



## Effect of two selected herbicides (Nicosulfuron + Atrazine and Dimethylammonium Acetate) on microbial activities and physicochemical properties of soil samples

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### Keywords:

*Microbial biomass carbon,*  
*Available phosphorus,*  
*Total nitrogen,*  
*Organic matter,*  
*Mineral content*

**Abstract** — This study investigated the effect of Dimethylammonium Acetate and Nicosulfuron + Atrazine on soil microbial activities and physicochemical properties, applied at X1, X2, and X3 rates. Dehydrogenase activity, Microbial biomass carbon, available phosphorus, total nitrogen, organic matter, mineral content, electrical conductivity, exchangeable acidity and pH were determined in the soil samples. There were significant reductions ( $P \leq 0.05$ ) in the dehydrogenase activities of soil samples treated with the two herbicides compared to the control. Treatment with the herbicides also resulted in significant increases ( $P \leq 0.05$ ) in the total nitrogen and available phosphorus content of soil samples compared to the control soil samples. Dimethylammonium Acetate treated soils had the lowest dehydrogenase activity ( $26.33 \pm 0.9 \mu\text{gg}^{-1}\text{h}^{-1}$ ) of the two herbicides. Soil treatment with Dimethylammonium Acetate resulted in significant increases ( $P \leq 0.05$ ) in calcium, magnesium, sodium, potassium, manganese, copper, and zinc content of soil samples. The manganese content of the soil samples was highest ( $7.29 \pm 0.20 \text{ mg/kg}$ ) in soils treated with Dimethylammonium Acetate. Dimethylammonium Acetate treated soils also had the highest exchangeable acidity and electrical conductivity values of  $0.85 \pm 0.08 \text{ Cmol kg}^{-1}$  and  $1.75 \pm 0.14 \text{ Cmol kg}^{-1}$ , respectively, at X3. Conclusively Dimethylammonium Acetate caused a noticeable increase in dehydrogenase activity and microbial biomass carbon content, while application of Nicosulfuron + Atrazine resulted in an increase in dehydrogenase activity and a decrease in microbial biomass carbon.

### Subject Classification (2020):

## 1. Introduction

Herbicides are used in mechanized farming to kill weeds. The use of herbicides, despite their obvious advantages, is fraught with many disadvantages that may pose a serious threat to crop production. Accumulation of these herbicides in soils may also lead to a severe reduction in crop yield. The application of herbicides at concentrations in excess of the manufacturer's instructions can cause

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essential alterations in the function and structure of soil microbial communities and cause a negative impact on the terrestrial ecosystem, thereby lowering soil fertility and quality [1]. Application of the weed control measures depends extensively on their effectiveness, availability and cost. Pre or post-emergent herbicides would make the herbicidal weed control more acceptable to farmers since it does not change the existing agronomic practices but will allow for complete control of weeds. The presence of herbicide residues in soils could have direct impacts on soil microorganisms. This is a matter of great concern.

When herbicides are applied at manufacturers recommended rates, there is no major or long-term effect on microbial populations. Sebiomo et al. [2] reported that some microorganisms could break down and utilize some selected herbicides as a nutrient source, while some other microorganisms were negatively affected depending on the application rates and the type of herbicides used. When Dimethylammonium Acetate and Nicosulfuron + Atrazine herbicide are applied at normal manufacturers rate, they controlled a wide spectrum of weeds in crops such as wheat, oats and barley in a pre- or post-emergence application. Dimethylammonium (2,4-dichloro phenoxy) Acetate has low toxicity for human beings and low-to-moderate toxicity to soil ecology. According to Holloway et al. [3], the major pathways to utilising Dimethylammonium Acetate and Nicosulfuron + Atrazine are microbial degradations and chemical hydrolysis, influenced by soil pH, clay content, organic and inorganic fertilizers, and climatic conditions (rainfall and temperature).

