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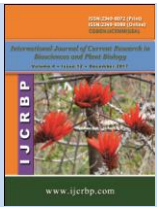

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Seasonal Influence of Glyphosate Herbicide Application on Soil Bacteria in Benue State, North Central, Nigeria

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Abstract

Glyphosate herbicide is among the most commonly used herbicide globally. Despite their value to agriculture, they posed direct or indirect threats not only to human health but also to beneficial organisms in the delicate web of nature. The soil samples were collected randomly from selected plots located in three towns in Okpokwu LGA of Benue State during wet and dry seasons (July and November respectively). Each of experimental plots sample were randomly selected from three different locations and exposed to various concentrations of glyphosate herbicide in three treatments (in replicate) 0, 0.84, and 840 kg/ha and four sampling days (1, 3, 7 and 30). The impacts of glyphosate herbicide application on soil bacteria were observed using standard laboratory procedures. The result revealed that there were no significant differences among the mean number of bacteria isolated in Ugbokolo town with the different concentrations of glyphosate applied on day 1. The same trend was established for days 3, 7, and 30 respectively for the remaining two towns, Ogene and Opialu. The bacteria *Acetobacter* sp., *Agrobacterium* sp., *Agromyces ramosus, Arthrobacter* sp., *Azotobacter* sp., *Bacillus* sp., *Clostridium* sp., *Pseudomonas* sp. and *Rhizobacter daucus* were found in all the locations studied. However, *Acinetobacter* sp. was only found in Ogene and Opialu while *Citrobacter* sp. was only found in Ugbokolo and Opialu. *Agrobacterium* sp., *Bacillus* sp. and *Pseudomonas* sp. increased in all the locations with increased concentration of glyphosate application. The increase in the number of bacteria colonies were more in the month of November (dry season) than in the month of July (wet) in all the locations. The indiscriminate use of glyphosate herbicide by rural farmers is a common practice among rural settlers in Benue State; therefore, public understanding and knowledge of the recommended dosage of glyphosate herbicide is of utmost important.

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Introduction

In recent times, the intensive use of herbicides has increasingly become a matter of environmental concern or controversy, largely because of the adverse impact of these chemicals on soil bacteria and other microorganisms in general. The use of herbicides have increased 50 fold since 1950 and about 2.5 million tons of industrial herbicides are now used each year to secure food supply for the growing

global population (Miller and Tyler, 2002). In the tropical regions of the world, agricultural intensification has led to higher pesticide utilization (Racke et al., 1997; Shipitalo et al., 2008).

Glyphosate based herbicide is a broad-spectrum, non-selective, post-emergence herbicide that is widely used and noted for its broad effectiveness in agriculture. The mild effect of herbicides on conifers, rapid inactivation in soil, and low mammalian toxicity confirmed its effectiveness and wide usage (DiTomaso 1997).The commercial success of glyphosate as a highly effective herbicide has stimulated several studies on its behavior and persistence in soil (Krzysko-Lupicka and Orlik, 1997; Forlani et al., 1999; Jonge and Jonge, 1999). Bacteria and other microbial degradation are considered to be the most important of the transformation processes that determine the persistence of herbicides in soil (Souza et al., 1999).

Glyphosate has been shown to drastically alter the balance of soil microbial populations and metabolites, by enhancing mycobacteria or decreasing bacteria population and other photosynthetic microorganisms (Araujo et al., 2003; Bahig et al., 2008). By altering the balance of soil ecology in thismanner, glyphosate herbicides poses hazard to one or more foundational branches of the microbial food chain. Accinelli et al. (2002) showed that glyphosate herbicide applied *in* agricultural and commercial doses can increase bulk microbial activity in agricultural soils. Soil which had been exposed to glyphosate herbicide for several years had a strong response in microbial activity due to available nutrient such as carbon, nitrogen and phosphorous. However, after 32 days of incubation with glyphosate, the result showed that bacteria population was slightly reduced. Though glyphosate herbicide appeared to be rapidly degraded by soil bacteria and other microbes regardless of soil types even at high application rates without adversely affecting microbial activity (Ficke et al., 2000). But Motavalli et al. (2004) observed that minor alterations are evident in the total diversity of the soil bacteria. Such appearances or disappearance of certain bacteria (e.g., rhizobacteria) could affect soil health and ecosystem function.

