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Effects of Glyphosphate and Alachlor on Microbial Respiration, Ammonification, Nitrification and Phosphorus Mineralization in a Sandy Loam Soil

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Abstract

Glyphosate (N–phosphonomethyl) glycine) and alachlor (2–chloro2^1,6^1–diethyl–N–(methoxymethyl) acetanilide) were added at the recommended and twice the recommended rates, viz, 3.0 and 6.0 and 2.5 and 5.0kg/ha active ingredients respectively to a sandy loam and incubated at 60% of the soil's moisture holding capacity for 8 weeks at 30°C in the laboratory. Microbial respiration, ammonium nitrogen (NH^+ –N), nitrate nitrogen (NO^-_3–N) and available phosphorus (P) production were determined weekly.

The herbicide treatments did not stimulate microbial respiration but rather had a negative effect (P = 0.05) during the early stage (2 – 4 weeks) of incubation. Ammonification, nitrification and phosphorus mineralization were not significantly affected also. However, the rate of nitrification was temporarily enhanced in soil samples that received twice the recommended rates of glyphosate and alachlor. High rates of the herbicides could therefore lead to increased loss of NO^-_3–N under humid conditions.

Introduction

Weed infestation is probably the most important factor limiting crop production in agriculture. This is because weeds compete with crops for radiant energy, available nutrients and moisture. The high cost of labour has made the use of herbicides, for example alachlor and glyphosate, an attractive alternative in Nigerian agriculture. Glyphosate (N–(phosphonomethyl)glycine) is a broadspectrum post-emergence organophosphorus compound effective for control of perennial grass and broad-leaved species. Alachlor (2–chloro–2^1,6^1 –diethyl–N (methoxymethyl) acetanilide), on the other hand, is an anilide and a selective pre-emergence herbicide for controlling annual grasses and broadleaved weeds among such crops as maize, beans, cotton, soybeans, surgarcane and peanuts (Fletcher and Kirk, 1982).

Pesticides being toxic and antibiological agents, can adversely affect microbial populations and biochemical activities such as ammonification, nitrification, denitrification and urea hydrolysis (Russell, 1973; Bollag and Henninger, 1976; Alexander, 1977; Greaves and Malkomes, 1980). While Tu (1978) found that WL24073 {0–2–chloro–1–2, 5–dichlorophenyl) vinyl) 0–methylethylphosphonothioate} increased nitrification of incorporated $(NH_4)_2SO_4$, the herbicide sethoxydim produced inconsistent stimulation and retardation of microbial respiration (Roslycky, 1986). However, Adebayo *et al.* (1979) assessed cyanazine and atrazine in cropped and uncropped soil and found that nitrification was not significantly affected. In a latter study, Daramola and Adebayo (1981) found that preforan, dacthal and dual exerted varying degrees of inhibition on Rhizobium strains in pure culture, and on nodulation, nodule and plant biomass and nitrogen content when applied to natural soil. The chlorinated phenols and chlorinated aliphatic acid herbicides have been reported to be moderately and fairly toxic, respectively, to the nitrifying bacteria, namely, *Nitrosomonas* and *Nitrobacter* (Otten *et al.,* 1957).

Although herbicides can be cost-effective, there is concern about their possible adverse effects on nutrient cycling and soil fertility especially in peasant agriculture which depends mainly on native soil organic matter and manures as primary sources of nutrients. There has been limited research into the effect of glyphosate (Roundup) and alachlor (Lasso) on microbial activity and nutrient mineralization in Nigerian soils. It is therefore the aim of this investigation to evaluate the effects of these herbicides on microbial respiration, ammonification, nitrification and phosphorus mineralization in soil.

Materials and Methods

The soil sample employed for this investigation was obtained from the plough later (0–15cm) of a plot that had no recent herbicide application at the Obafemi Awolowo University Teaching and Research Farm, Ile-Ife, Nigeria. The bulk sample was air-dried and sieved to pass 2mm sieve and later used for the laboratory incubation experiment. The physical and chemical properties of the soil are shown in Table 1.

The laboratory incubation assay was carried out in static microcosm at 30^{0}C over a period of 8 weeks. Three hundred gram (300g) portions of airdry soil were weighed into each of 500–ml incubation flasks. The samples were wetted with suspensions of glyphosate and alachlor (Monsanto) to achieve 60% of the soil's moisture holding capacity.

