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EFFECTS OF SELECTED HERBICIDES ON SOIL BENEFICIAL BACTERIA AND DEHYDROGENASE ACTIVITY

[1]Toluwani Samuel Faith, *[1]Olusegun Richard Adeoyo, [2]Abiola Olanike Adesina, [1]Marcus Oluyemi Bello, [1]Kehinde Tope Adegbehingbe and [1]Timothy Olubisi Adejumo

[1]Department of Microbiology, Adekunle Ajasin University, P.M.B. 001, Akungba-Akoko, Ondo State, Nigeria.
[2]Department of Chemical Sciences, Adekunle Ajasin University, P.M.B 001, Akungba-Akoko, Ondo State, Nigeria.

*Corresponding authors' email: olusegun.adeoyo@aaua.edu.ng

ABSTRACT

Herbicides are weeds control agents but their misuse can negatively affect soil beneficial microorganisms and dehydrogenase activity. This study evaluated the effects of herbicides on soil bacterial populations and dehydrogenase activity (DHA). Glyphosate, isopropylamine, paraquat dichloride, atrazine, and dimethylamine salt were employed. An 8000 g composite soil sample was collected using soil a soil auger from 10 different points on the botanical farm of Adekunle Ajasin University, Akungba-Akoko with no herbicide application for at least five years. The physicochemical properties of the soil were determined using standard methods. Each 500 g soil sample was treated weekly with varying herbicide concentrations [manufacturer's specification (X): ½X, X or 2X] for 21 days. Bacteria were isolated and identified using cultural, morphological, and biochemical characterization techniques. DHA was measured using a spectrophotometric assay and all data were subjected to analysis of variance (ANOVA). The findings revealed that untreated soil had the highest bacterial counts (14.67x106±0.882 CFU/mL), while glyphosate, isopropylamine, paraquat dichloride, atrazine, and dimethylamine salt exhibited significant reductions to 4.33x106±0.333, 3.67x106±0.333, 4.33x106±0.333, 3.67x106±0.667, and 4.33x106±0.333, respectively after 21 days of treatment. Similarly, glyphosate, isopropylamine, paraquat dichloride, atrazine, and dimethylamine salt reduced soil DHA to 11.02, 13.66, 22.94, 17.78, and 9.30 µg TPFg-1h-1, respectively compared to untreated soil (31.75 µg TPFg-1h-1). The results contribute to a broader understanding of herbicide-soil interactions and highlight how herbicide misuse can negatively affect soil health. Therefore, farmers are encouraged to always follow manufacturer's specification, avoid prolong herbicides usage, and adopt practices that promote overall soil health.

Keywords: Dehydrogenase activity, Herbicides, Plant growth-promoting traits, Soil bacteria

INTRODUCTION

Herbicides are chemical substances used to kill or control unwanted weeds and plants. The mechanism of their action involves disrupting essential biological processes in weeds and plants, such as photosynthesis or amino acids synthesis, ultimately leading to plant death. Despite their effectiveness in weed control, herbicides can negatively impact the environment, especially soil and its beneficial microorganism (Sebiomo *et al.,* 2011; Zain *et al.,* 2013; Adomako & Akyeampong, 2016). Over the past few decades, a number of herbicides have been employed as pre-emergent and post-emergent weed eradicators in various countries. Herbicides offer clear advantages in labour efficiency and productivity, their excessive and often indiscriminate use raises concern about long-term ecological consequences. Environmental sustainability is seriously challenged by the possibility of pesticide residues persisting in soil and water, as well as by off-target movement through leaching, runoff, and drift (FAO, 2019). The awareness of these herbicides use has also grown due to their widespread application by farmers locally and globally. Bacteria live in the soil, support plant growth, and maintain the ecological balance. Soil bacteria contribute to in nitrogen fixation, nutrient uptake through organic matter decomposition, suppression of plant diseases and pests, and improvement of soil structure and water retention. Examples include *Rhizobium* (Fahde *et al.,* 2023), which forms a symbiotic relationship with leguminous plants, *Bacillus* and *Streptomyces* also function as pant growth promoting rhizobacteria (PGPR) and biocontrol agents (Adeoyo, 2019; 2025).

