

Full Length Research Paper

Effect of higher concentrations of herbicides on bacterial populations in tropical soil

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It is a common practice for local farmers in the tropics to apply high concentrations of herbicides. Studies were carried out on the effect of high concentrations ($\times 1.5$ and 2.0 recommended rates) of two commonly used herbicides (glyphosphate and paraquat) - on soil pH, bacterial counts and species in a tropical soil. Treatment herbicides resulted in a general reduction of soil pH from 6.22 to as low as 5.28 when compared to the control soil treatment day (day 0). Glyphosphate (at $\times 1.5$) resulted in initial lowering of bacterial counts from 3.2×10^5 cfu/ml to 2.5×10^5 cfu/ml at week one and 3.0×10^5 cfu/ml at $\times 2.0$ recommended rates. While paraquat (at $\times 1.5$) resulted in an initial bacterial increase from 2.0×10^5 cfu/ml to 2.4×10^5 cfu/ml at week one; paraquat ($\times 2.0$) resulted in an initial increase from 2.2×10^5 cfu/ml to 2.5×10^5 cfu/ml. High herbicides concentrations generally resulted in bacterial population reductions when compared to the control soil. *Bacillus sp* (glyphosphate - 39.7%; paraquat - 20.8%) and *Pseudomonas sp* (glyphosphate - 23.5%; paraquat - 31.7%) were the most frequently isolated bacteria from herbicides treated soils.

Key words: Glyphosphates, paraquat, high concentrations, *Bacillus sp.*, *Pseudomonas sp.*

INTRODUCTION

Several tones of pesticides are applied annually in modern agriculture to increase food production by controlling harmful effects caused by pest organisms including insects, microorganisms as well as grasses growing in between economical crops (Liu and Xiong, 2001). However, less than 5% of these chemicals are estimated to reach the target organisms (Somerville, 1987). Most of the pesticides therefore reach the non-target parts of the agricultural ecosystems. The quality of soils, ground water, continental and coastal water as well as the air, is therefore compromised by pesticide contamination (Surekha et al., 2008).

Thousands of herbicides molecule are made every year (Cunningham et al., 1996). Modern pesticides are almost all completely new synthetic chemicals, previously unknown in nature.

They are designed to be biologically active, and while a remarkable degree of selectivity has been achieved in some materials, as in the case of sensitive herbicides and insecticides, it is not surprising that pesticides may produce undesirable effects particularly if they are used especially at high concentrations.

Another adverse effect of herbicides use in the changes they may bring on microbial populations in the soil and the activities of species of microorganisms (Ayansina and Oso, 2006). As a biologically active chemical is applied to ecological systems, it is inevitable that the systems will alter in response to the interference (Alexander, 1995). It is a common practice among local illiterate farmers to use high concentrations of pesticides with the hope to promote effectiveness (Mathews, 1992). In this report, the effect of two commonly used herbicides

Table 1. Mean pH values of herbicides treated soil samples at x 1.5 and x 2.0 recommended rates and untreated control soil.

Sample treatment	Weeks							
	0	1	2	3	4	5	6	7
Control	6.51 ^a	7.67 ^a	8.06 ^a	7.38 ^a	7.87 ^a	7.07 ^a	7.08 ^a	7.17 ^a
G1	5.50 ^b	6.08 ^a	6.88 ^a	6.68 ^a	7.09 ^a	6.44 ^a	6.82 ^a	7.28 ^a
G2	5.26 ^b	7.08 ^a	7.01 ^a	6.49 ^a	6.78 ^a	6.59 ^a	6.73 ^a	7.12 ^a
P1	5.57 ^b	7.36 ^a	7.12 ^a	6.65 ^a	7.03 ^a	6.51 ^a	6.91 ^a	7.18 ^a
P2	5.26 ^b	7.37 ^a	7.09 ^a	6.32 ^a	6.87 ^a	6.59 ^a	7.10 ^a	7.37 ^a

Values with same alphabet are not significantly different ($P < 0.05$). G1 ----- glyphosphate at x 1.5 recommended rate; G2 ----- glyphosphate at x 2.0 recommended rate; P1 ----- paraquat at x 1.5 recommended rate; P2 ----- paraquat at x 2.0 recommended rate.

(glyphosphate and paraquat) in Nigeria at high concentrations in a tropical soil has been presented.

MATERIALS AND METHODS

Sampling and soil treatments

Top soil samples (0 - 20 cm deep) were collected from the agriculture farm located within the campus of the Bowen University, Iwo, Osun State, Nigeria. The soil had no recent history of exposure to the herbicides. The soil samples were sieved through a 2.0 mm wide mesh to remove stones and plant debris.

Two commonly used herbicides - glyphosphate (G) and paraquat (P) were applied at $\times 1.5$ and $\times 2.0$ of the recommended doses. The use of higher concentrations of the herbicides was made to correspond approximately to the practice of the local peasant farmer as reported by Mathews (1992). One kilogramme each of the soil samples (in experimental bowls) was treated with each of the herbicides and another set was treated with 100 ml sterile distils water (control). The treatments were replicated. Soil samples from each of the treatments were taken for analysis one hour after application (day 0) and subsequently on a weekly basis for 7 weeks.

