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EFFECTS OF GRAMOXONE HERBICIDE ON SOIL MICROBAL POPULATION AT OBIO AKPA, SOUTHEAST, NIGERIA

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Abstract

This study was conducted to determine the effects of Gramoxone herbicide on microbal populations in the soil. The experiment was laid out using Randomized Complete Block Design containing four (4) levels (0, 3, 4 and 5l/ha) of Gramaxone with three (3) replications and each replication contained four (4) plots giving a total of twelve (12) plots of 5mx5m each. Samples were taken before and after depths and analyzed by routine methods. Treatment was applied, sample were taken before and after treatment applications. Results show that total heterotophic bacterial count increased on plots that received 0 L/ha rate of treatment by 70%. While 3, 4 and 5L/ha rates decreased by THBC 55% and 65 respectively. Total hetertrophic fungal count increased only at OL/ha by 20% while 3L/ha,/4L/ha, and 5l/ha. Rate decrease by 74%, 50% and 53% respectively. Bacteria isolated from the plots before the experiment were bacillus spp and staphylococcur aureus, after treatment application were Bacililus spp, staphylococcus spp; pseudomonas and achromaycates spp. Fungal isolated from the plots before the experiment were aspergillus niger and mould. After the treatment applications; aspergillus niger, mould and aspergillus falvus. The implications of these finding suggest that the application of herbicides should be understood in other to preserves soil micro-organism.

***Keywords**: Herbicide, microbial population, soil properties*

Introduction

Over the years soils have faced various degrees of perturbation due to man's incessant interference with them. Agricultural practices including herbicide application are among the common anthropogenic activities that impact on soil environments. Most herbicide applications affect the microbial communities and enzymes in the soil, particularly in the tropics where environmental conditions facilitate intense weathering and rapid organic matter decomposition (Ganifreda and Bollag, 1996). Soil microbial community composition is of great importance because they play a crucial role in carbon flow, nutrient cycling and with decomposition which in returns, affects soil fertility and plant nutrition and occupy a unique position in terrestrial habitats biological cycles (Pandey *et al.*, 2007; Tripathi *et al.*, 2006). The activities, numbers and diversity of soil microbes serve as bio-indicators of changes in soil properties following soil contamination. The stability of soil ecological system is a function of a healthy population of soil microbes which has the ability to regenerate nutrients to support plant growth. Any change in microbial population (and activity) may affect nutrient cycling as well as viability of nutrients, which indirectly affects productivity and other soil functions (Chauran *et al.,* 2006).

Herbicides are agrochemicals used to control weeds on farms, though they can also be used for non-farming purposes. They are classified according to their activities, timing of applications, method of application, mechanism of action and chemical family (Hush, 2001). Gramoxone herbicide is a contact and non-selective agrochemical with paraquat or N, N'- deinthy 1-4, 4 'bipyridum dichloride ($(C_6H_7N)_2Cl_2$) as the active ingredient (Revkin, 1983). It is fast-acting, rain-fast within minutes of application, stable for at least two years from the date of manufacturing (provided, kept under normal storage conditions) on contact with soil make the herbicide a very flexible product that fits into a wide range of farming practices (Sigma-Aldrich, 2015).

There is paucity of information on the effects of herbicide on soil microbial populations within the study area, it is for this reason that this study was thought out and conducted hoping that its finding would contribute to knowledge and help improve crop production and environmental health, besides creating public awareness on the danger of indiscriminate application of the herbicide.

Material and methods

Study area. The study area was Akwa Ibom State University and Research Farm, Obio Akpa with

geographical coordinates of $80^{0}00^{1}$E of GMT. The area has a humid tropical climate with the mean annual temperature of 23-27^{0}C. The area has a mean annual rainfall of 2500-300-mm with bimodal maxima called August break occurring in July or August and high relative humidity ranging from 75-79% during the rainy season (NIMET, 2018). Soils in the area are termed acidic (Enwerzor *et al.*, 1990). The geology of the study area is coastal plain sands. Its vegetation is mostly secondary forest (Ekong and Uduak, 2015).

Field studies. The study was conducted between March 2019 and June 2019. The experiment was laid out using a Randomized Complete Block design with three (3) replications and four plots per replications each representing the treatment rates. Each plots measured 5m x 5m. The distance between replications was 1.5m while the distance between treatment plots within a replication was 1m. Gramaxone herbicide was applied at the rates of 0, 3, 4 and 5l/ha. Each treatment was mixed with 750ml of clean water in a knapsack sprayer and sprayed on the soil of its corresponding plots. The specific plot application rates were worked out based on the Gramaxone manufacturer specification (i.e. Sigma-Aldrich, 2015) were 7.5, 10 and 12.5ml for 0, 3. 4 and 5l/ha rates of application respectively. Besides the manufacturer's recommendation of 30,000 ml of mixing water percentage, the specific amount of water used in mixing the herbicide applied on each treatment plot was 750ml. After thorough mixing, the herbicide was carefully and evenly applied on each treatment plot with a hand filed sprayer (2l capacity).