A valuable indicator for detecting the deleterious impact of herbicide treatments on the soil microbial biomass is soil dehydrogenase activity (DHA). Dehydrogenases are a class of enzymes present in all live microbial cells, supporting critical oxidation-reduction events required in energy metabolism (Liu *et al.,* 2017). DHA represents the integrated activity of a wide range of enzymes and is subject to changes in environmental circumstances, including the presence of stressors such as herbicides. Reductions in DHA imply a drop in microbial biomass, metabolic activity, or both, giving a convenient indicator for monitoring the potential toxicity of herbicides and other environmental stressors (Kaur and Kaur, 2021). Dehydrogenase enzymes produced by these microorganisms are involved in the process of respiration, which generates energy for the cells. Generally, DHA is often used as an indicator of soil microbial activity and overall soil health. Higher DHA is typically associated with greater microbial biomass and activity (Sebiomo *et al.,* 2011; Pertile *et al.,* 2020; Siddagangamma *et al.,* 2021).

Several studies have investigated the impact of herbicides on soil microbial communities. For instance, Hernandez (2025) found that glyphosate reduced bacterial population of *Bacillus, Bradyrhizobium, Devosia, Phenylobacterium, Pseudomonas, Rhizobium, Sphingomonas,* and *Stenetrophomonas*. Pyroxasulfone" had been shown to reduce the diversity and abundance of soil bacteria (Yu *et al.,* 2024). Higher concentrations of herbicides had been reported to lowering of microbial counts when compared to recommended concentrations (Ayansina & Oso, 2006). Some authors observed decline in the abundance of actinobacteria

population, which are important groups of beneficial bacteria due to glyphosate (He *et al.,* 2023). Sulfosulfuron was observed to have a reducing effect on *Bacillus* and *Streptomyces* (Dennis *et al.,* 2018), while diuron has shown to exert strong negative effect on plant growth-promoting rhizobacteria (PGPR) (Vacheron *et al.,* 2013; Renoud *et al.,* 2022). Similarly, a post-emergent, broad-spectrum herbicide bispyribac-sodium has been reported to negatively affect DHA (Mathiyalagan *et al.,* 2015; Srividhya *et al.,* 2020). The herbicide glyphosate had a negative impact on both soil microbial biomass and soil DHA in a wheat field (Pertile *et al.,* 2020; Mei *et al.,* 2024). Soil microbial diversity and soil enzyme activities under inorganic input sources on maize and rice ecosystems (Bharathi *et al.,* 2024) have asserted soil diversity.

PGPR generally enhance plant development through nitrogen fixation, phosphate solubilization, and the production of vital phytohormones like indole acetic acid (IAA) (Adeoyo, 2019). Root exudates are essential because they provide carbon sources that enhance bacterial colonization and activity, resulting in more productive and healthy plants (Chen & Liu, 2024). These bacteria also provide nutrients to crops, promote plant growth through hormone synthesis, control or inhibit plant pathogen activity, improve soil structure, and even bioaccumulate or microbially leach inorganics (Sun *et al.,* 2024). The healthy relationships between plants and bacteria in the rhizosphere are essential to the production of sustainable crops. During nutrient transformation, mobilization, and solubilization of nutrients from limited soil pools, plants are able to absorb essential components and develop to their full genetic potential (García-Berumen *et al.,* 2025). Biological methods are increasingly being considered for use in addition to chemical fertilizers/herbicides in order to boost crop yields in integrated plant nutrient management systems,

PGPR operate through three main mechanisms: i) aiding plants in synthesizing specific compounds, ii) facilitating nutrient uptake from the soil (Etesami and Adi, 2020), and iii) mitigating or preventing plant diseases (Vocciante *et al.,* 2022). The exact ways through which PGPR enhance plant growth and yield across various crops are still being elucidated (Bhat *et al.,* 2020), the potential explanations include the following: Symbiotic nitrogen fixation; production of plant hormones like IAA (He *et al.,* 2024) and cytokinins (Sosnowski *et al.,* 2023); synthesis of enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces ethylene levels in developing plant roots (Gamalero *et al.,* 2023); antagonism against phytopathogenic bacteria through the production of siderophores (Azeem, 2020; Adeoyo, 2025); production of water-soluble B-group vitamins such as niacin, thiamine, riboflavin, and biotin (Barghavi *et al.,* 2024); enhanced resistance to various abiotic stresses like drought, salinity, waterlogging and oxidative stress (Zulfiqar and Ashraf, 2023); and solubilization and mineralization of nutrients (Rawat *et al.,* 2021).