Soil pH determination

Soil: water ratio of 1:1 was used for analysis. Twenty grammes (20 g) of soil samples from each treatment were mixed with 20 ml of sterile distil water and stirred thoroughly in 100 ml baker. The soil water mixture was then allowed to stay for 30 min with intermittent stirring. The coarse particles were allowed to settle after the last stirring before the pH readings were taken using the Jenway pH meter (Model 3150).

Total viable bacteria courts and characterization

The conventional serial dilution and pour plate method

was used to estimate bacterial populations and subsequently assess the effect of herbicides treatments on the survival of bacteria in the soils. Incubation was done at 35°C for 24 - 48 h. Major Bacterial isolates were characterized based on cultural characteristics, staining reactions and biochemical reactions. Identification was further made with reference to the Bergey's manual of Systematic Bacteriology (1984).

Statistical analysis

Data generated from the study were subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Soil pH measurements from treated soil samples showed that herbicides treatments resulted in a general reduction of pH right from the first day of treatment (week 0) from 6.22 to... as 5.28 samples G2 and P2 as shown in Table 1. Major factors that can affect the action of herbicides in soil include pH, moisture, organic matter, temperature and amendments (Ayansina and Oso, 2006). Herbicides treatments resulted in soil pH reduction as compared to the control but the values obtained still fell within critical soil pH values as described by Adeoye and Agboola (1985). Other reports have suggested that soil pH is a very important factor in pesticides degradation (Wharton and Mathieson, 2005; Singh, 2008).

Mean viable bacterial courts from control and herbicides treated soil samples are presented in Figures 1 and 2. Glyphosphate treated soil at $\times 1.5$ recommended rates resulted in reduction of bacterial counts from an initial of 3.2×10^5 cfu/ml to 2.5×10^5 cfu/ml at week 1, but as the weeks progresses a final bacterial counts of 7.8×10^5 cfu/ml was obtained. From glyphosphate treated soil at $\times 2.0$ recommended rates of bacterial counts of 3.0×10^5 cfu/ml was obtained at week 1 and final bacterial counts of 5.6×10^5 cfu/ml.

Soil treated with paraquat at $\times 1.5$ recommended rates

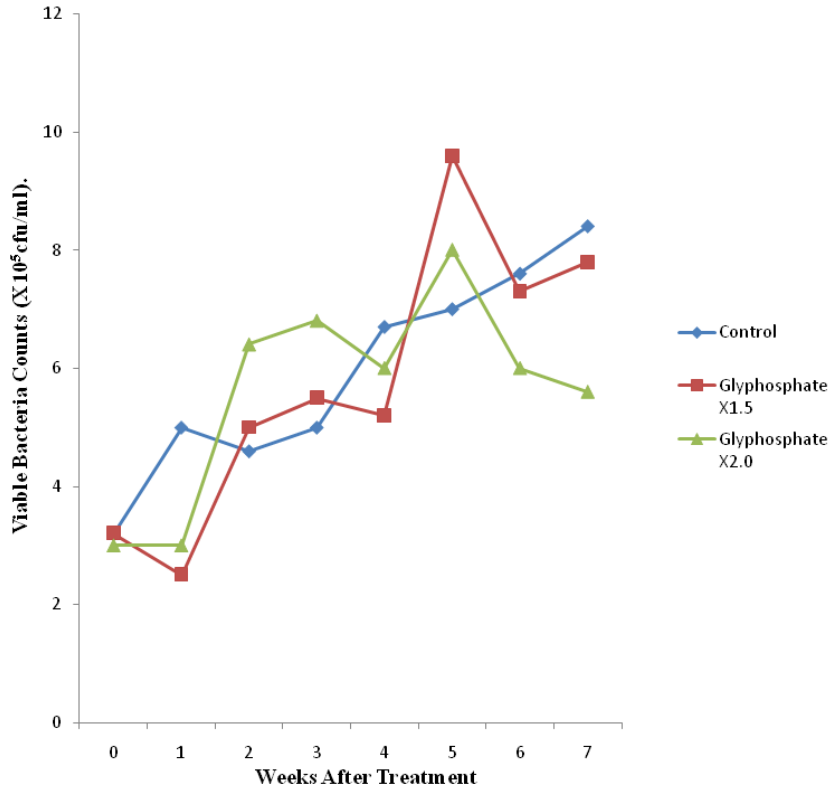


Figure 1. Mean viable bacterial counts at $\times 1.5$ and $\times 2.0$ of glyphosate concentrations.

