RESPONSES OF SOIL NUTRIENT AND MICROBIAL POPULATION TO THE APPLICATION OF DIURON FOR WEED CONTROL IN MAIZE IN AN ALFISOL.

Adejoro, S. A
Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure, Ondo State, Nigeria.

Correspondence Author's email: saadejoro@futa.edu.ng.com

Abstract
Diuron, is one of the commonly used herbicides for weed control in cereals in most parts of the world, but continuous use may leave residues in the soil, which are likely to alter soil biological and chemical properties. This study evaluates the influence of diuron treatments at 1.2, 1.8, 2.4, 3.0, 3.6 and 4.2 kg a.i. / ha including a weedy and weed-free control on soil chemical properties and microbial population. A field experiment was conducted using randomized complete block design at the Crop Type Museum of the department of Crop, Soil and Pest Management in the Teaching and Research Farm of the Federal University of Technology, Akure (FUTA). FUTA is located in the rainforest vegetation zone of Nigeria. Results showed that bacterial population was significantly reduced by diuron irrespective of rate of application compared to the untreated plots, and this decrease in bacterial population appeared to be directly related to the rate of application of the herbicide. Diuron was also found to reduced fungal population by 60.8, 58.8, 56.7, 68.0, 66.0 and 67.0 % for diuron application at 1.2, 1.8, 2.4, 30. 3.6 and 4.2 kg a i/ha respectively. Results further showed that soil pH, organic matter and the major nutrients were also reduced by the application of the herbicide. Results also indicated that diuron at 3.6 and 4.2 kg a.i. / ha provided effective weed control and significantly ($p<0.05$) increased maize grain yield over the weedy control at almost a similar level with the weed-free control. It is therefore concluded that application of diuron though has the potential to boost maize yield may not be favorable to the soil microbial community. Practices that can enhance diuron degradation should therefore accompany its application so as to reduce toxicity to non-target organisms.

Key words: Diuron, chemical properties, soil microbial population, maize

IJAFS 2018 (8), 8:1210-1219
Accepted for publication
August 2018

INTRODUCTION

Diuron, commonly sold under the brand names Karmex, Direx, and Diuron, is widely used for vegetation control along rights of way. Other significant uses include weed control in citrus orchards and alfafa fields (Cox, 2003). In Nigeria, the herbicide is used for post emergent control of weeds in maize and cassava. Diuron has however been found to be moderately to highly persistent in soils, and the commonly reported average field dissipation half-life is 90 days (USDA, 1995). Soil persistence of diuron is presumably dose dependent. Phytotoxic residues generally dissipate within a season when applied at low selective rates. At higher application rates, residues may persist for more than one year (Kidd and James, 1991). While persistence is a good indicator of weed control effectiveness in some herbicides, the carryover effects of such herbicides on soil productivity indicators should also be emphasized. When sustainable crop production is a priority, herbicides should be scored on their ability to conserve or improve both biotic and abiotic soil productivity indicators.

Much has been reported on the responses of soil microbial and enzymatic activities to diuron applications. Diuron is able to disrupt the complex ecological community of soil microorganisms, including algae and fungi. These effects have been demonstrated in a variety of ecosystems around the world. Biologists at the University of Havana (Cuba) showed that the dominant soil fungus in sugar cane fields did not occur on a diuron treated field and was replaced by another genus of fungi (Cox,2003). Scientists at the University of Regina (Canada) found that treatment of soil with diuron in concentrations equivalent to those used by farmers reduced algae populations by 99 percent in the top layer of the soil, and this reduction occurred in both clay and sandy loam soils (Pipe and Cullimore, 1980; 1984). Researchers at the Instituto de Química (Brazil) showed that diuron inhibits microbial activity in soil, even at concentrations as low as several parts per million, causing "conditions adverse to restoring soil fertility" (Prado and Airoldi, 2001). In addition, diuron reduces the activity of phytase, an enzyme that mineralizes the plant nutrient phosphorus in soil. (Cervelli and. Perna, 1985).

Nothing has however been reported on the effects of diuron on soil microbial properties in the afrisol soil of south western Nigeria. The present study therefore aims at gaining an insight into the weed control effectiveness of varying application rates of diuron in maize, as well as the effects of their residues on soil chemical properties and microbial population in south western Nigeria.

