



Atrazine-induced alteration in soil microbial activities and plant nutrient uptake efficiency in the soil

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Abstract

The use of herbicides such as atrazine has gained wide acceptance by farmers because of their ability to control numerous weeds, which has led farmers to apply it without considering their recommended dose. This work aimed to assess its effects on nutrient status, nutrient uptake by maize plants and microbial activities as indicators of soil health. The soil nutrient status, nutrient uptake by plants, soil enzyme activities and microbial population in the soil were determined via the standard method. The results of the analysis revealed no significant difference in the nutrient status of the soil or nutrient uptake by the plants compared with those of the control. The average P content of the soil was greater (7.12 ppm) in the soil treated with the recommended dose and lowest (1.45 ppm) in the plot sprayed with two times the recommended dose. The results of the enzyme activity assays revealed an increase in amylase activity in the soil treated with twice the recommended dose, with an amylase activity of 28.2 µg glucose/g soil, compared with that of the other treatments. The lowest concentration was observed in the soil without any herbicide treatment (12.8 µg glucose/g soil). A similar trend was observed in the phosphatase activity of the soil, with the highest phosphatase activity of 13.20 µg p-nitrophenylphenol/g soil recorded in the soil after the recommended dose was doubled. The results of these studies revealed that atrazine had a relatively small effect on the soil and plants when the recommended dose was applied.

Introduction

A significant constraint to agricultural production, which represents a substantial cost to agriculture globally, is weed control. Crop losses from weed interference have a significant effect on the net returns of producers (Soltani *et al.*, 2017). Globally, weeds cause crop losses of billions of dollars annually, with the global cost of controlling weeds running into billions of dollars (Kraehmer and Bauer, 2013). The most common method of weed control in sub-Saharan Africa, including Nigeria, is manual weeding; this method is backbreaking, labor intensive, expensive and strenuous and offers little hope for expanding farm size. Approximately 25–55% of the total cost of production is spent on labour and weeding operations (Ekeleme, 2009); these operations alone consume time and money and

thus tend to limit crop production activities in Nigeria. As a result, the use of chemical weed control with herbicides has become prominent; however, its indiscriminate use with respect to the appropriate dosage has several potential problems, such as environmental pollution, soil fertility, water bodies, humans, soil microbial diversity, crop safety and overall soil health.

Conventional agriculture relies heavily on the use of agrochemicals to control weeds and pests to increase crop yield and feed the growing population (Clemence *et al.*, 2019). While the effects of fungicides and insecticides on soil organisms have been reported (Paoletti *et al.*, 1998), very little is known about the impacts of herbicides on soil health (Zaller *et al.*, 2018). Several laboratory studies have shown that various nontarget organisms are affected (van Hoesel *et al.*, 2017). Diverse populations of microorganisms are needed for plant health, growth and productivity (Zarraonaindia *et al.*, 2015), and

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unfortunately, the activities and populations of these organisms are affected by the excessive use of herbicides. The main issue with the overuse of herbicides is that they may have unintended consequences for soil microorganisms (organisms) (Mijangos *et al.*, 2009). While herbicides can be crucial for weed control in agriculture, it can have significant impacts on both the chemical and biological properties of soil. These impacts can range from altering soil pH and nutrient availability to affecting microbial populations and enzyme activities. The various impacts that have been documented on the biology and ecology of rhizosphere microorganisms, as well as their interactions with plant roots and the area of soil that surrounds them and is influenced by plant roots, are a source of concern (Kremer and Means, 2009). Foods that have been frozen, dried, or processed may still have residues for a year or longer (EFSA., 2009). Animals and people absorb pesticide residues from water and plant products, which they then expel in their urine and feces. Major obstacles, such as the careless use of herbicides, must be considered in strategies to feed the expanding population of sub-Saharan Africa (Powles and Shaner, 2001). While some herbicides and formulations have been approved by regulatory bodies worldwide, concerns about their effects on humans and the environment persist (Pochron *et al.*, 2022). The German Federal Institute for Risk Assessment toxicology review in 2013 revealed that the available data are contradictory and far from convincing with respect to correlations between exposure to herbicides and the risk of developing non-Hodgkin lymphoma (NHL) and other malignancies. Jie *et al.* (2021) noted that the application of herbicides led to a reduction in soil microbial activity, and ÁlvarezBayona *et al.* (2022) discovered traces of herbicides in drinking water.