Zhong and Cai [4] reported that soil organisms cause important changes in the physical soil properties such as structure, porosity, aeration and water infiltration through the formation and stabilization of soil aggregates. Soil microbial community helps detoxify (bioremediation) soils contaminated with toxins and undesirable components due to human activities [5] and the biocontrol of plant pathogens. Soil organic matter improves a soil's chemical and physical properties, promoting biological activity and maintaining environmental quality, and this is why organic fertilizers, such as manure, promote the activities of soil microbial communities. Plants and microorganisms are important in the soil ecosystem and are responsible for many important soil cycling processes, such as C mobilization and N mineralization. On the other hand, land use influences soil microbial processes and the structure of microbial communities. Therefore, this study determined the effect of Dimethylammonium Acetate and Nicosulfuron + Atrazine on soil microbial activities and physicochemical properties.

## 2. Material and Methods

### 2.1. Herbicides

The herbicides Dimethylammonium Acetate and Nicosulfuron + Atrazine were purchased locally in Ijebu Ode, Nigeria. These herbicides are commonly used in the southwestern region of Nigeria to kill weeds. Often, they are applied in extreme amounts beyond the manufacturer's recommendations (According to manufacturer's instruction, Nicosulfuron + Atrazine is applied at 35-70 g ha<sup>-1</sup> while Dimethylammonium Acetate is applied at 225 ml - 1.4 L ha<sup>-1</sup>).

### 2.2. Soil sampling

The soil samples were collected from the research field of the Biological Sciences Department, Tai Solarin University of Education, Ijagun, Ogun State, Nigeria, using the soil augur at 5cm depth. The samples were sieved with wire mesh (size<2mm), sorted to remove stones, plant debris and any visible soil fauna and then thoroughly mixed.

The soil samples were allowed to settle for seven days by incubating at 27°C to permit the disturbances caused by sampling and sieving to subside. Soil treatments were replicated three times using a complete

randomized block design. The herbicides were then applied according to the manufacturer's instruction (X1), at twice (X2) and thrice (X3) the manufacturer's rates and incubated in different plastic pots filled with 5 kg of treated and untreated soil samples (control). The soil samples were then incubated for 14 days at 27°C, after which the soil samples were then taken from the pots (at 5cm depth of soil surface) for analysis.

### **2.3. Determination of the dehydrogenase activity using the tetrazolium salts (TTC) method.**

The method provided in [6] was used to determine the dehydrogenase activity of soil samples. Five grams of the soil samples treated with Dimethylammonium Acetate and Nicosulfuron + Atrazine and non-treated soil samples were dispensed into a 250 mL conical flask. Tris buffer (2.5 mL) and TTC-glucose solution (1 mL) were added to the herbicide treated soil samples. One mL of distilled water was then dispensed into the control flasks. The pH was adjusted to 7 using 1.0 N HCl, and the flasks were swirled gently to mix the contents. The mixtures were incubated at 30 °C for 24 hours. After incubation, the soil samples were treated with methanol, transferred into a funnel, and sieved with Whatman No 42-filter paper placed on a 100 mL graduated cylinder. Additional portions of methanol were passed through the soil until 50 mL of methanol, containing the formazan, was collected in the graduated cylinder. The red methanolic solutions of the formazan were determined using a UV/Vis Spectrophotometer [6].

### **2.4. Determination of microbial biomass carbon of the soil**

The method of Vance et al. [7] was used in this experiment to determine the microbial biomass carbon of soil samples. Five grams of Dimethylammonium Acetate and Nicosulfuron + Atrazine treated soil samples were treated with 50 mL of 2:1 chloroform-ethanol in a vacuum desiccator for 24 hrs. The soils that were not treated were used as blank. Soil sample extraction was carried out with 40 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> for 30 min in an oscillator at 300 rpm. The soil samples that were not treated were also extracted with the 0.5 M K<sub>2</sub>SO<sub>4</sub>, and the resulting extracts were filtered through Whatman No 42 Filter paper into a 250 mL conical flask. The filtrates were then used to determine microbial carbon on a UV/Vis Spectrophotometer. 0-10 µg/mL ethanolic acidified potassium dichromate solutions were used as working standards.

$$\text{Microbial respiration} = \frac{\text{Absorbance of sample} \times \text{Gradient Factor} \times \text{Dilution factor}}{\text{Wt of sample}}$$

### **2.5. Determination of electrical conductivity and exchangeable acidity of soil samples**

Electrical conductivity was determined using the method described by Rhodes [8]. 50 g of soil was mixed with 50 mL of deionized water, shaken at 200 rpm for 2 h, and then filtered through a Whatman filter paper. The electrical conductivity of the filtrate was then determined with an ionic probe. The exchangeable acidity of the sample was determined in KCl extracting solution by titration.