MATERIALS AND METHODS

Study area

The study was conducted at the Crop Type Museum of the department of Crop, Soil and Pest Management of the Federal University of Technology, Akure (7°16′N, 5°12′E) located in the rain forest vegetation zone of Nigeria during the early (April-July) cropping seasons of 2011. The average annual rainfall is about 1300mm with a mean temperature of 27°C and the climate is of the sub-humid type. The soil at the experiment site was a sandy clay loam. Chemical analysis of the top soil at 5cm depth before sowing maize are given in Table 1.

Experiment plan and crop establishment

A field trial involving application of diuron in maize at 1.2, 1.8, 2.4, 3.0, 3.6 and 4.2kg a.i. / ha was laid out in a randomized complete block design (RCBD) with three replications per treatment. Treatments also included a weed-free control (where weeding occurred once per week till harvest at 12 WAP) and a weedy control (where no weeding occurred during the period of the experiment). Two seeds of Downy mildew resistant (DMR), and early maturing maize variety were sown per stand but later thinned to one seedling per stand at 2 weeks after planting (WAP). Planting was done on plots measuring 4 × 4 m at a spacing of 75 × 25 cm with 1 m alleyway between plots. Diuron (80DF), a dry flowable formulation, was applied pre-emergence to both weed and crop at the foregoing rates with a knapsack sprayer fitted with Polijet nozzles calibrated to deliver 250 l / ha of the spray solution at a pressure of 2.5 kg / cm^2.

Data Collection

Weed assessment was conducted twice at 3 weeks after treatment (WAT) and at harvest to determine the weed spectrum, density and weight of total weed species using two fixed 50 × 50 cm quadrats along a diagonal in each plot from which weed samples were collected and analyzed for the foregoing parameters. Collected weed samples were bulked and subsequently weighed. At harvest, all the plots were clean-weeded regardless of previous weeding treatments, and the weeds weighed, thus allowing a comparison to be made of the total weed growth from the time of sowing till harvest for the different treatments. Soil samples were also collected at harvest at 0-15 cm depth in all the plots to determine the soil pH and nutrient status using standard methods (AOAC, 1990). Enumeration of soil microbial population was also done using standard methods.

Soil chemical Analysis

Composite soil samples collected after land clearing and soil samples collected at harvest from treatment plots were air-dried, ground and sieved using 2 mm sieve mesh. They were chemically analysed as described by Tel (1984). Organic matter was determined by wet oxidation method through chromic acid digestion. Nitrogen was determined by microkjeldahl approach; P was extracted by Bray-P1 solution and determined using the spectrophotometric method. Exchangeable K, Ca and Mg were extracted using ammonium acetate; K was determined using flame photometer, and Ca and Mg by EDTA titration method. Soil pH in ratio 1:2 water suspension was determined using a glass electrode.

Determination of soil microbial population

Numbers of microflora were estimated by soil dilution technique on Nutrient and
Potato Dextrose Agars as isolation media for bacteria and fungi respectively.
To achieve serial dilution, 5 grams of soil was suspended in 150 ml Erlenmeyer flask containing 95 ml of sterilized distilled water to obtain a 10^{-1} dilution and was kept under shaking conditions at 120 rpm for 15 minutes. From the flask 1 ml of suspension was transferred to 9 ml water blank to make 10^{-2} dilution. The water blank was vortexed and then again 1 ml of the suspension was transferred to a new water blank (9 ml) tube to obtain 10^{-3} dilution. In the similar manner dilutions were made up to 10^{-8}. The nutrient agar medium was composed of peptone 5 g, meat extract 3 g, agar agar 15 g and 1000 mL distilled water. For bacterial count 0.1 ml aliquot of the dilution to 10^{-8} was spread plated on Nutrient Agar medium petri plates in triplicates. Then the plates were incubated in an inverted position at 28˚C for 2 days. The constituents of the Potato Dextrose Agar (gL^{-1}) were Peptone 5.0, potato extract 5.0, dextrose 10.0, Agar 20.0, and Distilled water 1000.0 ml at pH 6.5. A mixture of 1g soil and 10mL of saline solution was shaken on a mechanical shaker for 10 minutes to dislodge fungal propagules into the solution.

This was followed by serial dilutions to the concentrations of 10^{-5}. 0.5 mL of the aliquot was spread on Potato dextrose extract agars to isolate fungal spores and this was incubated at 28^{0C} for 4 days. Dilution factors of 8 and 5 were used to determine the bacterial colony and fungal spore units respectively. Yield was assessed from fifteen randomly selected plants per plot. The harvested cobs were shelled, weighed and grain weight adjusted to 13% moisture. Data collected from the experiments were subjected to an analysis of variance while treatment means were compared using the standard error of mean differences. Simple linear correlation and regression analysis between increasing dose of diuron (X) and soil pH, soil nutrient content, growth or yield parameters (Y) of maize was performed with a scientific calculator (Casio *fx*-7400G PLUS POWER GRAPHIC Model).