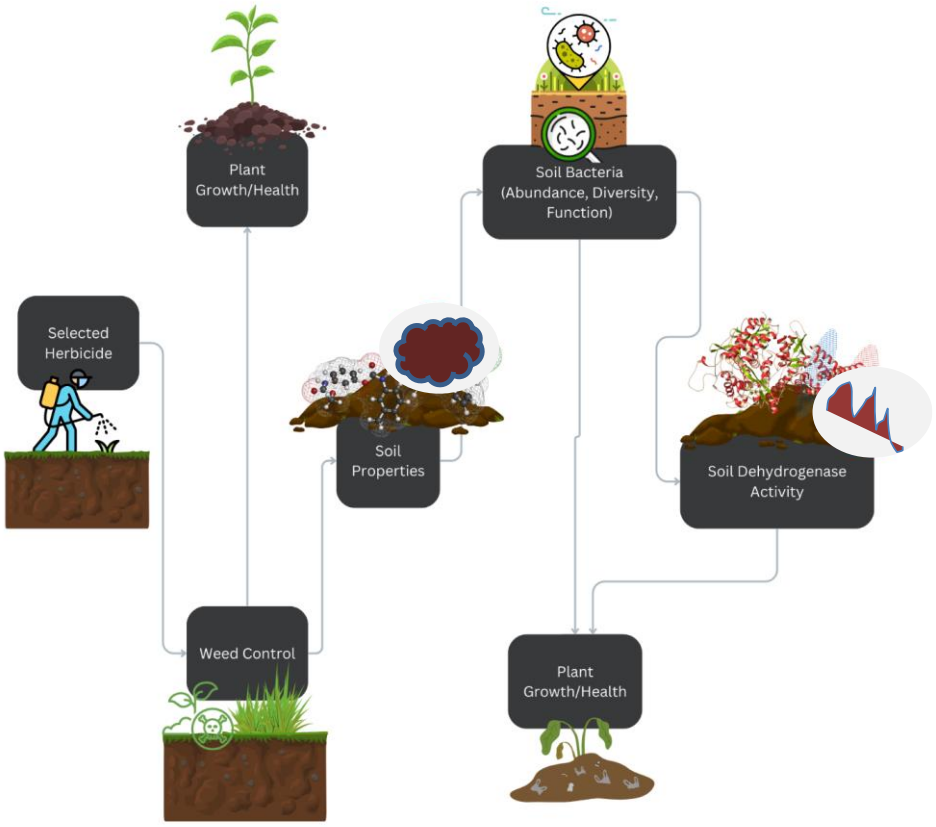

Figure 1: Conceptual framework elucidating the effects of herbicide on soil bacteria and dehydrogenase activity

Herbicides can have significant negative impacts on both beneficial bacteria and soil DHA in agriculture. Such effects may have implications for soil health, crop productivity, and ecosystem function (Figure 1). Therefore, it is important to carefully manage the excessive use of herbicides in agricultural systems and to explore alternative approach such as the use of biofertilizers that minimizes negative impacts on soil biodiversity and function. It is imperative to note that the practice of applying herbicide in keeping out weeds is good; the problem posed by its excessive use/misuse not just on

abiotic environment but also on soil beneficial bacteria is a call for concern. Moreover, herbicides have potentials to interfere with soil DHA which is closely linked with microbial biomass and metabolic processes. Hence, this study determined the effects of selected herbicides on beneficial soil bacteria and DHA.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from the botanical farm of Adekunle Ajasin University, Akungba-Akoko. The study area lies between latitude 7° 22' 27' N – 7° 22' 30' N and longitudes 5° 45' 20" E - 5° 45' 35" E (Oyeshomo, 2024). A Composite soil sample was collected from ten (10) different points on botanical farm with no prior herbicide application to ensure the representativeness of the data. Top soil of up to 5 cm depth was collected using a soil auger and placed in a sterile zip lock bags to prevent contamination. The bags were labeled with the sample number, location, depth, and the date of collection. After which it was stored in sectioned trough and placed in a cool and dry place throughout the study period.

Determination of Physio-chemical Properties of Soil Samples

Organic matter (%), pH, water content (%), texture, temperature (°c), ammonium content (ppm), phosphate content (ppm), electron conductivity (ds/m), porosity (%), and organic carbon (%) were determined using the standard methods (Dandwate, 2020).