Soil sample size and sampling technique: Ten (10) composite soil samples collected at 0-15cm and 15-30cm depths from the experimental field were used for the study. Two of the samples were taken before treatment application, while the rest were taken 30 days after treatment application (end of experiment). Four (4) composite soil sample (each representing a treatment level) were collected 30 days after application for the evaluation of treatment effects on the heterotrophic bacterial and fungal populations. At each time, soil sampling for microbial assay was done aseptically with the use of a sterilized soil augur, after which the soil samples were taken to the laboratory in clean and well-labeled polythene bags in an ice-cold pack for analysis.

Soil analysis: Two composite soil samples were air dried, crushed and passed through a 2mm-mesh sieve (Udo *et al*., 2009), to obtain fine earth separates before they were subjected laboratory analysis. Soil properties determined were particle size distribution, texture, P^{H}, EC, organic matter content, total

nitrogen, available phosphorus, exchangeable bases (Ca, Mg, K and Na), exchangeable acidity, effective cation exchange capacity (ECEC) and base saturation (BS). Particle size distribution was determined by the Bouyoucos hydrometer method after dispersing the soil samples with sodium haxametaphosphate (Calgon) solution (Klute, 1986). Soil pH was determined by using digital pH meter in water suspension of 1:2:5 soil/water ratio (FAO, 2002). Organic matter content was determined by Walkey-Black wet oxidation method (Nelson and Summers, 1996). Total nitrogen was determined by Macro-Kjedahl digestion and distillation method (Bremner and Mulvaney, 1982). Available phosphorus was determined by the Bray P-1 extraction method (Bray and Kurz, 1945) and its concentration in the extract was determined by the blue colour method of Murphy and Riley (1962). Exchangeable cations (Ca, Mg, Na and K) were determined by $IMNH_4OA_c$ extraction using EDTA titration method. Na and K were obtained using flame photometer, while Mg and Ca were obtained using Atomic absorption Spectrophotometer (AAS) as outline by Udo *et al.*, (2009). Electrical conductivity was determined in 1:2:5 soil water extract using a conductivity meter or bridge as described by Udoh *et al* (2009). Exchangeable acidity was determined by the KCI extraction method as described by Agbede (2009). Effective cation capacity (ECEC) was determined by summing up total exchangeable base (TEB) and exchangeable acidity (EA) as described by Udo *et al* (2009). Base Saturation was determined using the relationship: BS=100 TB/ECEC as described by *Udo et al* (2009).

Each composite soil sample was cultured to determine heterotrophic bacterial and fungal population densities which give the microbial assay. 1gram of each of the sample was weighed and serially diluted to 10^5. The last dilutions were cultured on Nutrient Agar for total Heterotrophic fungal counts (THFC). Plates of Nutrient Agar were incubated at 37^0c for 24-48 hours, while plates of Subrand Dextrose Agar for 3-4 days at room temperature. The emergent colonies were enumerated and sub-cultured. Pure culture of bacterial isolates was obtained by repeated sub-culturing. The pure bacterial isolates were obtained by repeated sub-culturing. The pure bacterial isolates were stored in slants of nutrients Agar for characterization and identification. The bacterial isolates were examined for colonial morphology, microscopic appearance and biochemical characteristics using standard identification procedures outlined by Cowan and Steels (1994).

Statistical Analysis: Data generated were subjected to analysis of variance (ANOVA) to assess the effects of the treatment rates of Gramoxone herbicide on soil microbial populations. Significantly different treatments were separated using the least significant difference (LSD) at 5% probability.

Results and discussions

Table 1: soil physicochemical properties of the experimental soil before treatment application

Soil properties	Soil depth 0-15cm	Soil depth 15-3ocm
Sand (%)	89.80	85.80
Silt (%)	3.80	5.40
Clay (%)	6.40	8.80
Texture	Loamy sand	Loamy sand
P^H	5.30	5.20
Electrical conductivity	0.04	0.55
Organic matter (%)	2.89	2.79
Total nitrogen (%)	0.07	0.07
Available phosphorus (mg/kg)	16.23	11.86
Exchangeable bases ($cmolkg^{-1}$)		
Ca	3.20	4.00
Mg	1.90	1.80
K	0.12	0.11
Na	0.06	0.06
Exch. Acidity ($cmolkg^{-1}$)	3.84	3.08
Effective Cation Exchange Capacity ($cmolkg^{-1}$)	9.12	9.61
Base saturation (%)	57.89	67.9

The result of the mechanical analyses of the pre-experimental soil revealed that the top soil (0-15cm depth) has 89.80% and 3.80% silt and 6.40% clay respectively. The distribution of the various separates showed that the soils were predominantly loamy sand in texture (Ekong and Uduak, 2015). This finding agrees with those reported by earlier researcher (Brady and Weil, 2002; Ibia, 2012) on similar soils.