### **2.6. Determination of organic matter in the soil**

Percentage organic matter was determined by the method described by FAO [9]. Soil samples treated with Dimethylammonium Acetate and Nicosulfuron + Atrazine and untreated soil samples were collected and sieved through 0.5 mm sieve. One gram of treated and untreated soil samples was dispensed into 250 mL Erlenmeyer flasks, and 10 mL of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution was allowed to dissolve into each flask. 20 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added and swirled until the soil samples were properly mixed with the reagents. The mixture was then swirled vigorously for one minute; the flasks were

allowed to stand in a sheet of asbestos for about 30 minutes. One hundred millilitres of distilled water were added to each flask, followed by 3-4 drops of indicator (ferroin) and titrated with 0.5 N FeSO<sub>4</sub> solution to the endpoint, from greenish or dark green to red (maroon colour), in reflected light against a white background. The organic matter was then calculated according to using the following formula,

$$\% \text{ Organic matter} = \frac{(\text{me K}_2\text{SO}_4 - \text{me FeSO}_4) \times 0.003 \times 100 \times f \times 1.729}{w}$$

w = Weight of air-dried soil

Correlation factor "f" = 1.33

me = Normality of solution × millilitres of solution used

## 2.7. Determination of total nitrogen content of the soil

Five grams of soil samples treated with Dimethylammonium Acetate and Nicosulfuron + Atrazine were digested with H<sub>2</sub>SO<sub>4</sub> in the presence of CuSO<sub>4</sub> H<sub>2</sub>O as a catalyst and K<sub>2</sub>SO<sub>4</sub>, which raises the digestion temperature. The ammonium content of the digest was analysed by distilling with excess NaOH, and the evolved NH<sub>3</sub> was absorbed in standard HCl. The HCl was then titrated against standard NaOH using methyl red as an indicator. The endpoint was then determined by a change of colour from pink to yellow.

## 2.8. Determination of available phosphorus in soil using Bray No. 1 Method

Available phosphorus was determined using the method of Houba et al. [10]. Five grams of soil samples treated with Dimethylammonium Acetate and Nicosulfuron + Atrazine and untreated soil samples were sieved with a 2 mm sieve, weighed into a centrifuge tube, and 20 mL of extracting solution were added. The mixture containing the treated and untreated soil samples were then shaken for 1 min on a mechanical shaker and centrifuged at 2000 rpm for 15 minutes. Two millilitres of clear supernatant were then dispensed into 20 mL test tube with pipettes. Five millilitres of distilled water and 2 mL of ammonium molybdate solution were then added. The contents were mixed, and 1 mL of SnCl<sub>2</sub>.2H<sub>2</sub>O dilute solution was added and remixed. Percentage transmittance was measured on spectronic-20 electro photometer at 660 nm wavelength.

## 2.9. Chemical properties

The samples' concentrations of Sodium, potassium, calcium, zinc, manganese, copper and magnesium were determined using the atomic absorption spectrophotometer fitted with a hollow cathode lamp and a fuel-rich flame (air acetylene). Sample extract solutions from the herbicide treated and untreated soil samples and standard solution for each mineral were injected into the atomic absorption spectrophotometer, and the mean signal response was recorded for each element at their respective wavelength. The concentration of the elements was calculated [11].

## 2.10. Soil pH determination

Twenty grams of treated (with Dimethylammonium Acetate and Nicosulfuron + Atrazine) and untreated soil samples were weighed into a 50 mL beaker; 20 mL of distilled water was then added and allowed to stand for 30 min and occasionally stirred with a glass rod. The soil pH in a soil: water ratio of 1:1 was determined using the method of Rhodes [8].

## 2.11. Statistical Analysis

Data generated from this study were subjected to analysis of variance (ANOVA). Means were compared at a 5% level of significance using Duncan's multiple range tests.