Atrazine, (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), is one of the selective herbicides commonly used in south western Nigeria. It is a chlorinated systemic herbicide used to control weeds in crops like maize and other crops (Fabria, *et al.*, 2017). It can persist in the soil for long time with an average half-life of 13 to 261 days in the soil (Jiangwei *et al.*, 2019). It has shorter half live in microbial active soil than sterile soil. The use of atrazine enhances weed control which will increase economic gains of farmer but its hazardous effect to

non-target organism endangers some essential soil organisms (ÁlvarezBayona *et al.* 2022). The short term and long term toxicity of this herbicide and its effects on essential soil organism have lead Several researchers to investigate the short and long term impact of atrazine on soil microbe, however the results have been inconsistent, while some reported positive response (Yang *et al.*, 2023) others reported negative response (Denis *et al.*, 2023), This calls for more research on the role of different doses of atrazine on soil microbial activities and uptake of nutrients by plants. Hence this study was conducted to determine the right dosage that will minimize these negative effects of atrazine and enhance nutrient uptake in the plant.

Materials and Methods

Experimental location and design

The experiment was conducted at the screen house section of the Institute of Agricultural Research and Training Moor Plantation, Ibadan. The experimental set up was allowed to stand for eight week in a controlled environment. An atrazine-based herbicide commonly used in southwestern Nigeria was used for this study. The soil samples were sprayed with atrazine herbicides via three spraying regimens—the recommended dose (3 l/ha), below the recommended dose (1.5 l/ha) and twice the recommended dose (6 l/ha) while unsprayed soil samples served as controls. Five kilograms of the spiked soil was weighed into plastic pots and used for the screen house studies. The experiment was conducted via a completely randomized design with three replicates. The impacts of the herbicide on microbial activities, the chemical composition of the soil and nutrient uptake by the plant were analysed.

Sample collection

Soil samples were collected at the beginning and end of the experiment and transferred to the laboratory immediately for microbial analysis. The chemical components of the soil as well as plants tissues were also analysed.

Determination of the chemical components of the soil.

The physical and chemical analyses of the soil samples were carried out via standard operating procedures. Standard techniques were used to measure the chemical properties of the soils, including the pH in a 1:2.5 soil/water suspension via

a pH meter, the amount of organic carbon via the Walkley–Black method (Allison, 1965), the amount of total nitrogen using the Kjeldahl method (Bremner and Mulvaney, 1982), and the amount of available P via the Olsen et al. (Olsen *et al.*, 1954) method. The exchangeable K was determined using 1 M NH₄OAC (pH 7.0) and then measured via a flame photometer (Jackson, 1958).

Effects of the treatments on soil microbial activities

The soil samples collected were analysed for the following microbial enzyme activities:

Amylase activity: 1.5 ml of toluene was added to 5g of the sample in a 50 ml Erlenmeyer flask. The mixture was then gently shaken and left to stand for 15 minutes before being mixed with 10 ml of distilled water and 5 ml of a 2% soluble starch solution. Following Dinder et al. (2015), amylase activity was assessed.

Dehydrogenase activity: The dehydrogenase activity of the soil samples was assessed. The TTC reduction technique developed by Dinder et al. (2015) was employed. A fresh soil sample weighing one gram was placed in a test tube and mixed with 0.1 g of calcium carbonate (CaCO₃) and 1 ml of 1% TTC solution. After shaking, the mixture was sealed with a rubber stopper and incubated at 30°C for 24 hours in an incubator. Three replicates were maintained for each sample. The resulting slurry was transferred to Whatman filter paper No.1 and extracted using successive aliquots of concentrated methanol. The filtrate was adjusted to a volume of 50 ml with methanol, and the optical density was measured at 485 nm using a Hitachi spectrophotometer (220), with the methanol extract used as a reference.