The P^H was 5.3 at 0-15 depth and 5.2 at 15-30cm depth indicating strong acidity of the soils. The electrical conductivity of the soils at 0-15cm depth was 0.042dsm^{-1} and at 15-30cm depth was 0.55dsm$^{-1.}$ The organic matter level was 2.89% at 0-15cm depth and 2.79% at 15-30cm depth, indicating moderate level. Total nitrogen from the two soil samples was 0.070% indicating a very low statue. Available P at 0-15cm depth was 16.23mhkg^{-1} and at 15-30cm depth (3.20Ca, 1.90mg, 0.06 Na and 0.12k cmolkg^{-1}), while at 15-30cm depth (4.00Ca, 1.80mg, 0.06Na and 0.11k cmolkg^{-1}). The exchangeable acidity (EA) levels were; at 0-15cm depth (3.84) and at 15-30cm (3.08). Soils ECEC in the soils of the study were: at 0-15cm depth (9.12) and at 15-30cm (9.61). Base saturation at 0-15cm depth was 57.89% and at 15-30cm depth was 67.95%.

Table 2: Heterotrophic fungal Count (THFC) (cfu/g) and Total Heterotrophic Bacterial count (THBC) (cfu/g) as influenced by different rates of Gramoxone herbicide.

Treatment (L/ha)	Total Heterotrophic Bacteria count			Total Heterotrophic Fungal count		
	Before application	After application	%	Before application	After application	%
0	$1.1x10^6$	$3.3x10^6$	70	$1.5x10^6$	$0.6x10^6$	20
3	$6.7x10^6$	$3.0x10^6$	55	$8.3x10^6$	$2.1x10^6$	74
4	$1.1x10^6$	$2.5x10^6$	127	$0.8x10^6$	$0.4x10^6$	50
5	$4.0x10^6$	$1.4x10^6$	65	$6.4x10^6$	$3.0x10^6$	53

This table shows that the populations of bacteria before the experiment were 1.1 x 6.7 x 10^6, 1.1 x $l0^6$ and 4.0 x 10 in plots that received 0, 3, 4 and 5 L/ha rates of herbicide respectively. It had corresponding values of 3.3 x 10^6, 3.0 x 10^6, 2.5 x 10^6 and 1.4 x 10^6 respectively after the application of the herbicide. the result further shows that 0 and 4 L/ha rate increased the total heterotrophic bacterial count (THBC) by 70% and 127% respectively, while 3 and 5 L/ha rates decreased the total heterotrophic bacterial count by

55% and 65% respectively. the increase in Total Heterotrophic bacterial Count in plots that receive 0 and L/ha compared to 3 and 5 L/ha could be due to variation in some soil properties among the plots as well as effect of rainfall that perhaps decrease the effect of 3 and 5L/ha as recorded by Ufot (2012).

This table shows that the populations of fungi before the experiment were 0.5 x 10^6, 8.3 x 10^6, 0.8 x 10^6 and 6.4 x 10^6 in plots that received 0, 3, 4 and 5 L/ha rates of herbicide respectively. After application of the herbicide, it had corresponding values of 0.6 x 10^6, 2.1 x 10^6, 0.4 x 10^6 and 3.0 x10^6, respectively. The result further shows that only 0L/ha rate increased the total heterotrophic fungal count by 20%, while 3,4 and 5 L/ha rates decreased the total heterotrophic fungal count by 74%, 50% and 53% respectively. The reduction in total heterotrophic fungal count in plots that received the different herbicide treatment relative to control (0L/ha) indicates the deleterious effect of herbicide application to fungal population in the soil. This observation is in agreement with Wu *et al* (2008). Note, numbers/kinds of microorganisms in soils depends on soil moisture, aeration, $P^{H,}$ texture, etc. (Preycott *et al*., 1999)

Table-3 Bacterial and fungal Isolates obtained from each of the samples before and after application as influenced by different rates of Grarmoxone herbicides.