### 3. Results and Discussion

Presented in Table 1 are the biochemical and physicochemical properties of stabilized and un-stabilized soil samples.

**Table 1.** Microbiological, biochemical and physicochemical properties of soils at the experimental site

Soil characteristics	Soil Sample
Soil type	
Total nitrogen (%)	0.12
Available phosphorus (ppm)	12.51
Organic matter (%)	1.736
Soil electrical conductivity ( $\mu\text{S}/\text{cm}$ )	253.00
pH	6.20
Soil moisture ( $\text{g}\cdot 100\text{g}^{-1}$ )	18.80
Sand ( $\text{g kg}^{-1}$ )	94.00
Silt ( $\text{g kg}^{-1}$ )	4.00
Clay ( $\text{g kg}^{-1}$ )	2.00

In Table 2, there were significant reductions ( $P \leq 0.05$ ) in the dehydrogenase activities of soil samples treated with the two herbicides compared to the control. Treatment with the herbicides also resulted in significant increases ( $P \leq 0.05$ ) in the total nitrogen and available phosphorus content of soil samples compared to the control soil samples. Soil samples treated with Dimethylammonium Acetate had the lowest dehydrogenase activity ( $26.33 \pm 0.9 \mu\text{g g}^{-1} \text{h}^{-1}$ ) of the two herbicides. There were no significant differences ( $P \geq 0.05$ ) in the dehydrogenase activities of soil samples treated with different doses of herbicides at X1, X2 and X3. Soil samples treated with Dimethylammonium Acetate recorded the lowest total nitrogen and available phosphorus values of  $0.68 \pm 0.01\%$  and  $6.65 \pm 0.01\%$ , respectively, in Table 2.

There were significant changes ( $P \leq 0.05$ ) in the total nitrogen (this might be as a result of the ammonium content of the herbicides) and available phosphorus contents of X1, X2 and X3 treated soil samples. Soil samples treated with Nicolsufuron+Atrazine had significantly higher ( $P \leq 0.05$ ) microbial biomass carbon (MBC) values compared to control, while the soil samples treated with Dimethylammonium Acetate recorded significantly lower ( $P \geq 0.05$ ) MBC value compared to control. Meanwhile, there were no significant changes in the MBC values of X1, X2, and X3 treated soil samples of the two herbicides. There was a significant increase ( $P \leq 0.05$ ) in the organic matter content of the herbicide treated soil samples compared to the control. The control soil sample recorded the lowest value of  $1.74 \pm 0.08\%$ .

**Table 2.** Effects of selected herbicides on soil dehydrogenase activity, microbial respiration, total nitrogen and available phosphorus content of soil samples