RESULTS

The soil at the study site was slightly acidic, low in organic matter, N, K and Mg (Table 1). Exchangeable Ca was also marginal.

Table 1. Nutrient status and soil pH of experimental sites before treatment application

Parameters	Soil concentration
pH	5.52
Organic matter (%)	2.02
Nitrogen (%)	0.15
Available P ($mgkg^{-1}$)	3.18
K ($cmolkg^{-1}$)	0.32
Ca ($cmolkg^{-1}$)	2.11
Mg ($cmolkg^{-1}$)	1.20

Significant differences were recorded amongst the treatments in soil pH and nutrients other than potassium (Table 2). The weedy control resulted in significantly higher soil pH than the weed-free control and the diuron treatments, among which there were no significant differences. Hand weeding however lowered soil pH significantly compared to all the other treatments. Organic matter content was significantly higher in the weedy and weed-free controls than observed in the diuron treatments. Significant reductions in N content were noted for diuron treatments compared to the remaining treatments. Weedy control treatment significantly increased phosphorus values compared with the diuron treatments. K was not significantly influenced by any of the treatments at the end of the experiment. Mg and Ca were also significantly affected by the treatments but not in any consistent manner. Regressing soil nutrient parameters (Y) against increasing dose of diuron (X) indicated positive relationships (Table 3) except N that appeared to be inversely related to the increasing rates of the herbicides.

Table 2 Organic matter and nutrient composition of the soil sample at maize harvest

	Soil pH	OM (%)	N (%)	P (mg/kg)	K (cmol/kg)	Mg (cmol/kg)	Ca (cmol/kg)
Diuron at 1.2kg a.i/ha	5.74b	2.67b	0.18b	2.22d	0.31a	1.96a	2.76ab
Duiron at 1.8kg a.i/ha	5.76b	2.51c	0.19b	3.39c	0.38a	1.45ab	2.47b
Diuron at 2.4kga.i/ha	5.85ab	2.56b	0.13c	3.89b	0.36a	1.31b	2.24b
Diuron at 3.0kg a.i/ha	5.80b	2.29bc	0.18b	3.80b	0.39a	1.56ab	2.72ab
Diuron at 3.6kg a.i/ha	5.89b	1.72d	0.17b	3.56b	0.35a	1.36ab	2.39b
Diuron at 4.2kg a.i/ha	5.74b	0.61e	0.17b	3.85b	0.36a	1.42ab	2.94a
Hand weeding	5.20c	2.90a	0.22a	3.87b	0.2a	1.02c	2.33b
Weedy check	6.2a	3.2a	0.25a	5.00a	0.40a	1.30b	2.96a

Means in a column with the same letter (s) are not significantly different by Tukey's test ($p \leq 05$)

Effects of soil microbial population as influenced by the varying rates of diuron application are presented in figure 1. Bacterial population was significantly reduced by diuron irrespective of rate of application compared to the untreated plots. The highest bacterial population was recorded in the weedy control followed by the hand weeded plots. Among the diuron treatments, bacterial population appeared to decrease with increase in the rate of application of the herbicide. Hand weeding reduced bacterial population by 27.5% whereas percentage reduction caused by diuron application was above 80% irrespective of the rate of application.

Table 3: Linear correlation and regression analysis between increasing rates of diuron (X) and soil nutrient content (Y) at harvest

Soil parameter	Correlation coefficient(r)	Regression equation
Soil pH	0.30	Y=5.7+0.016X
Organic matter (%)	0.42	Y=5.83+0.07X
N (%)	-0.15	Y=0.18-0.003
P(mg/kg)	0.69	Y=27.13+2.96
K (cMol/kg soil)	0.41	Y=33.55+1.02X
Ca (cMol/kg soil)	0.19	Y=4.48+0.01X
Mg (cMol/kg soil)	0.28	Y=4.15+0.17X
Na (cMol/kg soil)	-0.45	Y=15.62-0.27X

The soil fungal population did not respond to diuron treatment in any consistent manner with respect to rate of application. Diuron application however reduced fungal population with respect to the weedy control irrespective of application rate, and percentage reduction in fungal count were lower than those recorded for bacteria. Diuron reduced fungal population by 60.8, 58.8, 56.7, 68.0, 66.0 and 67.0 % for diuron application at 1.2, 1.8, 2.4, 30. 3.6 and 4.2 kg a i/ha respectively, whereas hand weeding reduced fungal population only by 15.5% compared to the weed check control.