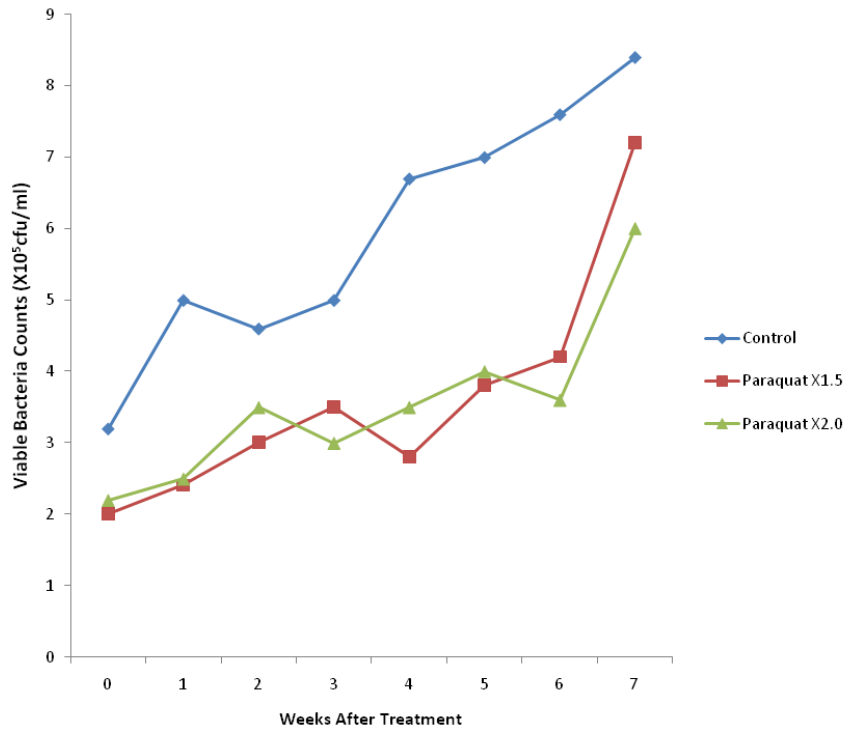


Figure 2. Mean viable bacterial counts at $\times 1.5$ and $\times 2.0$ of paraquat concentrations.

Table 2. Bacterial isolations and frequencies of isolations from control and herbicide treated soils.

Bacterial isolation	Control	Glyphosphate	Paraquat
	Number and frequencies	Number and frequencies	Number and frequencies
<i>Bacillus</i> sp	130 (34.8%)	81 (39.7%)	21 (20.8%)
<i>Pseudomonas</i> sp	60 (16.0%)	48 (23.5%)	32 (31.7%)
<i>Flavobacterium</i> sp	50 (13.4%)	21 (10.3%)	-----
<i>Actinomycetes</i>	50 (13.4%)	14 (6.9%)	21 (20.8%)
<i>Staphylococcus</i> sp	22 (5.9%)	12 (5.9%)	9 (8.9%)
<i>Proteus</i> sp	41 (10.9%)	21 (10.3%)	10 (9.9%)
<i>Lactobacillus</i> sp	11 (2.9%)	-----	-----
Unidentified	10(2.7%)	7 (3.4%)	8 (7.9%)
Total	374	204	101

resulted in an initial increase in bacterial counts from 2.0×10^5 cfu/ml to 2.4×10^5 cfu/ml. As the weeks progresses a final bacterial counts of 6.0×10^5 cfu/ml was obtained. Paraquat treatment soil at $\times 2.0$ recommended rates also resulted in an increase in bacterial counts from an initial of 2.2×10^5 cfu/ml to 2.5×10^5 cfu/ml after week 1. As the week progresses a final bacterial count of 6.0×10^5 cfu/ml was obtained. While compared to the control soil paraquat treatment at the two concentrations resulted in a general reduction of bacteria counts.

Higher herbicides concentrations resulted in significant reduction of bacterial counts. This in agreement with the report of Moorman et al. (2001) and Ayansina and Oso (2006) who reported that herbicides have greater tendencies of toxicity to microbial populations in soils contaminated with high levels of herbicide applications. It has been reported that the misuse of herbicides (as occasioned by high concentrations) do result in decrease in microbial counts and the elimination of some species (Singh and Walker, 2006).

A summary of major bacteria isolated from control and herbicides treated soils and frequency of occurrence is presented in Table 2. *Bacillus* sp. (glyphosphate - 39.7%; paraquat - 20.8%) and *Pseudomonas* sp. (glyphosphate - 23.5%; paraquat - 31.7%) were the most frequently isolated bacteria from the herbicides treated soils. *Flavobacterium* sp. was not encountered in paraquat treated soil though some (13.4%) was isolated from glyphosphate treated soil. *Actinomycetes* were encountered in control soil (13.4%); glyphosphate (6.9%) and paraquat (20.8%). While *Lactobacillus* sp. was encountered in control soil (2.9%), none was encountered in the two herbicides treated soils.

Bacterial isolations encountered in this report were those common to tropical soils (Table 2). *Bacillus* sp. and *Pseudomonas* sp. were the predominant bacterial species. The occurrence of the two bacteria in other herbicides treated soils has been reported by Ayansina and Oso (2008). These two bacteria are important in degradation of many organic compounds. Another report

has shown that *Actinomycetes* and *Bacillus* sp. have great potentials for the transformation and biodegradation of pesticides (Vander, 2003). According to Rueppel (2010) complete biodegradation of herbicides involves the oxidation of parent compound to form carbon dioxide and water; a process that provides both carbon and energy for the growth and reproduction of microorganism.

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