		X1	X2	X3
DA( $\mu\text{gg}^{-1}\text{h}^{-1}$ )	2, 4 D A	26.33 $\pm$ 0.9 <sup>a</sup>	26.30 $\pm$ 1.8 <sup>a</sup>	26.03 $\pm$ 0.6 <sup>a</sup>
	NA	28.80 $\pm$ 0.6 <sup>b</sup>	29.20 $\pm$ 0.6 <sup>b</sup>	29.23 $\pm$ 0.9 <sup>b</sup>
	Control	33.30 $\pm$ 0.6 <sup>c</sup>	33.23 $\pm$ 0.9 <sup>c</sup>	33.01 $\pm$ 0.1 <sup>c</sup>
MBC (KgC m <sup>-2</sup> )	2, 4 D A	6.33 $\pm$ 0.9 <sup>a</sup>	6.30 $\pm$ 0.6 <sup>a</sup>	6.50 $\pm$ 0.6 <sup>a</sup>
	NA	8.33 $\pm$ 0.3 <sup>c</sup>	8.07 $\pm$ 0.3 <sup>c</sup>	8.13 $\pm$ 0.3 <sup>c</sup>
	Control	7.23 $\pm$ 0.3 <sup>b</sup>	7.43 $\pm$ 0.9 <sup>b</sup>	7.23 $\pm$ 0.9 <sup>b</sup>
TN (%)	2, 4 D A	0.68 $\pm$ 0.01 <sup>b</sup>	0.78 $\pm$ 0.01 <sup>b</sup>	1.63 $\pm$ 0.01 <sup>c</sup>
	NA	1.24 $\pm$ 0.01 <sup>c</sup>	1.20 $\pm$ 0.01 <sup>c</sup>	0.55 $\pm$ 0.01 <sup>a</sup>
	Control	0.53 $\pm$ 0.01 <sup>a</sup>	0.50 $\pm$ 0.01 <sup>a</sup>	0.53 $\pm$ 0.01 <sup>b</sup>
AVP (%)	2, 4 D A	6.65 $\pm$ 0.01 <sup>b</sup>	6.57 $\pm$ 0.01 <sup>b</sup>	6.62 $\pm$ 0.01 <sup>c</sup>
	NA	6.89 $\pm$ 0.01 <sup>c</sup>	6.40 $\pm$ 0.01 <sup>a</sup>	6.10 $\pm$ 0.01 <sup>a</sup>
	Control	5.46 $\pm$ 0.01 <sup>a</sup>	6.62 $\pm$ 0.01 <sup>c</sup>	6.42 $\pm$ 0.01 <sup>b</sup>
OM (%)	2, 4 D A	3.78 $\pm$ 0.27 <sup>c</sup>	2.58 $\pm$ 0.54 <sup>b</sup>	3.39 $\pm$ 0.42 <sup>c</sup>
	NA	2.99 $\pm$ 0.41 <sup>b</sup>	4.14 $\pm$ 0.45 <sup>c</sup>	2.65 $\pm$ 0.38 <sup>b</sup>
	Control	1.74 $\pm$ 0.08 <sup>a</sup>	1.74 $\pm$ 0.08 <sup>a</sup>	1.74 $\pm$ 0.08 <sup>a</sup>

DA= Dehydrogenase Activity, MBC=Microbial Biomass Carbon, TN= Total Nitrogen, AVP= Available Phosphorus, OM= Organic Matter. Values followed by the same superscript along the same vertical column are not significantly different ( $P \geq 0.05$ ), while values followed by different superscripts along the same vertical column are significantly different ( $P \leq 0.05$ ). X1= manufacturers specification, X2= twice manufacturers specification, X3= thrice manufacturers specification.

Table 3 shows the effect of the Nicolsufuron + Atrazine and Dimethylammonium Acetate herbicides at the manufactures rate of application (X1) on soil physicochemical properties. The result reveals a significant difference ( $P \leq 0.05$ ) between the pH of the control and herbicide treated soils.

Soil treatment with Dimethylammonium Acetate resulted in slight increases in calcium, magnesium, sodium, potassium, manganese, copper and zinc content. The manganese content of the soil samples was highest ( $7.29 \pm 0.20 \text{ mg kg}^{-1}$ ) in soils treated with Dimethylammonium Acetate. However, treatment of the soil samples with Nicolsufuron + Atrazine revealed a slight drop in the Ca, Mg, Na, K, Ma, Cu, and Zn contents. The electrical conductivity and exchangeable acidity values of the soil samples increased significantly ( $P \leq 0.05$ ) after treatment with Dimethylammonium Acetate compared to the control. The electrical conductivity and exchangeable acidity values were also highest ( $0.88 \pm 0.49 \text{ Cmol kg}^{-1}$  and  $1.90 \pm 0.12 \text{ Cmol kg}^{-1}$  respectively) in Dimethylammonium Acetate treated soils.

**Table 3.** The Effect of Nicolsufuron + Atrazine and Dimethylammonium Acetate herbicides applied at the manufacturer's application rate on physicochemical soil properties