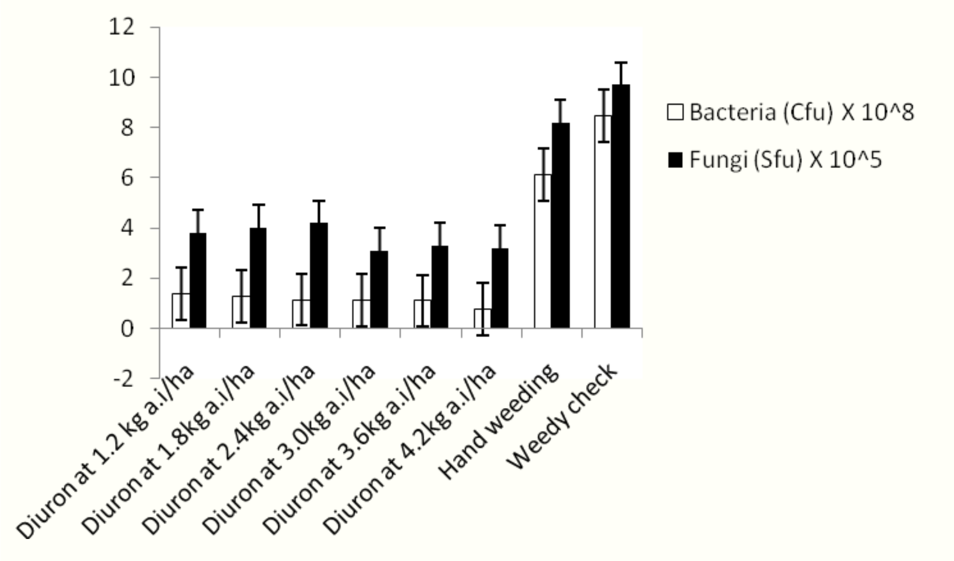

Figure 1. Effects of increasing rates of diuron on the bacterial and fungal population

Figure 2 shows the effects of the treatments on the plant height of maize measured in dynamics from 2 to 10 WAP. There were no significant differences noticed among the various treatments in the first 4 weeks after treatment application.

Diuron treatments however started to affect the heights of maize plants as from the eight week after planting (WAP), and these variations among the varying rates of plant height continued until the 9^{th} week. The tallest plants were obtained from the hand weeding treatment at maize harvest. Among the herbicide treatments, rates from 3.0ka a I / ha and above performed excellently well in terms of plant growth enhancement. The lowest rate of diuron treatment reduced plant height at week 10 after planting compared to the weedy control. Average number of leaves per plant was however affected by the herbicides treatments in different manner (figure 3). For this parameter, significant differences were only noticed among the diuron treatments at 2 and 5 weeks after application of diuron to the soil. At harvest, the tallest maize plants were found in the hand weeded plots, and among the diuron treatments.