Soil Treatment with Herbicides

Three (3) different concentrations of selected herbicides were prepared and applied for period of 21 days (at 7 days interval). Three various concentrations used include; double (2X), normal (X) and half (½X) of herbicide manufacturers' recommendation. An untreated soil was used as a control. Herbicide treatments were carried out at recommended rates of 6 L/ha (at 300 mL in 10 L sprayer) for isopropylamine liquid, glyphosate, and atrazine; 3 L/ha (at 150 mL in 10 L sprayer) for paraquat dichloride; 2.24 L/ha (at 112 mL in 10 L sprayer) for 2,4-dimethylamine salt. All soil treatments were carried out in triplicates (Sebiomo *et al.*, 2011).

Bacterial Counts

Nutrient agar medium was used for count of total heterotrophic bacteria. Using a dilution factor of 10^3, after incubation, colonies were counted and the number of viable bacteria expressed as colony forming units per gram dry weight of soil (CFU/g) (Adeoyo, 2019).

Soil Bacterial Identification

Microbial analysis techniques such as cultural, morphological, and biochemical characterization were used for bacterial identification. These include Gram staining, microscopic examination, and motility test, and biochemical tests (catalase, citrate, urease, indole, H_2S, lactose, sucrose, dextrose, glucose fermentation, methyl red, Voges Proskeur, and oxidase) (Ruangpan & Tendencia, 2004).

Determination of Soil Dehydrogenase Activity (DHA)

Tris-HCl was prepared in a 50 mL centrifuge tube with a pH of 7.4, with 0.5% of Tetrazolium Bromide (TTB). To 1 g of sieved soil, 5 mL of Tris-HCl was added along with 1 mL of TTB. The mixture was incubated for 60 minutes and centrifuged at 4000 rpm for 5 minutes. DHA was measured by using a spectrophotometer at 484 nm. The assay was based on the reduction of tetrazolium salt by the dehydrogenase enzyme, which produced a colored product from lemon green color to a range of reddish brown colour. The rate of colour development is proportional to the DHA in the soil extract (Adomako & Akyeampong, 2016). The DHA of the treated and untreated soil samples were compared. A 0.03 g triphenyl formazan was dissolved in 500 mL ethyl alcohol. Then, a set of 8 solutions with triphenyl formazan concentrations of 3.0, 6.0, 9.0, 12.0, 15.0, 18.0, 21.0 and 24.0 µg/mL (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 µmol/50 mL) were prepared. Finally, the optical densities of the prepared solutions were measured (in duplicate) using spectrophotometer (Spectronic 601, Fisher Scientific, Montreal, Quebec, Canada) at a wavelength of 484 nm and plotted against the known TF concentrations (µg/mL). A blank sample was used to zero the spectrophotometer (Adomako & Akyeampong, 2016: Wang *et al.,* 2023).

Ammonia Production

Each bacterium was grown in peptone broth (10 mL) at 28°C for 48 hours. After incubation, 0.5 mL of Nessler's reagent was added to the bacterial suspension and observed for the development of brown to the yellow colour (Devi *et al.,* 2018).

Indole Acetic Acid (IAA) Production

Each nutrient broth containing L-tryptophan (100 mg/L) was separately inoculated with a bacterium and incubated at 28°C for 48 hours in the dark followed by centrifugation for 15 min, and cell-free supernatant was assayed for IAA content according to Devi *et al.* (2018),

Heamolysis Assay

Each bacterial colony was transferred to a blood agar plate (containing sheep blood). The formation of a transparent heamolytic halo (beta-hemolysis) surrounding each colony signified biosurfactant production, development of a dark and greenish hue in the agar beneath the colony was indicative of alpha-hemolysis, which is often induced by the bacterium's hydrogen peroxide, which showed partial erythrocyte destruction; gamma-hemolysis left the agar medium unchanged in terms of colour and opacity (Buxton, 2005).

Phosphate Solubilisation Assay

All bacterial isolates were screened by culturing at 30°C on a Pikovskaya medium (PVK medium). When the colonies appeared in 4 days, those with clear phosphate-solubilizing zones were recorded (Ejeagba *et al.,* 2023).

$$\text{Phosphate Solubilisation Index} = \frac{\text{Clear zone diameter (mm)}}{\text{Colony diameter (mm)}} \quad (1)$$

Data Analysis

The data obtained from the experiments were subjected to analysis of variance (ANOVA) at $p < 0.001$ level of significance and correlation coefficient analysis.