Each herbicide was applied at the recommended and twice the recommended rates, viz: 3.0 and 6.0, and 2.5 and 5.0kg/ha active ingredients respectively. The control received distilled water only. A vial containing 10ml of 1N KOH was placed in each flask which was then tightly stoppered. The following treatments were triplicated and arranged in a completely randomized design:

(01) Control
(02) Soil + recommended rate of glyphosate (GL_1)
(03) Soil + twice the recommended rate of glyphosate (GL_2)
(04) Soil + recommended rate of alachlor (AL_1)
(05) Soil + twice the recommended rate of alachlor (AL_2)

At the end of each incubation period, the vials were retrieved and the contents poured into wide-mouthed 250–ml conical flasks. Excess OH^- was first titrated against 0.5N H_2SO_4 using phenolphthalein as indicator. The $CO_3^=$ formed was then titrated in the presence of bromophenol blue indicator. The exchangeable NH_4^+ and NO_3^- were determined by steam distillation after extraction using 2N *KCl* with 1h shaking (Bremner and Kenney, 1966). Available P was extracted using the Bray–1 procedure (Bray and Kurtz, 1945) and read colorimetrically at 660nm after the development of molybdenum blue colour.

Results and Discussion

Effects of herbicides on microbial respiration and ammonification

The mean weekly and cumulative amounts of carbon(C) evolved and NH_4^+–N contents obtained from the treatments are presented in Table 2. Microbial respiration was not significantly affected (P = 0.05) by the recommended and twice the recommended rates of glyphosate and alachlor at the end of the 1st, 3rd and 5th – 8th weeks of incubation. While this finding is contrary to that of Tu (1978) who found that phorate stimulated microbial respiration, it is similar to the non-significant effects of carbofuran, deltamethrin and dimethoate applied at the recommended and ten times the recommended rates reported by Germida *et al.* (1987). Significant depressions of microbial respiration were obtained at the end of the 2nd and 4th weeks. This cannot be attributed to partial sterilization effect as observed for pentachlorophenol (Sato, 1983) since the herbicides did not reduce C evolution at the end of the 1st week. Such a pattern of inconsistent retardation of microbial respiration had also been found with sethoxydim (Roslycky, 1986). Hence, this trend might be due to the combined effects of undegraded herbicides and their degradation products on the heterogenous microbial population in soil. Soil microorganisms are known to adjust and adapt physiologically with time to chemical agents added to the soil (Russell, 1973; Alexander, 1977; Roslycky, 1986). The absence of a significant difference (P = 0.05) in C evolution between the 5th and 8th weeks of incubation is an evidence of the adaptation of the soil micro-organisms to the presence of the degradation products of the herbicides. Although the cumulative amount of C evolved from the control at the end of 8 weeks of incubation was significantly higher (P = 0.05) than in the herbicide treatments, this difference was due to the higher microbial activitity in the control at the end of the 2nd and 4th weeks.

Ammonium N is produced from soil organic matter by neterotrophs during the process of ammonification. As with microbial respiration, the trend obtained with NH_4^+–N content was inconsistent. There was no significant difference (P = 0.05) in the contents of NH_4^+–N at the end of the 1st, 4th – 6th and 8th weeks of incubation. Hence, the herbicide treatments did not adversely affect the process of ammonification during this period. Sato (1983) and Somda *et al.* (1991) had similar findings with pentachlorophenol, and benlate and captan respectively. With GL_2 and AL_2, although there was no significant difference (P = 0.05) in the levels of NH_4^+–N compared with the control, the values were generally lower (2nd – 6th weeks) with the result that the cumulative amounts were significantly lower (P = 0.05) at the end of 8 weeks of incubation (Table 3). This trend could be attributed to an increased rate of nitrification of the NH_4^+–N, discussed below.