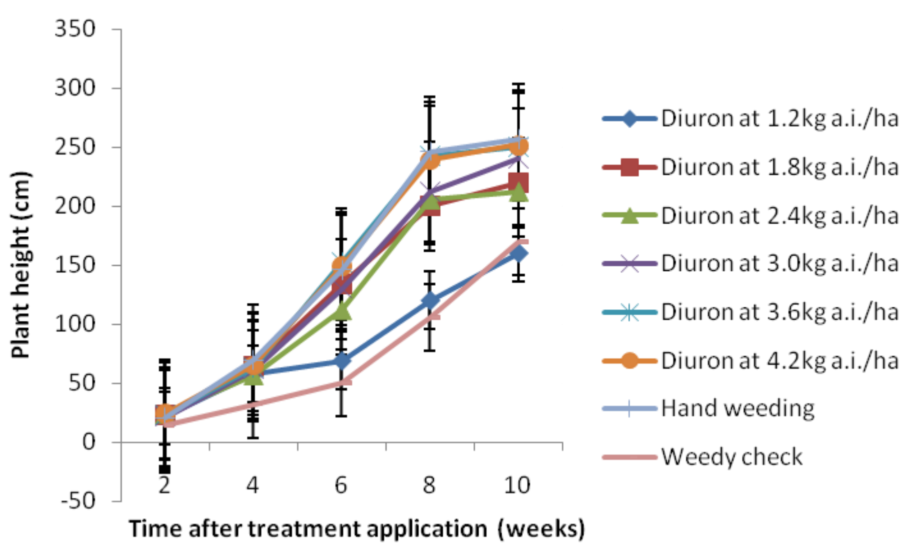

Figure 2. Effects of diuron treatments on maize plant height

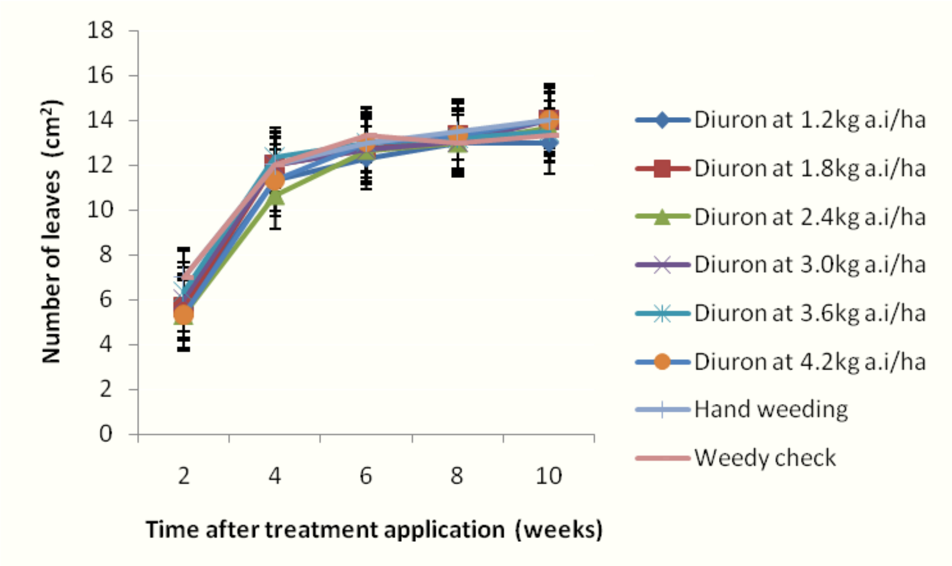

Figure 3. Effects of diuron treatments on average number of leaves of maize

There were significant differences amongst treatments regarding their influences on maize grain yield recorded at harvest as shown in table 6. Among the treatment, total grains weight obtained from hand weeded plot was the highest compared to other treatments. Maize grain weight obtained from weedy check plots were lower than that obtained from diuron treated plots. There were no significant difference between diuron at 1.2kg a.i/ha and 1.8kg a.i/ha in term of maize grain yield. Also, there were no significance difference between diuron at 3.6kg a.i/ha and 4.2kg a.i/ha in term of maize grain yield but hand weeded was significantly higher when compared to other treatments in terms of maize grain yield. Among the diuron treatments, increasing dose of the appeared to increase maize yield.

Table 4. Effects of diuron treatments on maize yield

Treatment	Yield
Diuron at 1.2 kg a.i/ha	868.33d
Diuron at 1.8kg a.i/ha	891.33d
Diuron at 2.4kg a.i/ha	975.00c
Diuron at 3.0kg a.i/ha	1038.33c
Diuron at 3.6kg a.i/ha	1115.00b
Diuron at 4.2kg a.i/ha	1175.67b
Hand weeding	1333.33a
Weedy check	744.33e

Source: Laboratory analysis results

There were significant ($p<0.05$) differences amongst treatments in weed weight recorded at 3 WAT and at harvest including the cumulative weed weights (Table 2). In both years, total weed growth as measured by cumulative weed fresh weight was significantly reduced in the weed-free control, when weeds were removed once per week till harvest at 10 WAP compared with the weedy control and the diuron treatments. Specifically, application of diuron at 1.2 kg a.i. /ha resulted in higher total weed weight removed compared with the other treatments including the weedy control, while weed weight removed were similar for diuron from 2.4 to 3.6 kg a.i. / ha and the weedy control in. Regressing weed fresh weight (Y*)* against increasing dose of atrazine (X) showed negative relationships with prediction equations shown in Table 3.