RESULTS AND DISCUSSION

Physio-chemical Properties of the Soil

Table 2 shows the physicochemical properties of the soil samples (A, B, C, D, and E). The soil texture across all samples was consistently loam. The pH values of the soils ranged between 6.57 and 6.77 (slightly acidic to neutral). Water contents varied from 41.13% to 41.87% (this shows a relatively consistent moisture level across the samples used). Organic matter contents ranged from 4.3% to 5.1% while ammonium contents ranged from 3.03 ppm to 3.47 ppm. Phosphate contents ranged from 7.03 ppm to 7.23 ppm.

Electron conductivity ranged from 0.33 dS/m to 0.37 dS/m, indicating low salinity levels across the soil sample. Porosity of the soil samples ranged from 48.5% to 48.87%, reflecting a relatively porous soil structure while organic carbon contents ranged from 3.07% to 3.23%. Overall, the physicochemical properties of the soil samples were generally similar, suggesting that the soil (composite soil sample collected from Akungba-Akoko) was relatively homogenous.

Table 1: Physio-chemical Properties of the Soil

S/N	Parameter	NA	NB	NC	ND	NE
1	pH	6.63±0.033	6.77±0.033	6.57±0.120	6.73±0.033	6.73±0.067
2	Water Content (%)	41.13±0.067	41.50±0.058	41.87±0.029	41.4±0.058	41.77±0.145
3	Texture	Loam	Loam	Loam	Loam	Loam
4	Temperature (°C)	22.17±0.088	22.43±0.033	22.73±0.133	22.27±0.088	22.2±0.115
5	Organic Matter (%)	5.10	5.03±0.033	4.87±0.067	5.07±0.067	4.30±0.153
6	Ammonium Content (ppm)	3.07±0.033	3.47±0.033	3.43±0.033	3.03±0.033	3.43±0.067
7	Phosphate Content (ppm)	7.10	7.10±0.058	7.03±0.033	7.17±0.088	7.23±0.067
8	Electron Conductivity (dS/m)	0.33±0.033	0.33±0.033	0.37±0.033	0.33±0.033	0.33±0.033
9	Porosity (%)	48.87±0.033	48.53±0.088	48.87±0.033	48.50±0.058	48.60±0.058
10	Organic Carbon (%)	3.13±0.033	3.23±0.033	3.07±0.067	3.07±0.067	3.10±0.058

Key: NA = Soil in container A, NB = Soil in container B, NC = Soil in container C, ND = Soil in container D, and NE = Soil in container E

Effect of Herbicides on Soil Bacterial Populations

The effect of the selected herbicides on soil bacterial populations was monitored over a period of 21 days, and the resulting colony counts (CFU) are illustrated in Figures 2 to 5. Bacterial populations were quantified as colony-forming units (CFU) per gram of dry soil. A consistent trend of decreasing bacterial counts was observed across all tested herbicides and their respective concentrations throughout the experimental period. Notably, atrazine and 2,4-dimethylamine Salt appeared to exhibit a less pronounced reduction in bacterial colony counts, particularly during the first week at both half and the manufacturer's recommended application rates, when compared to the effects of glyphosate and isopropylamine. Furthermore, the highest concentration of each herbicide, which was twice the manufacturer's recommended specification, consistently resulted in the lowest bacterial colony counts at each of the three weekly assessment. Figure 5 provides a consolidated view of the bacterial counts across the three weeks for all herbicide treatments.