Parameters	NA	2,4 DA	Control
pH	6.71 ± 0.27	5.88 ± 0.29	7.20 ± 0.20
Calcium (Cmol kg <sup>-1</sup> )	3.53 ± 0.13	3.81 ± 0.12	3.61 ± 0.19
Magnesium (Cmol kg <sup>-1</sup> )	1.34 ± 0.15	1.61 ± 0.60	1.47 ± 0.13
Sodium (Cmol kg <sup>-1</sup> )	0.56 ± 0.04	0.66 ± 0.17	0.52 ± 0.07
Potassium (Cmol kg <sup>-1</sup> )	1.68 ± 0.14	1.88 ± 0.90	1.79 ± 0.14
Manganese (mg kg <sup>-1</sup> )	5.65 ± 1.09	7.29 ± 0.20	6.96 ± 0.41
Copper (mg kg <sup>-1</sup> )	0.67 ± 0.12	0.69 ± 0.74	0.67 ± 0.06
Zinc (mg kg <sup>-1</sup> )	2.10 ± 0.20	2.28 ± 0.78	2.15 ± 0.15
Exchangable-Acidity (Cmol kg <sup>-1</sup> )	0.69 ± 0.08	0.88 ± 0.49	0.72 ± 0.12
Electrical-Conductivity (Cmol kg <sup>-1</sup> )	1.56 ± 0.10	1.90 ± 0.12	1.65 ± 0.24

Values are expressed as mean ± standard error of three replicate. NA= Nicolsufuron + Atrazine, 2,4 DA=Dimethylammonium Acetate

In Table 4, herbicide treatment resulted in a significant reduction ( $P \leq 0.05$ ) of pH. The lowest pH of  $6.34 \pm 0.13$  was obtained in Dimethylammonium Acetate treated soils. Treatment of soil samples, in Table 4, resulted in a slight increase of Mg, Na, K, Ma, Cu and Zn content of soil samples compared to the control. However, the calcium content of soil samples was slightly reduced compared to the control. Soils treated with Dimethylammonium Acetate recorded the highest manganese content value of  $7.35 \pm 0.50$  mg kg<sup>-1</sup>. There was also a slight increase in the exchangeable acidity and electrical conductivity of soil samples. Soils treated with Dimethylammonium Acetate had the highest exchangeable acidity and electrical conductivity values of  $0.80 \pm 0.41$  cmol.kg<sup>-1</sup> and  $1.76 \pm 0.49$  Cmol kg<sup>-1</sup> respectively.

**Table 4.** The Effect of Nicolsufuron + Atrazine and Dimethylammonium Acetate herbicides applied at double the manufacturer's rate on soil physicochemical properties

Parameters	NA	2,4 D A	Control
pH	6.46 ± 0.80	6.34 ± 0.13	7.20 ± 0.20
Calcium (Cmol kg <sup>-1</sup> )	3.54 ± 0.09	3.50 ± 0.11	3.61 ± 0.19
Magnesium (Cmol kg <sup>-1</sup> )	1.37 ± 0.11	1.58 ± 0.73	1.47 ± 0.13
Sodium (Cmol kg <sup>-1</sup> )	0.59 ± 0.04	0.66 ± 0.51	0.52 ± 0.07
Potassium (Cmol kg <sup>-1</sup> )	1.79 ± 0.13	1.88 ± 0.24	1.79 ± 0.14
Manganese (mg kg <sup>-1</sup> )	7.27 ± 0.05	7.35 ± 0.50	6.96 ± 0.41
Copper (mg kg <sup>-1</sup> )	0.78 ± 0.08	0.79 ± 0.06	0.67 ± 0.06
Zinc (mg kg <sup>-1</sup> )	2.27 ± 0.05	2.27 ± 0.00	2.15 ± 0.15
Exchangable-Acidity (Cmol kg <sup>-1</sup> )	0.78 ± 0.04	0.80 ± 0.41	0.72 ± 0.12
Electrical-Conductivity (Cmol kg <sup>-1</sup> )	1.64 ± 0.08	1.76 ± 0.49	1.65 ± 0.24

Values are expressed as mean ± standard error of three replicate. Values are expressed as mean ± standard error of three replicate. NA= Nicolsufuron + Atrazine, 2,4 DA=Dimethylammonium Acetate

Table 5 shows the effect of the Nicolsufuron + Atrazine and Dimethylammonium Acetate herbicides at three times the manufacturer's rate of application (X3) on soil physicochemical properties. The result revealed a slight drop in pH after treatment with the herbicides compared to the control. Treatment of soil samples, in Table 4, resulted in a significant increase of Mg, Na, K, Ca, Cu and Zn content of soil samples compared to the control. However, the calcium content of soil samples was slightly reduced compared to the control. However, there was a little increase in exchangeable acidity, and electrical conductivity of the herbicide treated soils compared to the control soil samples. Soil samples treated with Dimethylammonium Acetate recorded the highest exchangeable acidity and electrical conductivity values of  $0.85 \pm 0.08$  Cmol kg<sup>-1</sup> and  $1.75 \pm 0.14$  Cmol kg<sup>-1</sup> respectively.