The aim of the work presented in this paper was to evaluate the seasonal influence of glyphosate based herbicide on bacterial activity in the soil with the history of absence of glyphosate application on the soil.

Materials and methods

Experimental design and field sampling

The samples were selected randomly each from three farm plots located at three different areas namely; Ugbokolo, Ogene and Opialu in Okpokwu Local Government Area of Benue State during wet and dry seasons. All the farm plots had no previous history of any herbicidal application. The experiment was a completely randomized design with three replicates per treatment. Treatment included three levels of glyphosate application which were 0, 0.84 and 840 kg ai/ha and four sampling days (1, 3, 7 and 30) after application. The field rate concentration of the herbicide is 0.84 kg ai/ha but the highest concentration used (100-fold) was selected to test effects since farmers sometimes use this or even a higher concentration other than the recommended field rate. A control treatment with no glyphosate application was included for each soil. Therefore, comparison with glyphosate addition to each soil would measure the positive or negative influences on bacterial cell (Haney et al., 2002). The different days were selected to test the relative short and long term effects of the glyphosate herbicide since the biodegradation of the herbicide is about 32 days (Bahig et al., 2008). The glyphosate was applied to the plots at the rates expressed above using Carbon dioxide pressurized knapsack sprayer as describe by Parions et al. (2003). The soil samples were collected from the upper 10 cm of the soil profile as proposed by Lal and Saxena (1982). After the removal of living materials and debris (such as mosses, roots, etc.) including objects > 2 cm in diameter, about 500g of fresh soil was obtained using a hand trowel and cutlass. The samples were taking to the laboratory in polyethylene bags. The samples where air dried by spreading it on a sterile carbon paper on a flat bench for 72 hrs and afterward was put in polyethylene bags and kept in the refrigerator at 4°C for subsequent analysis (Haney et al., 2000).

Laboratory analysis

Bacterial culture and isolation

The media were prepared according to the manufacturer's instructions. Before inoculation of samples, the prepared media were incubated over-night to check for their sterility (CoIlins et al., 1989).

Culturable bacteria were extracted by agitating a 3g sub-

sample of soil (oven dry equivalent) in a 10-fold dilution of 0.15m NaCI with 3mm glass beads for 10 minutes. Serial dilutions were plated in replicates on tryptic soy agar and nutrient agar for culturable bacteria. Bacterial colonies were counted after 24 hrs of incubation at 28°C (Alice et al., 2006). Bacterial colonies were counted on dilution plates containing 50 - 200 colonies. For the culture of anaerobic bacteria, anaerobic jar was used. The jar was incubated at 28°C for 24 hrs. Individual colonies of bacteria which vary in shape and colour were picked up and purified by streaking on nutrient agar. The bacteria isolates were kept on nutrient agar at 4°C and recultured every four weeks. The bacteria isolates were identified on the basis of classification schemes published in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

Identification of bacteria

The bacteria isolates were identified on the basis of classification schemes published in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994), using the following characteristics :Gram reaction, Cell shape, Motility in liquid medium, colony pigmentation, Oxidase and Catalase test, and Indole production test.

Statistical analysis

Treatments were replicated three times in a completely randomized design. Statistical analysis was conducted using the Turkey-Kramer multiple comparisons test at the 5% probability level.

Results

The result of the soil samples revealed that there were no significant differences among the mean number of bacteria obtained in Ugbokolo town with the different concentration of glyphosate herbicide applied on day 1. The same trend was established for days 3, 7, and 30 respectively for sample obtained from Ogene and Opialu town (Tables 1 and 2).