Table 5: Effects of varying rates of diuron on fresh weight in g/m^2 of weed removed in maize plots

Treatment	Fresh weight at 4 WAT	Fresh weight at harvest	Cumulative weight
Diuron at 1.2kg a.i/ha	38.67a	45.00a	83.67a
Diuron at 1.8kg a.i/ha	28.33bc	36.67b	65.00b
Diuron at 2.4kg a.i/ha	20.00abc	30.67c	50.67bc
Diuron at 3.0kg a.i/ha	18.67cd	25.67d	44.34c
Diuron at 3.6kg a.i/ha	15.67d	20.33e	36.00d
Diuron at 4.2kg a.i/ha	11.67de	17.67f	29.34d
Hand weeding	3.67e	9.66f	13.33e
Weedy check	29.00b	42.33a	71.33a

Source: Laboratory analysis results

Table 3: Linear correlation and regression analysis between increasing rates of diuron (X) and fresh weight (Y)

Weed fresh weight	Correlation coefficient(r)	Regressions equation
At 3WAP	-0.97	Y= 37.3 - 6.3x
At harvest	-0.99	Y= 52.58 – 8.9x
Cumulative	-0.99	Y= 89.89 – 15.16x

Source: Laboratory analysis results

DISCUSSIONS

This study has clearly demonstrated that the diuron herbicide exerts divers' influences on the soil chemical and microbial status, and this confirmed the conflicting nature of results obtained from previous studies on the influences of herbicide treatment on soil chemical properties. For instance, several triazine herbicides have been found not to exert any significant influence on soil nutrient content in field trials conducted in established crop rotations (Gruzdyev, 1974). In contrast, significant increases in soil nutrient content over the unsprayed plots have been recorded following repeated applications of triazine herbicides in the field (Kruglov *et al*., 1975; Sidorov, 1974). The discrepancies in responses of soil nutrient content to atrazine application often reported in the literature appear to be related to, among other factors, differences in soil edaphic and climatic conditions under which these studies were conducted and any lack of uniformity in the physical conduct of the experiments (Lebedeva *et al*., 1990). The relatively low soil pH recorded in this experiment in the weed-free and the diuron-treated plots at maize harvest presumably arose from water erosion of the exposed soil in the early period of crop growth which led to leaching out of much of the base-forming cations, leaving the exchange complex dominated by aluminum and hydrogen ions (Brady & Weil, 1999). This might also be responsible for the low values recorded for these exchangeable bases (K, Mg and Ca) at the time of sampling. The higher pH recorded in the weedy check control might have resulted from accumulation of liters during the course of the experiment because the plot was left unheeded. Decomposition of these liters had definitely added organic matter to the soil, and this would have in turn raised the pH of the soil.

This may also be adduced as the reason the untreated weedy control had higher OM concentration compared with the weeded plots either with diuron or hand weeding. Addition of organic manure has been found to move soil pH towards neutrality in acidic (Benke *et al*. 2009) and alkaline (Chang *et al*. 1990; Hao and Chang 2002) soils, thus improving nutrient availability especially for P and micronutrients. This may also be responsible for the boost in OM, N and P in weedy check compared to the other treatments. N and P are among the nutrients known to be furnished by the soil organic matter. Furthermore, diuron has been found to reduce the populations of certain strains of nitrogen fixing bacteria including algae (Flores, and Barbachano 1992) as well as ammonia oxidizing bacteria (*Nitrosomonas*) (Gadkari,1988), thus grossly reducing the possibilities of external N fixation to the soil. In addition, diuron reduces the activity of phytase, an enzyme that mineralizes the plant nutrient phosphorus in soil (Cervelli and Perna, 1985). Decrease in the levels of soil organic matter and basic soil nutrients in the diuron treated plots could be as a result of the decrease in soil microbial population earlier discuss. The delay in activity and the decrease of the total microbial activity of soil affects the decomposition of crop residues and the soil organic matter that are an essential contribution of the nutrient cycling process in the soil (Prado and Airodi, 2001). In general, pesticides that have toxic effects on soil microbial activity, such as diuron, give rise to the possibility of adverse consequences on long-term soil fertility (Moorman, 1989).

Reduction in soil bacterial and fungal population irrespective of the rate of diuron application to soil indicated that a fraction of the soil microbial community did not adapt to the herbicide. Similar results were obtained by Adejoro (2016) when soil samples were treated with cypertmethrin, a synthetic pyrethoid insecticide. This is in consonance with previous findings that diuron is able to disrupt the complex ecological community of soil microorganisms, including algae and fungi (Prado and Airoldi , 2001) in a variety of ecosystems. Diuron in concentrations equivalent to those used by farmers has also been reported to reduce algae populations by 99 percent in the top layer of the soil in both clay and sandy loam soil (Pipe and Cullimore, 1980; 1984). The herbicide has also been shown to inhibit microbial activity in soil, even at concentrations as low as several parts per million, causing conditions averse to restoring soil fertility (Prado and Airoldi, 2001).

