% but steeply declined to 6.4% from the 10DAT to 15DAT. The pattern of change may vary as a result of differences in exposure period, the concentration of the active ingredient in the formulation, time of exposure, and so many environmental factors. This is supported by the view that microbial response to herbicides manifests itself in a variety of ways depending on factors including the herbicide itself, inherent micro-organism populations,

herbicide concentration, exposure time, and chemical and physical characteristics of the soil. Almost all the herbicide inhibited the growth of some specific fungi; the reason was that some fungi which were found in the baseline determination were not seen after the herbicide treatment and vice versa. In this experiment the % organic matter seems not to be influenced by the herbicide exposure and its concentration but studies have already shown that continuous application of herbicide leads to an increased organic matter. Again, any change in the microbial structure will have a proportional change in the % organic matter since dehydrogenase activities level in the soil is an indication of how fertile a particular soil is. However, the significance of these herbicides in modern agriculture should not be relegated to the background when issues of productivity and food security are at stake.

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