Phosphatase activity: Hundred ml (100 ml) conical flasks containing five grams of air-dried soil were filled with 1.5 ml of toluene, which was then mixed thoroughly by shaking before being left to stand for 15 minutes. First, 5.0 ml of 0.013 M disodium phenyl phosphate in Tris-HCl buffer (pH 7.0) and 10 ml of 0.1 M Tris-HCl buffer were added. The flask was sealed and left at 37°C for three hours. The phosphate activity was then determined using Dinder et al. method (Dinder *et al.* 2015),

Soil respiration: Subsamples (50 g) were weighed and adjusted to 60% of the water holding capacity in a plastic tube and placed at the bottom of a Durham

bottle; 25 ml of 0.05 N sodium hydroxide was pipetted and suspended on the soil samples to trap evolved CO₂, which was subsequently incubated at 30°C for seven days. After incubation, the bottles were opened, and beakers containing the NaOH solution were emptied into the jar. Five-millilitre barium chloride solution (0.5 M) and several indicator drops were added. The CO₂ evolution was then determined by titrating the solution with HCL (0.05 M) under continuous stirring until the color changed to colorless (Alef and Nannipieri, 2013)

Determination of the microbial population

The bacterial and fungal populations were determined using plate count method. The samples were serially diluted and plated on nutrient agar and potato dextrose agar and incubated for 24–48 h. The bacterial population was determined on nutrient agar after 24h of incubation using via a colony counter, while the fungal population was determined after 48h of incubation. The results were recorded as colony forming units per ml (Cfu/ml).

Effects of atrazine on nutrient uptake by plants (maize): Nutrient uptake by maize plants was determined using the method of Gaije et al. (2020). Data were also analyzed using Analysis of Variance (ANOVA) to determine significant differences using SPSS. Post-hoc tests (Tukey's HSD) were used to compare mean differences at $p < 0.05$ significance level.

Results

Effects of atrazine on soil chemical properties

The chemical properties were not significantly different from those of the control. The average P content of the soil was greater (7.12 ppm) in the soil treated with the recommended dose and lowest (1.45 ppm) in the plot sprayed with two times the recommended dose. A similar trend was observed for the K content of the soil and other parameters, except for some micronutrients. The results of the determination of the soil chemical properties are presented in Table 1.

Effects of atrazine on soil enzyme activities

The effects of atrazine on soil enzyme activities included an increase in the activities of some enzymes such as amylase, dehydrogenase and phosphatase activities. Amylase activity was highest in the soil treated with twice the recommended dose with an am-

Table 1: Effects of atrazine on some soil nutrients

Treatment	pH	Ca	Mg	Na	K	H ⁺	CEC	Av. P	Total N	Total OC	Cu	Zn	Fe	Mn
		cmol/kg						mg/kg	%		mg/kg			
Pre-cropping soil	5.50	2.61	1.97	0.24	0.47	0.14	5.43	4.57	0.50	5.00	3.00	2.30	21.20	21.00
Twice recommended dose	5.20	4.18	2.27	0.13	0.32	0.15	7.05	1.45	0.42	3.81	1.20	3.40	19.80	19.90
Half recommended dose	6.90	2.27	1.44	0.43	0.59	0.06	4.80	5.46	0.69	6.57	4.90	5.60	31.10	16.50
Recommended dose	7.78	6.45	3.07	0.48	0.62	0.02	10.65	7.12	0.72	6.78	3.20	1.10	22.20	21.30
No herbicide	5.60	2.60	1.98	0.25	0.48	0.15	5.46	4.60	0.66	5.00	3.50	2.40	21.30	19.00

ylase activity of 28.2 µg glucose/g soil compared with the other treatments. The lowest concentration was observed in the soil without any herbicide treatment (12.8 µg glucose/g soil). A similar trend was observed in the phosphatase activity of the soil, with the highest phosphatase activity of 13.20 µg p-nitrophenyl-phenol/g soil recorded in the soil with two doses and the lowest activity in the precropping soil, with a phosphatase activity of 9.05 µg p-nitrophenyl-phenol/g soil. The same trend was observed for the soil dehydrogenase activity. Compared with those of the control,