This decrease in the soil microbial population compared to the control treatment can therefore be associated with toxic effects of diuron on soil microorganisms. The higher microbial population recorded in the weedy check control was not unexpected. This may not be unconnected with the rhizosphere effect obtainable at the root zones of diverse weed species, and the colonizing organisms were likely to possess good rhizosphere competences (Kennedy, 2005). Microbial population in the hand-weeded plots was almost at pal with the unheeded plots. Microbial count might have been increased by hoeing in this study through increased aeration and water movement in the soil (Iremiren, 1988), which are good conditions for microorganisms to thrive. The observation that diuron exerted more toxicity to the soil bacterial than the fungal community in this study may not be unconnected with the fact that degradation of the herbicide in soil is carried out by fungi. Three fungal strains (*B. bassiana, C. elegans*, and *M. isabellina*) have been identified as being able to transform diuron to any extent (up to 50%) after 7 days of incubation (Bogarets *et al*., 2000). The two metabolites identified after the degradation (N- (3,4-dichlorophenyl)-N-methylurea and N-3, 4-dichlorophenylurea) was however reported to present a three times higher toxicity than that of diuron (Bogarets *et al*., 2000).

The weed free control and the higher rates of diuron significantly increased maize plant height and grain yield over the weedy control and the treatments involving lower rates of diuron. These were in response to the differential weed growth between the foregoing treatments. The highest maize grain yield occurred in the weed-free plots, indicating that it is necessary to protect the crop from weed competition throughout most of its growth to ensure maximum yield. As stated earlier, aeration and water movement caused by hand weeding engendered by hoeing might have been responsible for the yield increase. Diuron at 3.0 to 4.2 kg a.i/ha provided optimum weed control and improved maize grain yield close to that obtained in the weed-free plots. The significant differences observed amongst the diuron treatments in terms of weed weight at 3 WAT and at harvest including the cumulative weed weights indicate that the extent of weed growth in the treatments was dose-dependent.

Weed growth decreased more or less significantly in the order of increasing dose of diuron presumably because higher doses imply a higher quantity of active components available to cause control. The very poor weed control and consequent low maize yield from the lowest rate (1.2 kg a.i./ha) of the herbicide confirmed earlier observation that many herbicides commonly used for weed control show growth-stimulating or hormone-like properties at sublethal concentrations (hormesis) (Akobundu, 1987). Wiedman & Appleby (1972) screened several herbicides in the greenhouse and indicated that many of them from different herbicide groups stimulated plant growth at sublethal concentrations. Plant response to sublethal doses has been extensively reviewed by Ries (1976), who also discussed the possible implication of this phenomenon in agriculture and herbicide rotations (Akobundu, 1987).

CONCLUSIONS

Application of diuron at the standard rate of 3.0 kg a.i.ha and above compared favourably with the weed free control in terms of growth and yield enhancement in maize. This was because these rates of the herbicide provided optimum weed control in the crop. Soil nutrient and microbial status were however adversely affected by the herbicide irrespective of rate of application. It is therefore recommended that practices or inputs that can reduce the toxicity of diuron to soil microorganisms, which may in turn lead to improvement in soil nutrient status, be integrated into diuron weeding programmes as reduction in its rate of application may not achieve the desired weed control efficacy.

REFERENCES

Adejoro, S.A (2016) Interaction Effects of Glyphosate and Cypermethrin on Soil Basal Respiration and Carbon Mineralization Quotient. *Applied Tropical Agriculture* 21(1),: 7-14

Akobundu, I.O. (1987).*Weed Science in the Tropics: Principles and Practices*. Wiley. New York

AOAC. (1990). Official Methods of Analysis. 15th edition. Association of Official Analytical Chemists, Washington, DC, USA.

Benke, M. B., Hao, X., O'Donovan, J. T., Clayton, G. W., Lupwayi, N. Z., Caffyn, P. and Hall, M. (2009). Livestock manure improves acid soil productivity under a cold northern Alberta climate. *Can. J. Soil Sci. 90: 685-697.*

Bogaerts, P., Bohatier, J., Bonnemoy, F., Cuer, A., Sancelme, M., Tixier, C., Twagilimana, L., Veschambre, H. (2000). Fungal Biodegradation of a Phenylurea Herbicide, Diuron: Structure and Toxicity of Metabolites. Pest Management Science. 56:455-462.

Brady, N.C. & Weil, R.R. (1999). *The Nature and Properties of Soils.* 12th Edition, Prentice Hall, Upper Saddle River, New Jersey 07458.