The bacteria *Acetobacter sp., Agrobacterium sp., Agromyces ramosus, Arthrobacter sp., Azotobacter sp., Bacillus sp., Clostridium sp., Pseudomonas sp.* and *Rhizobacter daucus* were found in all the locations studied. However, bacteria *Acinetobacter sp.,* were only found in Ogene and Opialu while *Citrobacter sp.,* was only found in Ugbokolo and Opialu. *Agrobacterium sp., Bacillus sp.,* and *Pseudomonas sp.,* increased in all the locations with increased concentration of glyphosate application. The increase in the number of bacteria colonies were more in the month of November (dry season) than in the month of July (wet) in all the locations (Table 3).

Table 1. Effect of glyphosate application on the number of bacteria detected in Ugbokolo, Ogene and Opialu towns in Okpokwu LGA of Benue State in the month of July during the wet season.

Soil locations	Day1			Day3			Day7			Day30			SD	SE
	0kgha^{-1}	0.84kgha^{-1}	840kgha^{-1}	0kgha^{-1}	0.84kgha^{-1}	840kgha^{-1}	0kgha^{-1}	0.84kgha^{-1}	840kgha^{-1}	0kgha^{-1}	0.84kgha^{-1}	840kgha^{-1}		
Bacteria (cfu/ml)														
Ugbokolo	9.8×10^{6}a	1.2×10^{7}a	1.7×10^{7}a	8.0×10^{6}a	7.2×10^{8}a	9.0×10^{8}a	6.9×10^{6}a	9.4×10^{8}a	1.4×10^{9}	8.8×10^{6}a	6.3×10^{7}a	8.2×10^{7}a	4.987×10^{8}	1.440×10^{8}
Ogene	7.6×10^{6}a	9.4×10^{6}a	1.6×10^{7}a	8.2×10^{6}a	1.2×10^{8}a	1.8×10^{8}a	6.2×10^{8}	6.8×10^{8}a	8.8×10^{8}a	5.8×10^{6}	1.1×10^{7}a	1.3×10^{7}a	5.581×10^{8}	1.611×10^{8}
Opialo	1.7×10^{7}a	6.1×10^{7}a	8.8×10^{7}a	7.5×10^{7}a	6.6×10^{8}a	5.4×10^{8}a	1.4×10^{7}a	1.8×10^{8}a	8.6×10^{8}a	1.6×10^{7}a	6.2×10^{7}a	1.7×10^{8}a	3.108×10^{8}	8.971×10^{8}
SD	4916638	2.9×10^{7}	4.13×10^{7}	3.86×10^{7}	3.58×10^{8}	2.38×10^{8}	3.52×10^{8}	5.86×10^{8}	4.31×10^{8}	5242137	2.97×10^{7}	7.87×10^{7}		
SE	2838623	1.68×10^{7}	2.38×10^{7}	2.33×10^{7}	2.07×10^{8}	1.38×10^{8}	2.03×10^{8}	3.38×10^{8}	2.49×10^{8}	3026549	1.72×10^{7}	4.54×10^{7}		

*Values for each location are mean of triplicates sample per plots. Means followed by the same letter in each column are not significantly different at $p<0.05$ according to Tukey-Kramer multiple comparisons test. (SD= Standard Deviation, SE= Standard Error of Mean.

Table 2. Effect of glyphosate application on the number of bacteria detected in Ugbokolo, Ogene and Opialu towns in Okpokwu LGA of Benue State in the month of November during the dry season.