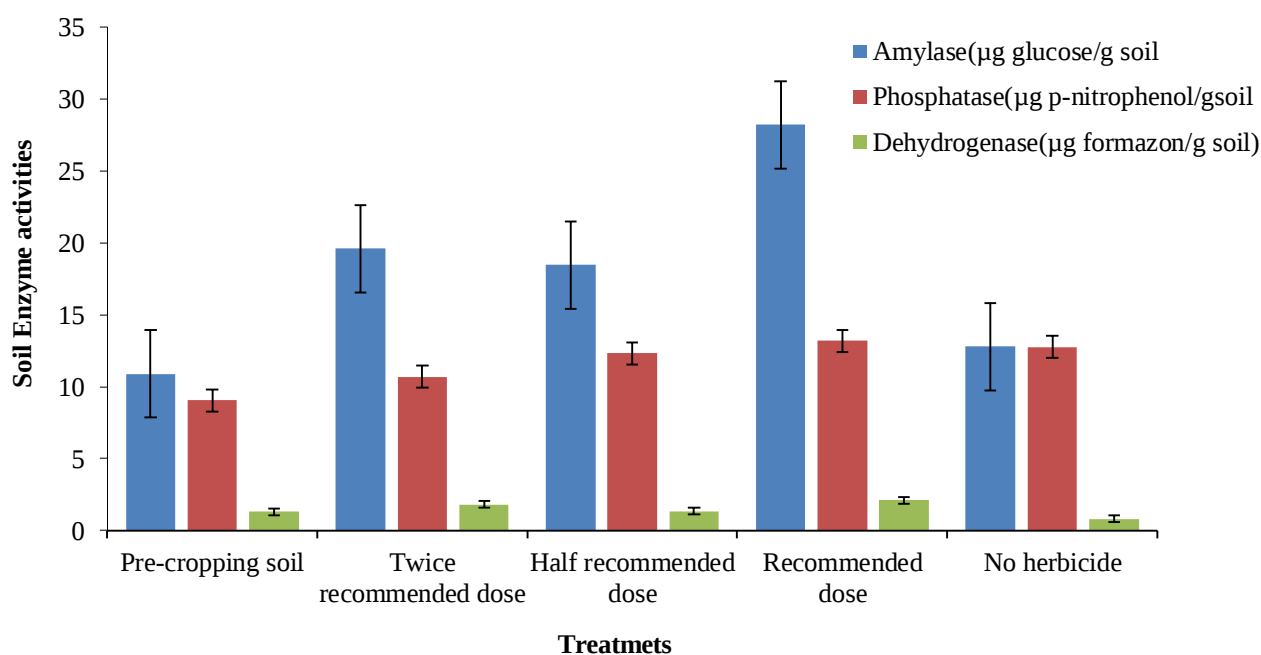
Effect of the Atrazine on soil respiration and microbial counts

The result of the effects of different doses of atrazine on the soil respiration and microbial population is presented in Figs. 2 and 3, respectively. The results of soil respiration showed a significant increase in the treated soil when compared with the

control. Though there was no significant difference in soil respiration rate among the treated soil but the soil treated with half of the recommended dose showed slight increase when compared to others. Similar trend were observed on soil microbial counts which also showed increased bacterial and fungal count in the treated soil.

Effects of atrazine on nutrient uptake by a plant (maize)

The effects of the herbicides on the uptake of nutrients by maize plants were also evaluated, and the results are presented in Table 2. The uptake of nutrients by maize at different doses of atrazine did not significantly differ from that of the control. The percentage uptake of iron (Fe) was greater in the soil without herbicide (0.0521%) than in the soil with two doses (0.0032). The uptake of nutrients such as Fe, Zn and P slightly decreased in the treated soil compared with that in the untreated soil.