**Table 5.** The Effect of Nicolsufuron + Atrazine and Dimethylammonium Acetate herbicides applied at thrice (X3) the manufacturer's rate on soil physicochemical properties

Parameters	NA	2,4 DA	Control
Ph	$6.66 \pm 0.33$	$6.37 \pm 0.07$	$7.20 \pm 0.20$
Calcium (Cmol kg <sup>-1</sup> )	$3.26 \pm 0.12$	$3.54 \pm 0.07$	$3.61 \pm 0.19$
Magnesium (Cmol kg <sup>-1</sup> )	$1.20 \pm 0.50$	$1.61 \pm 0.15$	$1.47 \pm 0.13$
Sodium (Cmol kg <sup>-1</sup> )	$0.48 \pm 0.03$	$0.70 \pm 0.09$	$0.52 \pm 0.07$
Potassium (Cmol kg <sup>-1</sup> )	$1.58 \pm 0.06$	$1.92 \pm 0.10$	$1.79 \pm 0.14$
Manganese (mg kg <sup>-1</sup> )	$4.78 \pm 1.18$	$6.80 \pm 0.67$	$6.96 \pm 0.41$
Copper (mg kg <sup>-1</sup> )	$0.61 \pm 1.12$	$0.80 \pm 0.05$	$0.67 \pm 0.06$
Zinc (mg kg <sup>-1</sup> )	$2.01 \pm 0.17$	$2.28 \pm 0.01$	$2.15 \pm 0.15$
Exchangable-Acidity (Cmol kg <sup>-1</sup> )	$1.65 \pm 0.05$	$0.85 \pm 0.08$	$0.72 \pm 0.12$
Electrical-Conductivity (Cmol kg <sup>-1</sup> )	$1.36 \pm 0.06$	$1.75 \pm 0.14$	$1.65 \pm 0.24$

Values are expressed as mean  $\pm$  standard error of three replicate. NA= Nicolsufuron+Atrazine, 2,4 DA=Dimethylammonium Acetate

This study shows an important correlation between the herbicides (Nicolsufuron + Atrazine and Dimethylammonium Acetate) and the enzymatic activities of microorganisms in the soil samples. Gianfreda and Sannino [12] reported that enzymatic activities are early and sensitive indicators of soil ecological pollution in the soil ecosystem. According to Gianfreda and Rao [13], herbicides interact directly and indirectly with soil enzymes, thus resulting in a positive or negative impact on microbial populations. In this study, treatment with Dimethylammonium Acetate and Nicolsulfuron + Atrazine resulted in significant differences in enzymatic activities. In soil treated with Dimethylammonium Acetate at the manufacturer's rate, dehydrogenase activities (DHA) decreased compared to the control. The decrease in the dehydrogenase activities of Dimethylammonium Acetate treated soils occurred because the herbicide depleted the microbial population in the soils and hence the dehydrogenase enzymes responsible for dehydrogenase activity reduce in the herbicide treated soil samples. However, after the adaptation of the microorganisms to the herbicide, microorganisms capable of utilising Dimethylammonium Acetate as a nutrient source develops. This will then result in a spike in the dehydrogenase activities of the soil samples. Bacmaga et al. [14] reported that enzymatic activities did not change immediately after applying the herbicides. They also reported that decreased dehydrogenase activity was noticed in soils treated with similar herbicides. In this study, the application of recommended doses of herbicides resulted in a noticeable decrease in the dehydrogenase activities of the soil samples. However, dehydrogenase activities did not change after the application of increased dosage. This might be due to the decrease in the microbial population with the possibility of hampering their carbon source.



However, according to Sebiomo et al. [2], *in vitro* studies reported that an increase in dehydrogenase activities became pronounced from the 4<sup>th</sup> to 20<sup>th</sup> day of incubation.