Soil locations	Day1			Day3			Day7			Day30			SD	SE
	0kgha^{-1}	0.84kgha^{-1}	840kgha^{-1}	0kgha^{-1}	0.84kgha^{-1}	840kgha^{-1}	0kgha^{-1}	0.84kgha^{-1}	840kgha^{-1}	0kgha^{-1}	0.84kgha^{-1}	840kgha^{-1}		
Bacteria (cfu/ml)														
Ugbokolo	4.2×10^{4}	4.4×10^{4}	4.6×10^{4}	4.4×10^{4}	4.6×10^{4}	4.1×10^{4}	4.3×10^{4}	6.0×10^{4}	4.5×10^{4}	4.5×10^{4}	4.8×10^{4}	4.8×10^{4}	2.549×10^{7}	7.359×10^{8}
Ogene	4.8×10^{4}	4.4×10^{4}	4.6×10^{4}	5.1×10^{4}	5.0×10^{4}	4.9×10^{4}	4.9×10^{4}	4.6×10^{4}	5.1×10^{4}	4.7×10^{4}	4.2×10^{4}	4.7×10^{4}	4.894×10	1.412×10^{8}
Opialo	4.8×10^{4}	4.4×10^{4}	5.0×10^{3}	4.5×10^{4}	4.6×10^{4}	4.5×10^{4}	4.7×10^{4}	4.3×10^{4}	4.8×10^{4}	4.9×10^{4}	4.8×10^{4}	4.7×10^{4}	3.022×10^{8}	8.72×10^{8}
SD	4305020	3.3×10^{7}	4.39×10^{7}	2803266	4.51×10^{7}	10×10^{8}	4235957	9.63×10^{7}	10×10^{8}	3592511	3.50×10^{8}	4.42×10^{8}		
SE	3485505	1.94×10^{7}	102.53×10^{7}	1618466	2.6×10^{7}	7.48×10^{7}	2445631	5.6×10^{7}	1.79×10^{8}	2074137	2.02×10^{8}	2.6×10^{8}		

*Values for each location are mean of triplicates sample per plots. Means followed by the same letter in each column are not significantly different at $p< 0.05$ according to Tukey-Kramer multiple comparisons test. (SD= Standard Deviation, SE= Standard Error of Mean.

Table 3. Bacterial colonies isolated from Ugbokolo, Ogene and Opialu towns of Okpokwu LGA in Benue State before and after gyphosate application during the wet and dry seasons.

Bacteria	Month of July in the wet season {No. of colonies (cfu/ml) × 10^4}						Month of November in the dry season {No. of colonies (cfu/ml) × 10^4}					
	Before glyphosate application			After glyphosate application			Before glyphosate application			After glyphosate application		
	Ugbokolo	Ogene	Opialu	Ugbokolo	Ogene	Opialu	Ugbokolo	Ogene	Opialu	Ugbokolo	Ogene	Opialu
Acetobacter sp.	9.0^{a}	7.0^{a}	10.5^{a}	15.0^{a1}	13.5^{a}	18.0^{a2}	6.5^{a}	5.0^{a}	8.0^{a}	23.5^{a3}	21.0^{a1}	26.5^{a3}
Acintobacter sp.	0.0^{a}	15.2^{a}	15.0^{a}	0.0^{a}	21.0^{a}	26.0^{a}	0.0^{a}	10.0^{a}	12.5^{a}	0.0^{a}	28.5^{a}	34.0^{a}
Agrobacterium sp.	11.5^{a}	9.0^{a}	13.0^{a}	18.0^{a}	14.5^{a}	23.0^{a}	9.0^{a}	8.5^{a}	11.5^{a}	30.5^{a}	32.0^{a}	31.5^{a}
Agromyces ramosus	19.0^{a}	16.0^{a}	14.5^{a}	25.0^{a}	21.0^{a}	22.0^{a}	17.5^{a}	14.0^{a}	13.0^{a}	29.5^{a}	29.0^{a}	29.5^{a}
Arthsobacter sp.	14.5^{a}	17.0^{a}	19.0^{a}	19.5^{a}	28.0^{a}	27.5^{a}	11.5^{a}	13.5^{a}	16.0^{a}	28.0^{a}	30.5^{a}	31.0^{a}
Azotobacter sp	8.5^{a}	10.0^{a}	13.5^{a}	11.0^{a}	14.5^{a}	18.0^{a}	7.0^{a}	8.0^{a}	11.5^{a}	21.0^{a}	24.0^{a}	29.5^{a}
Bacillus sp.	13.0^{a}	10.0^{a}	15.0^{a}	31.5^{a}	29.0^{a}	37.0^{a}	10.5^{a}	9.0^{a}	12.5^{a}	39.5^{a}	37.0^{a}	41.0^{a}
Citrobacter sp.	11.0^{a}	0.0^{a}	13.0^{a}	16.0^{a}	19.0^{a}	9.0^{a}	14.0^{a}	0.0^{a}	14.0^{a}	22.0^{a}	0.0^{a}	28.5^{a}
Clostridium sp.	12.5^{a}	13.0^{a}	16.0^{a}	22.0^{a}	20.5^{a}	25.5^{a}	11.0^{a}	10.5^{a}	13.5^{a}	26.5^{a}	24.0^{a}	30.0^{a}
Pseudomonas sp.	9.5^{a}	11.0^{a}	13.0^{a}	28.5^{a}	36.0^{a}	51.0^{a}	7.0^{a}	8.5^{a}	10.0^{a}	34.5^{a}	41.5^{a}	53.0^{a}
Rhizobacter daucus	15.0^{a}	9.0^{a}	12.5^{a}	18.0^{a}	11.0^{a}	14.0^{a}	11.5^{a}	7.0^{a}	9.5^{a}	28.5^{a}	21.5^{a}	26.5^{a}
SD	4.782	4.588	2.212	8.613	7.633	11.463	4.554	3.857	2.247	10.100	10.776	7.805
SE	1.442	1.383	0.6668	2.597	2.301	3.456	1.373	1.163	0.6776	3.045	3.249	2.353