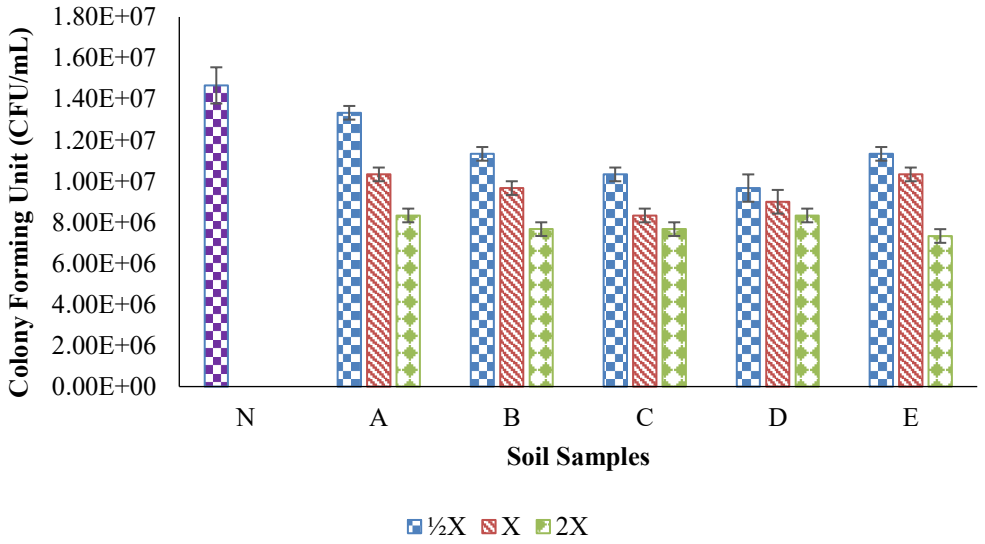

Figure 2: Colony counts of treated soil (Week 1). Keys: N = Untreated Soil Sample, A = 360g/L Isopropylamine Liquid, B = 360/L Glycosophate, C = 276g/L Paraquat Dichloride, D = 500g/L Atrazine, E = 720g/L 2,4 – Dimethylamine Salt, X = Manufacturer specification of herbicide treatment

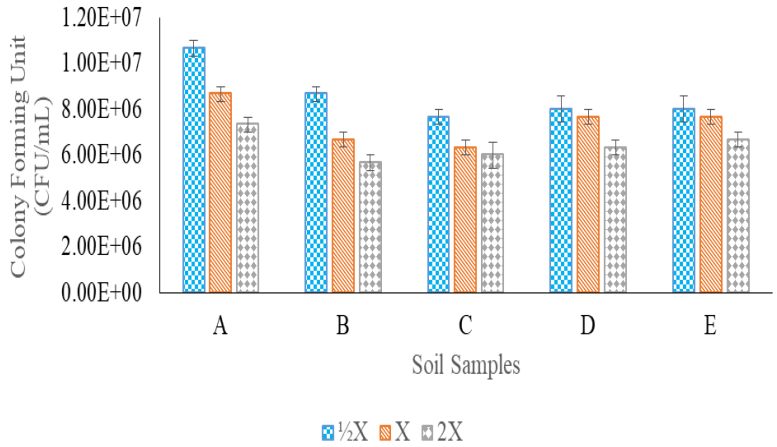

Figure 3: Colony counts of treated soil (Week 2). Keys: A = 360g/L Isopropylamine Liquid, B = 360/L Glycosophate, C = 276g/L Paraquat Dichloride, D = 500g/L Atrazine, E = 720g/L 2,4 –Dimethylamine Salt, X = Manufacturer specification of herbicide treatment

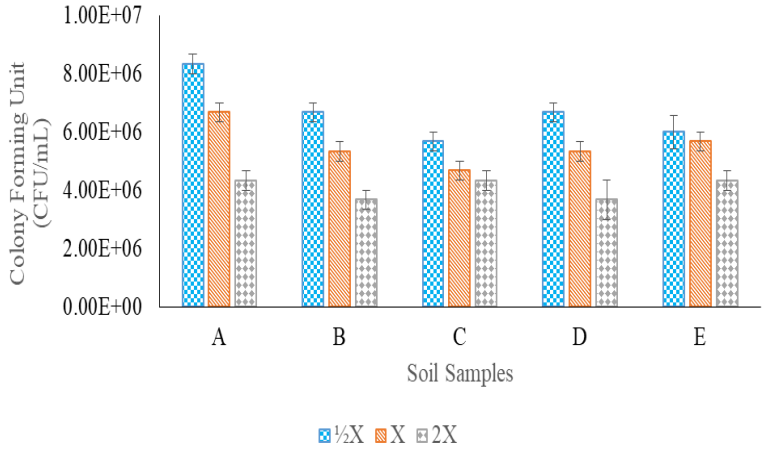

Figure 4: Colony counts of treated soil (Week 3). Keys: A = 360g/L Isopropylamine Liquid, B = 360/L Glycosophate, C = 276g/L Paraquat Dichloride, D = 500g/L Atrazine, E = 720g/L 2,4 –Dimethylamine Salt, X = Manufacturer specification of herbicide treatment.