Cervelli, S. and A. Perna. (1985). Phytase inhibition by insecticides and herbicides. Wat. Air Soil Pollution 24:397-403.

Chang, C., Sommerfeldt, T. G. and Entz, T. (1990). Rates of soil chemical changes with eleven annual applications of cattle feedlot manure. *Can. J. Soil Sci. 70: 673–681.*

Cox, C (2003) Herbicide factsheet –Diuron. *Journal of Pesticide Reform* Vol. 23 (1): 12-20

Flores M, Barbachano M (1992). Effects of herbicides Gramoxone, Diuron and Totacol on growth and nodulation of three strains of Rhizobium meliloti. Sci. Tot. Environ. 123/124:249-260.

Gadkari D. (1988). Assessment of the effects of the photosynthesis-inhibiting herbicides Diuron, DCMU, metamitron, and metribuzin on growth and nitrogenase activity of Nostoc muscorum and a new cyanobacterial isolate, strain G4. Biol. Fertil. Soils 6:50-54.

Gruzdyev, G.S. (1974). *Primenenie gerbitsidov v sevooborote.* Izdatel'stvo TSKHA. Vypusk 2,108-121. (In Russian)

Hao, X. and Chang, C. (2002).Effect of 25 annual cattle manure applications on soluble and exchangeable cations in soil. *Soil Sci. 167: 126–134.*

Harvey, J.J., Bray, W.E., Jones, A.G. & King, J.M. (1982). Weed control in row crops. In H.A..Roberts (Edited), *Weed Control Handbook*: *Principles* (7th Edition, pp.292-315).Oxford, Blackwell Scientific

Iremiren, G.O. (1988). Frequency of weeding for optimum growth and yield of okra (*Abelmoschus esculentus*). *Experimental Agriculture 24*, 247-252.

Kennedy, A. C. (2005). Rhizosphere.In Principles and Applications of Soil Microbiology; (SylviaD.M. Ed.) Pearson Educational, Inc. Upper Saddle River, *New Jersey.Pp 242- 262.*

Kidd, H. and James, D.R. (1991) The Agrochemicals Handbook. 3rd Edition, Royal Society of Chemistry Information Services, Cambridge.

Kruglov, Yu. V., Pertseva, A.I. & Galkina, G.A. (1975). Izminenie biologicheskoi aktivnosti pochvy pod vlianiem mnogoletnei sistematicheskoi obrabotki gerbitsidami plantatsii dioskorei kavkazhskoi. *Dokladi VASKHNIL 2*, 20-21. (In Russian)

Lebedeva, G.E., Agapov, V.L., Blagoveshchensky, Yu. N. & Samsonova, V.P. (1990). *Gerbitsidi i (Russian) Pochva: Ekologicheskie Aspekty Primenenia Gerbitsidov.* Izdatel'stvo Moskovskogo Universiteta.

Moorman TB (1989) A review of pesticide effects on microorganisms and microbial processes related to soil fertility. *J. Prod. Agric* 2: 14-23

Pipe AE, Cullimore DR. (1980). An implanted slide technique for examining the effects of the herbicide diuron on soil algae. *Bull. Environ. Contam. Toxicol*. 24:306-312.

Pipe AE, Cullimore DR. 1984. Influence of five phenylurea herbicides on the diatom Hantzschia in a sandy loam soil. *Bull. Environ. Contam. Toxicol*. 33:439-443.

Prado, AGS , Airoldi C. (2001). The effect of the herbicide diuron on soil microbial activity. Pest. Management. Sci.57:640-644.

Ries S. K. (1976). Subtoxic effects on plants. In L.J Audus,(Ed), *Herbicides physiology, Biochemistry, Ecolgy, volume 2,(2nd* Edition, pp. 313-334). London and New York. Academic Press

Sidorov, V.I. (1974). *Issledovanie dlitel'nogo primenenia gerbitsidov v zvene ovoshchekormovogo (Russian) sevooborota v usloviakh Moskovskoi oblasti*. Avtoreferat Kandidatskoi Dissertatsi. Moskva.

Tel, D. A. (1984). Soil and plant analysis. Department of Land Resources Science, University of Gnelph, Ontario, Canada: pp. 277.

U.S. Dept. of Agriculture. Agricultural Research Service. (1995). ARS pesticide properties database: Diuron. http://wizard/arsusda.gov/acsl/textfiles/DIURON.

Wiedman, S.J. & Appleby, A.P. (1972). Plant growth stimulation by sublethal concentrations of herbicides. *Weed Research 12*, 65-74.