**Fig 1: Effects of atrazine on enzyme activities in the soil**

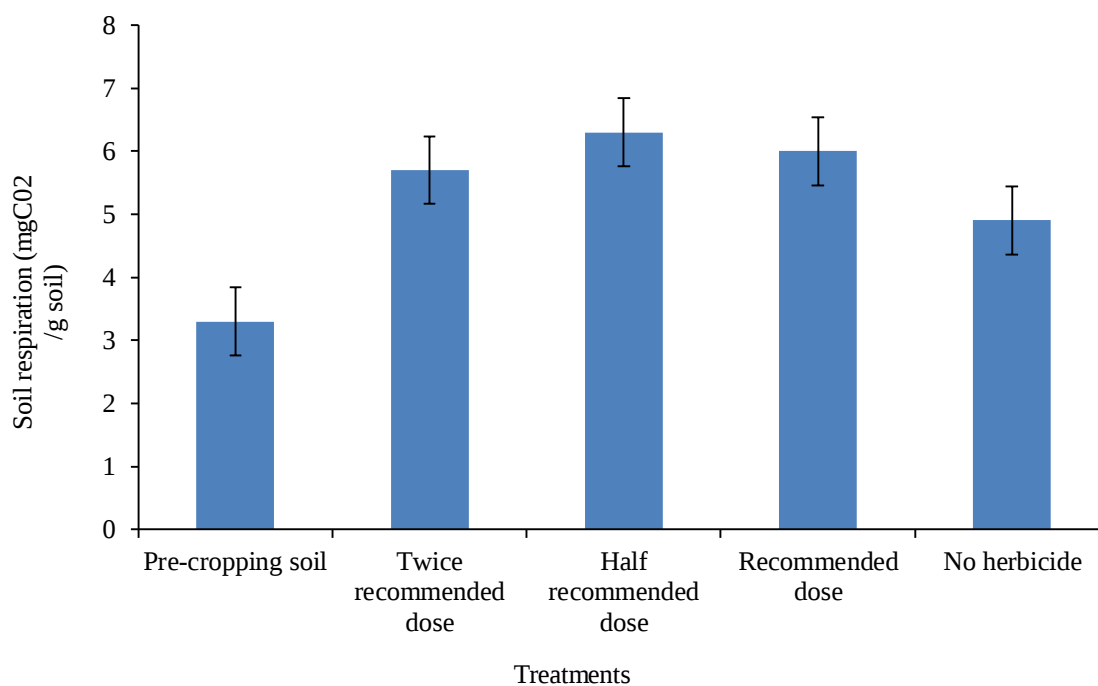


Fig. 2. Effects of atrazine on soil respiration

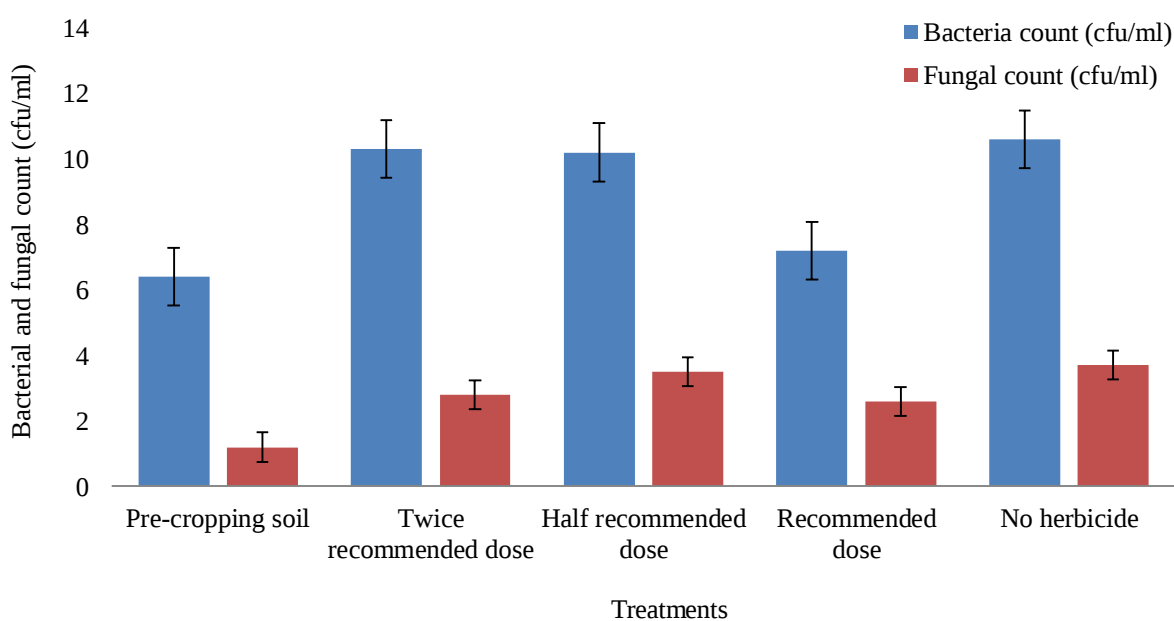


Fig. 3: Effects of atrazine on soil bacterial and fungal count

Table 2: Effects of atrazine on nutrient uptake by plants (maize)

Treatments	% Fe	% Zn	% Mn	% Cu	% Ca	% Mg	% P
Recommended dose	0.0424±0.01 ^a	0.0065±0.25 ^a	0.0572±0.04 ^a	0.0061±0.35 ^a	0.0635±0.91 ^a	0.0043±0.00 ^a	0.0188±0.28 ^a
Twice recommended dose	0.0088±1.20 ^{ab}	0.0073±0.07 ^a	0.0520±0.28 ^a	0.0012±0.76 ^b	0.0632±0.53 ^a	0.0040±0.00 ^a	0.0197±0.77 ^a
Half recommended dose	0.0032±0.58 ^b	0.0044±0.42 ^b	0.0556±1.05 ^a	0.0028±0.35 ^b	0.0614±0.77 ^a	0.0026±0.01 ^a	0.0214±0.32 ^a
No herbicide	0.0521±0.31 ^a	0.0082±0.92 ^a	0.0638±2.11 ^a	0.0122±1.26 ^b	0.0585±0.81 ^a	0.0052±0.52 ^a	0.031±0.02 ^a

Means with the same letters within a column are not significantly different ($P \leq 0.05$).