*Values for each location are Means of replicate samples per plot. Means followed by the same letter in each column are not significantly different at $p< 0.05$ according to Tukey-Kramer multiple comparisons test (SD= Standard Deviation, SE= Standard Error of Mean).

Discussion

In our present findings of the seasonal influence of glyphosate herbicide on soil bacteria established that bacteria cells increased with increasing concentrations of the glyphosate we herbicide. The increase could be attributed to the availability of nutrients (nitrogen, carbon and phosphorus) provided by the glyphosate herbicide. This is in conformity with the reports of Haney et al. (2000) who noted that glyphosate application can increase soil microbial biomass, respiration, carbon and nitrogen mineralization. This finding was also supported by the study of Barrett and McBride (2005) who observed that both biotic and abiotic oxidative degradation of glyphosate caused breakage of both with the C-P and C-N bonds thereby releasing carbon, phosphorous and nitrogen to the soil which could have contributed to the growth of the bacteria as observed in this study. The study further revealed that there was a decline in bacterial counts on day 30 after glyphosate application compared to day 7. The reason could not be far-fetch from the decline in the available nutrients due to rapid mineralization as suggested by Mamy et al. (2005) that glyphosate availability in soil declined between averages of 25 to 35 days.

The findings further established that *Acetobacter sp.*, *Agrobacterium sp.*, *Agromyces ramosus*, *Arthrobacter sp.*, *Azotobacter sp.*, *Bacillus sp.*, *Clostridium sp.*, *Pseudomoas sp.* and *Rhizobacter sp.* occurred in all the locations in both wet and dry seasons whereas *Acinetobacter sp* occurred only in Ogene and Opialu while *Citrobacter sp.* occurred in Ugbokolo and Opialu respectively. *Agrobacterium sp.*, *Bacillus sp.* and *Pseudomonas sp.* increased significantly after glyphosate application. Their increase could stem from the fact that they are organotrophic bacteria, meaning that they can use organic chemicals for energy and nutritional requirements. The viable bacterial cells isolated in all the locations in the month of November showed considerable increase in the number of bacterial cells as the concentration of the glyphosate herbicide increases. Unlike the result obtained in the month of July, where the number of bacterial cells witness a reduction from day 7, in contrast to bacterial cells obtained in the month of November which increases up to day 30. The reason for this observation was borne of the fact that the degradation of glyphosate was more rapid in the wet season than in the dry season which conforms to the finding of Smith and Aubin (1993) that the relative dryness of the soil in dry seasons may result to slow dissipation of glyphosate herbicide. Weaver et al. (2007), reported that glyphosate herbicide dissipates rapidly when applied to moist soil than dry soil.

In conclusion, glyphosate herbicide seems to have little or no detrimental effects on soil bacteria therefore its usage should not only be encouraged but also the recommended application rate should be followed in addition to the time of application of glyphosate herbicide following its persistent nature in certain seasons.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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