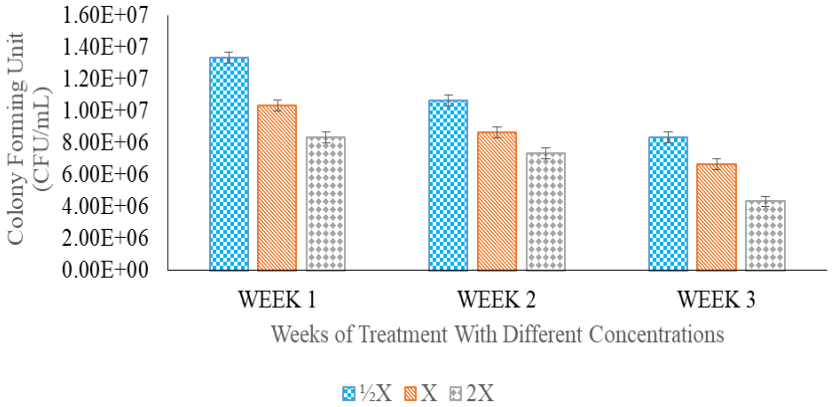

Figure 5: Colony counts within the three weeks of soil samples treatment with only 360g/L Isopropylamine Liquid. Key: X = Manufacturer specification of herbicide treatment

Identification of Bacterial Isolates

Table 2 shows the morphological and biochemical characteristics of the bacteria isolated from the soil samples. Eleven (11) bacterial genera were identified and they include the following: *Aerococcus, Bacillus, Enterobacter, Enterococcus, Klebsiella, Lactobacillus, Pediococcus, Pluralibacter, Pseudomonas, Staphylococcus,* and *Streptococcus.* The biochemical test such as catalase, citrate, urease, indole, motility, H_2S production, sugar fermentation, methyl red, Voges-Proskauer, and oxidase tests showed a wide range of metabolic capabilities among the identified bacteria. Gram staining revealed that both Gram-positive and Gram-negative bacteria were present.

Table 2: Morphological and Biochemical Characteristics of some Bacterial Isolates

S/N	Isolate code	Shape	Gram Stain	Catalase	Citrate	Urease	Indole	Motility	H_2S	Coagulase	Lactose	Mannitol	Sucrose	Dextrose	Glucose	Methyl Red	Voges-Proskauer	Oxidase	Organism
1	BH2	Cocci	+	-	+	-	-	+	+	-	+	+	+	+	+	-	-	-	*Aerococcus viridans*
2	AH4	Rods	+	+	+	-	-	+	+	-	-	-	-	+	+	-	+	-	*Bacillus cereus*
3	AN1	Rods	+	+	-	-	-	+	+	-	-	+	+	+	+	-	+	-	*Bacillus pumilus*
4	AX3	Rods	-	+	+	-	-	+	-	-	+	+	+	+	+g	-	+	-	*Klebsiella aerogenes*
5	AH1	Rods	-	+	+	+	-	+	-	-	+	+	+	-	+g	-	+	-	*Enterobacter cloacae*
6	AH5	Rods	-	+	-	+	-	+	+	-	+	+	-	-	+g	-	+	-	*Pluralibacter gergoviae*
7	AN3	Cocci	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	*Enterococcus faecalis*
8	AY1	Rods	+	-	-	-	-	-	-	-	+	+	+	+	+g	+	-	-	*Lactobacillus acidophilus*
9	EH1	Rods	+	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	*Lactobacillus plantarum*
10	AX1	Rods	+	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	*Lactobacillus* sp.
11	AX8	Cocci	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	-	*Pediococcus* sp.
12	AX2	Rods	-	+	+	-	-	+	-	-	-	+	-	-	-	-	-	+	*Pseudomonas aeruginosa*
13	AD1	Cocci	+	+	+	+	-	-	-	+	-	+	+	-	+	+	+	-	*Staphylococcus aureus*
14	AX7	Cocci	+	-	+	+	+	+	+	-	+	+	+	+	+g	-	+	-	*Streptococcus bovis*
15	AX6	Cocci	+	+	+	-	-	+	+	-	-	+	-	+	+	-	-	-	*Enterococcus faecium*
16	EN1	Cocci	+	-	+	-	-	-	+	-	+	-	+	+	+	-	-	-	*Streptococcus* sp