Effects of herbicides on nitrification, total inorganic N and available P

The weekly and cumulative contents of $N0_3^-$–N, total inorganic N (NH_4^+–N + $N0_3^-$–N) and available P are presented in Tables 3 and 4 respectively. Except for the total inorgaic N in $A1_2$ at the end of the 8th week of incubation, there were no significant differences (P = 0.05) in the periodic and cummulative levels of $N0_3^-$–N, total inorganic N and available P throughout the period of incubation. Overall, nitrification as indicated by levels of $N0_3^-$–N and available P were not significantly affected by the herbicide treatments. As earlier explained, however, the lower levels of NH_4^+–N obtained in GL_2 and AL_2 especially at the ends of the 2nd and 3rd weeks indicated an increased rate of nitrification. Contradictory results have been found by some other workers. While Tu (1978) found that WL24073 increased the nitrification of incorporated $(NH_4)_2SO_4$, Zomda (1991) had earlier found that zineb, parathion, DDT and furadan had no effect. Adebayo *et al.* (1979) also found that cyanazine and atrazine applied to two Nigerian soils tended to increase nitrification. In this experiment, however, such an enhancement is temporary since cumulative amounts of NO_3^- –N at the end of incubation were not significantly different. This temporary increase in nitrification rate can lead to greater leaching of NO_3^- –N from herbicide – treated soil if it coincides with the period of high rainfall.

Organic phosphorus mineralization was not adversely affected by the two herbicides at the recommended and twice the recommended rates. This can be attributed to the fact that P mineralization is carried out by heterotrophs which are not easily sensitive to foreign agents in soil (Alexander, 1977).

These results indicated that glyphosate and alachlor had no significant effects on microbial activity using microbial respiration, ammonification, phosphorus mineralization and gross nitrification as indices. However twice the recommended rates of both herbicides temporarily increased the rate of nitrification.

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Table 1: **Some Physical and Chemical Properties of the Soil used**

Soil Property	Mean Value
Sand	71%
Silt	13%
Clay	18%
Organic matter	1.04%
pH (0.01M $CaCl_2$)	5.5
NH_4^- –N	1.0 mg/g
NO_3^- –N	4.6 mg/g
Available P	0.5 mg/g

Table 2: **Mean weekly and cumulative amounts of (a) C (mg) and (b) NH_{4+}-N (mg/kg) obtained from each treatment at 30°C over an 8-week period of incubation**

(a) Treatments	1	2	3	4	5	6	7	8	Cumulative
	Weeks								
Controls	1.35*	0.42a	0.34a	0.29a	0.19a	0.26a	0.27a	0.12a	3.25a
GL1	1.29a	0.28b	0.29a	0.18b	0.21a	0.29a	0.16a	0.14a	2.86b
GL2	1.33a	0.27b	0.28a	0.21b	0.14a	0.23a	00.16a	0.14a	2.71b
AL1	1.28a	0.32b	0.29a	0.18b	0.18a	0.25a	0.25a	0.11a	2.88b
AL2	1.26a	0.28b	0.27a	0.21b	0.16a	0.24a	0.20a	0.20a	2.78b
Mean	1.30	0.31	0.29	0.22	0.18	0.25	0.21	0.12	2.92.90
SE	0.04	0.03	0.03	0.03	0.02	0.03	0.03	0.02	0.07
(b) Control	1.8a	1.2a	0.6a	1.8a	2.2a	1.3a	1.9a	1.8a	12.67a
GL1	1.5a	0.7b	0.6a	1.4a	1.6a	1.2a	1.9a	2.0a	11.53ab
GL2	1.5a	0.5b	0.5ab	1.0a	1.6a	0.97a	1.9a	1.9a	9.87c
AL1	1.6a	0.8a	0.3b	1.6a	1.9a	1.3a	1.3b	2.3a	11.23abc
AL2	1.8a	0.9ab	0.2b	1.1a	1.8a	1.4a	1.4b	2.0a	10.67ab
Mean	1.64	0.82	0.44	1.38	1.82	1.23	1.68	2.00	11.19
SE	0.16	0.13	0.10	0.20	0.17	0.18	0.12	0.24	0.45

Means followed y different letters are significantly different (Duncan's Multiple Range Test, P = 0.05).

Table 3: **Mean fortnightly and cumulative amounts of available P (mg/kg) obtained from treatments at 30°C over an 8-week period of incubation**

Treatments	2	4	6	8	Cumulative
		Weeks			
Control	1.5a*	1.3a	1.9a	2.6a	7.30a
GL1	1.3a	1.4a	1.6a	2.6a	6.90a
GL2	1.3a	1.5a	1.7a	2.3a	6.77a
AL1	1.5a	1.6a	1.8a	2.1a	7.00a
AL2	1.6a	1.5a	1.8a	2.9a	7.90a
Mean	1.44	1.46	1.78	2.50	7.17
SE	0.15	0.15	0.20	0.27	0.44

*Means followed by different letters are significantly different (Duncan's Multiple Range Testm P = 0.05)