Moreno et al. [15] reported that Atrazine caused a pronounced increase in dehydrogenase activity. However, in this present study, dehydrogenase activity decreased after treatment with Nicosulfuron + Atrazine. The decrease observed in dehydrogenase activities after treatment with Nicosulfuron + Atrazine occurred because the microflora had not initially received herbicide treatment, so the microflora will need to adapt to the herbicides, which will enable them to utilize the herbicides as a nutrient source. In this study, the results showed that there was an insignificant difference in their dehydrogenase activities at higher the concentration of Nicosulfuron + Atrazine and Dimethylammonium Acetate.

Microbial biomass carbon was significantly reduced when soils were treated with Nicosulfuron + Atrazine and Dimethylammonium Acetate. Application of herbicides to the soil can be both useful and negatively impact several soil microorganisms, which may, in turn, result in alterations to soil carbon cycling. Toxicity of herbicides to soil microorganisms may cause a depletion of microbial biomass, soil microbial respiration and microbial population. Panettieri et al. [16] stated that herbicides could benefit soil microbes by supporting their growth, thereby acting as nutrient sources. Zabaloy et al. [17] stated no observable differences in the total soil respiration between control soil samples and the herbicide treated soil samples. Gomez et al. [18] reported no significant differences in soil respiration after applying different doses of glyphosate. Araújo et al. [19] reported that an increase in soil respiration shows a correlation between the production of carbon dioxide and the breakdown of the herbicide by soil microorganisms, which is capable of using the herbicide as a carbon source. According to Panettieri et al. [16], the time course in the relationship between the application of glyphosate and the release of carbon dioxide is complex and indicative of soil adsorption mechanism that reduces the availability of herbicide to soil microorganisms.

Soil treatment with Nicosulfuron + Atrazine and Dimethylammonium Acetate herbicides at the manufacturer's dosage caused a pronounced increase in total nitrogen concentrations. These effects were also observed after treatment with increased concentration of the herbicides. In the work of Das et al. [20], microbial biomass carbon and total nitrogen increase became more pronounced in soils treated with herbicides compared to control. They also reported that the highest response for microbial biomass nitrogen was recorded with a single application. This study revealed that the application of Dimethylammonium Acetate and Nicosulfuron + Atrazine at X2 and X3 rates also caused increased percentage composition of total nitrogen.

After treatment of soil samples with nicosulfuron + Atrazine and Dimethylammonium Acetate, there were noticeable increases in the quantity of available phosphorus. This might have occurred as a result of a chemical reaction between the components of the herbicides and the available soil phosphorus in relation to soil microorganisms. This result is also similar to the report presented by Das et al. [21]. Earlier workers (Tahar et al., 2017) also reported that the application of herbicides resulted in increased solubilization of insoluble tricalcium phosphates by the microorganisms present in the soil.

The pH values of the herbicide treated soil samples at the manufactures rate of application was acidic, ranging from (5.88-6.78) and varied from the control soil samples, which were basic (7.20). This, however, contradicts the result of Tahar et al. [22]. Tahar et al. [22] reported that the pH value in their study was higher in soil samples treated with herbicides. At doubled the manufactures rate of application, the pH value showed only slight change; however, at three times the manufactures rate, the pH value of both Nicosulfuron + Atrazine and Dimethylammonium Acetate still showed no significant change. The percentage of sand, clay and silt in the soil samples of the treated and control plot were within the range of 90–92% (sand), 4-5% (silt) and 2-3% (clay), respectively.

The increase in the mineral content of the herbicide treated soils correlated with the work of Tahar et al. [22], who stated that most chemical properties are higher in herbicides treated soil than that of the untreated soil.

## 4. Conclusion

This study has shown that the application of herbicides to soils results in a slight reduction in pH. Application of Dimethylammonium Acetate herbicides to soil samples resulted in slight changes in soil minerals, whereas treatment of soil samples with Nicosulfuron + Atrazine caused a reduction in soil minerals. This study has also shown that applying Dimethylammonium Acetate causes a noticeable increase in dehydrogenase activity and microbial biomass carbon content while applying Nicosulfuron + Atrazine increased dehydrogenase activity and decreased microbial biomass carbon.

## Author Contributions

All authors contributed equally to this work. They all read and approved the last version of the manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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