Treatment (L/ha)	**Bacterial Isolates**		**Fungal Isolates**	
	Before application	**After Application**	**Before application**	**After Application**
0	*Bacillus spp*	*Bacillus spp*	*Aspergillus niger Mould*	*Aspergillus niger Mould*
3	*Staphylococcus aureus* and *Bacillus spp*	*Staphylococcus aureus, Bacillus spp, Staphylococcus aureus, Micrococcus spp and Actinomycete spp*	*Aspergillus niger Mould*	*Aspergillus niger Aspergillus flarvus mould*
4	*Bacillus spp Staphylococcus*	*Bacillus spp, Staphylococcus pseudomonas and Actinomycete spp*	*Aspergillus niger Mould*	*Aspergillus niger Mould*

5	*Bacillus spp Staphylococcus aureus*	*Bacillus spp Staphylococcus aureus and Micrococcus spp*	*Aspergillus niger Mould*	*Aspergillus niger Mould Aspergillus flarvus*

This table shows that bacteria isolated from the plot before the experiment were *Bacillus spp* and *Staphylococcus aureus* in all the plots. After application of the herbicide, *Bacillus spp* and *Staphylococcus aureus* were found in plot that received 0L/ha. *Bacillus spp*, *Staphylococcus spp*, *Micrococcus spp* and .*Actinomycetes spp* were found in plot that received 3 L/ha, while *Bacillus spp*, *Sraphylococt spp*. *Psuedomas* and *Actinomycetes spp* were identified in plot that received 4 J/ha *Bacillus spp*, *Staphylococcus aureus* and *Micrococcus spp* were found in plot that received 5L/ha. The result further shows that *Bacillus spp* and *Staphylococcus aureus* were isolated from all the treated plots. *Micrococcus spp* were isolated from plots that received 3 and 5 L/ha rates of herbicide. *Actinomycetes spp* were isolated from plots that received 3 and 4 L/ha rates of herbicide, whereas *Pseudomona*s was isolated from the plot that received 4L/ha rate of herbicide. The emergence of *Microcaccus spp* and *Actinomycete spp* isolates in 3L/ha, *Pseudomonas* and *Actinomycete spp* in 4L/ha, and *Micrococcus spp* in 5L/ha suggest availability of favorability environment for activities of these bacterial isolates. These findings are in consonant with Wu *et al* (2008).

This result shows that the fungal isolates from all the plots before the experiment were *Aspergillus niger* and mould. After the application of herbicide, *Aspergillus niger* and mound were obtained from plots that received 0 and 4 L/ha rates of herbicide, while *Aspergillus niger*, mould and *Aspergillus flavus* were obtained from plot that received 3 and 5 L/ha rates of herbicide. The result further indicated that *Aspergillus niger* and mould were isolated from all the treated plots, while *Aspergillus flavus* were isolated from plots that received 3 and 5 L/ha rates of herbicide. The emergence of *Aspergillus flavus* in plots that received 3 and 5 L/ha compared to 0 and 4 L/ha, could be linked to variation in soil micro-climate and nutrient availability I these plots compared to others. Fungi are found primarily in the top 10cm and are rarely found below 30cm depth. Fungi are most abundant in wetlands and acidic soils (Domsch *et al*., 1980) and most of them are opportunist, thriving only where conditions are favourable (where high concentration of utilizable substrate abound).

CONCLUSION

The observed variations in identified microbial population and activities could be due to the effect of the applied herbicide. Herbicides have been found to interact with soil organisms and thus influence their metabolic activities (Singh and Walker, 2006) which may alter the physiological and biochemical behavior of soil microbes. Microbial biomass is an important indicator of microbial activities and provides direct assessment of the linkage between microbial activities and the nutrient transformations and other ecological processes (Schultz and Urban, 2008). The adverse impacts of herbicides on soil microbial biomass and soil respiration had earlier been reported by Pampulha and Oliveira,(2006). It has also been found that some microbial groups are capable of using applied agrochemical as a source of energy and nutrients to multiply, whereas the pesticide may be toxic to other organisms. Likewise, application of herbicides reduces microbial diversity but increases functional diversity of microbial communities (Ayansina and Oso 2006) which sometimes demonstrate the tendency of reversible stimulatory/inhibitory effects on soil microorganisms (Pampulha and Oliveira, 2006). Herbicides application may also inhibit or kill certain group of microorganisms and outnumbered other groups by releasing them from the competition.

From the study, it is obvious that when herbicides are applied they have negative effects on soil microorganisms. Some microbial groups are capable of using herbicide as a source of energy and nutrients to multiply, whereas the herbicide may be toxic to other organisms. The highest number of heterotrophic bacteria and fungi counts was at the control (0L/ha) rates of treatment. This was due to non-interference with biological activities by foreign substances such as herbicide. The implications of these findings suggest that the application of herbicides should be understood in order to preserve soil microorganisms and enzymes for increased soil productivity.

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