Discussion

The challenges of weed management have led farmers to resort to the use of herbicides. This enables them to control the effects of weeds on the crop. Atrazine, which is a pre-emergence herbicide commonly used to control broadleaf weeds and some annual weed species, is a photosynthetic inhibitor that binds to the plastoquinone protein binding site (Williams *et al*, 2010). The findings of this research showed slight effect of atrazine on the soil pH. The slight variation in pH may be attributed to the dissociation ability of the components of the herbicide. This is in line with the findings of Tundararo-Aherobo and Ataikiru (2020) who reported no significant effect of atrazine on the soil pH and it is in contrast with the findings of Jafaru *et al* (2024) who reported a significant increase in soil pH in the soil treated with different dosage of atrazine. He also reported that the dissociation ability of herbicide was responsible for the variation in soil pH depending on the condition of the soil. The finding also differs from that of Sebiomo and Banjo (Sebiomo and Banjo, 2020) who observed increased pH, potassium, magnesium, calcium, organic matter, percentage organic matter and total nitrogen when compared with control. The results of the exchangeable ions such as Na, Ca and Mg were also not affected by the atrazine application when compared with the control and this might be due to previous exposure of the soil to atrazine. It is also consistent with the findings of Tundararo-Aherobo and Ataikiru (2020) and differs from that of Jafaru *et al* (2024). The total organic carbon content of the soil was lower on the soil with twice the recommended dose of atrazine and this agrees with that of Tundararo-Aherobo and Ataikiru *et al.*(2020) who reported a higher total organic carbon at a lower concentration of Atrazine in the soil. He also reported that the persistence and impact of atrazine depends on the soil organic matter because it aids its disperse. The lower organic matter on soil with high dosage of herbicides may be as a result of more active components of the inorganic fraction of the herbicide at a higher dosage. The findings is in line with the report of Jafaru *et al* (2024), Denis *et al* (2023) who reported a significant increase in organic carbon at higher concentration of atrazine. Though there is no significant effect of the herbicide on the P-content, the finding is in contrast with that of Sebiomo *et al*, (2020) who also reported a significant increase in P-content at different concentrations of Atrazine, this might be as results of persistence atrazine application which residues has accumulated on soil over time as well as the dissociation ability of the component of the herbicide.. In our findings also, Atrazine had slight effect on the total nitrogen and this might also be as a result of the consistent use of herbicide on the soil. The slight variation noticed may be as a result of interference of nitrogen cycle by the components of atrazine by affecting Plant-microbe interaction as well as nitrogen transformation.. This results is in line with the findings of Tundararo-Aherobo and Ataikiru (2020) and in contrast with that of Jafaru *et al*,(2024). The results of soil micronutrients revealed no significant effect of the atrazine application, this might be as results of relative abundant of these micronutrients in south western part of Nigeria and also continuous use of herbicide on studied soil. The results of this research revealed an increase in enzyme activity in the soil treated with herbicides but reduced activity in the soil not treated with herbicide, which shows that the application of atrazine to the soil triggers microbial activities and enhances the degradation and mineralization of starchy components in the soil. The higher activity in the soil with twice the recommended dose may be a result of the organisms trying to increase its activity to break down excessive herbicides. This result contrasts with the findings of Paul *et al.* (2023), who reported a decrease in the microbial population and activities as a result of atrazine application. He reported that the decrease might be a result of the lysis of the microbial cell by the herbicide, which altered the microbial load. Hatti *et al.* (2024) reported an improved microbial population after days of application, possibly because of the ability of the organisms to degrade the herbicides and utilize them as nutrient sources. Researchers have also reported that the application of herbicides causes lysis of microbial cells and alters the quantity of the microbial population (Baboo *et al*, 2013). However, the impacts of herbicides are usually short-lived, and temporal reductions in the microbial population have been reported. Similar scenarios were reported by Ramesh and Nadanassababady (2005). Our results agreed with those of Emurotu and Anyanwu (2016), who reported that the concentration of herbicides influences the microbial population and its activities. A 2023 meta-

analysis revealed that atrazine application significantly increased soil microbial biomass and respiration but decreased microbial diversity and Enzyme activities (Tudararo and Ataikiru, 2020), this is slightly similar to the result of our soil respiration which showed an increase in soil respiration on the soil treated with atrazine. This might be as a result of increased microbial metabolic activities resulting from addition of atrazine. The quest for the soil microbes to utilize atrazine as carbon or nitrogen source might have increased soil respiration in the treated soil. These findings suggest that atrazine's impact on soil health is complex and may vary based on the soil condition, other environmental conditions and application rates.

The uptake of nutrients by the maize plants was not significantly different, although there was a decrease in some nutrients in the soil sprayed with herbicides when compared the one without herbicides. This is slightly different from the findings of Paul et al. (2023), who reported an improved uptake of nutrients by crops sprayed with herbicides. He reported the maximum utilization of nutrients by weeds rather than crops as a result of a high degree of weed competition. Tollenaar et al. (1997) reported that more nutrients are available for crops in weed-free conditions, which leads to improvements in nutrient uptake and yield. Peeyush et al. (2024) also reported that the application of atrazine at a concentration of 0.25 kg/ha on maize farms improved nutrient uptake by plants, resulting in high stock yield. In contrast with our findings, Pratap et al. (2021) reported greater nutrient uptake in herbicide-treated soil than in the control. These research results have shown that atrazine has a relatively no effect on soil and plants when the recommended dose is applied. This study will serve as a guide for farmers in deciding the right dose of herbicides to apply. This study provides reasons to adhere to the recommendations to maintain soil health and enhance crop yield and food safety

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