Key: + = positive result, - = negative result, g = gas production

Soil Dehydrogenase Activity

Figure 5 shows the soil DHA (µg TPF $g^{-1}h^{-1}$) in the treated and untreated soil samples. The DHA in the untreated soil was 31.7 µg TPF $g^{-1}h^{-1}$. All herbicide treatments resulted in a reduction in DHA. Isopropylamine at half the manufacturer's specification had the least impact (17.7 µg TPF $g^{-1}h^{-1}$) while glyphosate and paraquat dichloride at double the manufacturer's specification showed the highest reduction of 13.7 and 22.9 µg TPF $g^{-1}h^{-1}$, respectively.

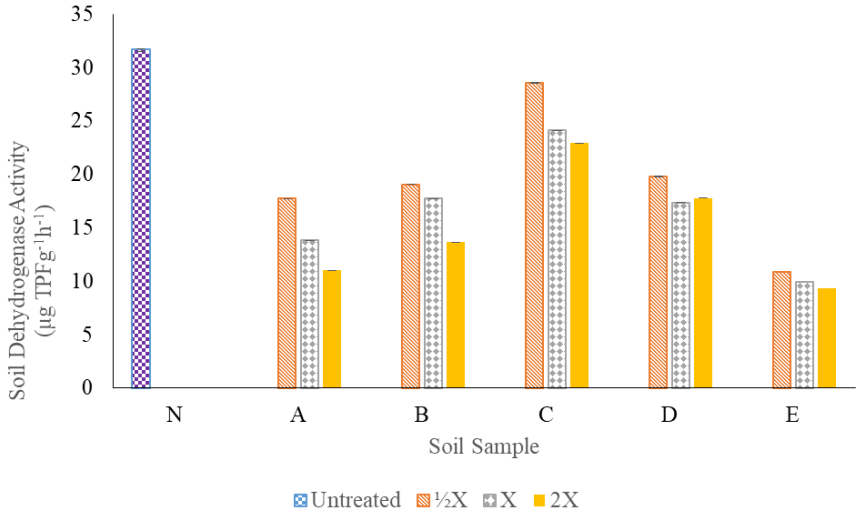

Figure 5: Dehydrogenase activity (µgTPF$g^{-1}h^{-1}$) of the treated and untreated soil samples. Key: N = Untreated Soil Sample, A = 360g/L Isopropylamine Liquid, B = 360/L Glycosophate, C = 276g/L Paraquat Dichloride, D = 500g/L Atrazine, E = 720g/L 2,4 –Dimethylamine Salt, X = Manufacturer specification of herbicide treatment

Plant Growth Promoting (PGP) Traits

Table 3 shows the results PGP traits. Several bacteria exhibited IAA production, including *Enterobacter cloacae, Pluralibacter gergoviae, Bacillus cereus, Bacillus pumilus, Lactobacillus acidophilus,* and *Lactobacillus plantarum*. Many bacteria were positive for the ammonia test while phosphate solubilization varied among the isolates, with *Enterobacter cloacae, Bacillus pumilus,* and *Lactobacillus* sp. showing high solubilization indices of 2.23, 2.15, and 1.84, respectively. Haemolysis tests revealed the presence of alpha, beta, and gamma haemolytic activities among the bacterial isolates. *Bacillus cereus, Bacillus pumilus, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus* sp showed β-haemolysis on the blood agar while only *Aerococcus viridans* (BH2) showed alpha haemolysis (Table 4).

Table 3: Plant Growth Promoting Traits

S/N	Organism	Phosphate Solubilisation (PS)			Indole Acetic Acid (IAA)	Ammonia Production	Haemolysis
		PS	Index	Strength			
1.	*Aerococcus viridans* (BH2)	+	0.67	Low	-	-	Alpha
2.	*Bacillus cereus* (AH4)	+	1.84	High	+	-	Beta
3.	*Bacillus pumilus* (AN1)	+	2.15	High	+	+	Beta
4.	*Klebsiella aerogenes* (AX3)	+	1.44	Moderate	-	-	Gamma
5.	*Enterobacter cloacae* (AH1)	+	2.23	High	+	+	Gamma
6.	*Pluralibacter gergoviae* (AH5)	+	1.45	Moderate	+	+	Gamma
7.	*Enterococcus feacalis* (AN3)	+	1.11	Low	+	+	Gamma
8.	*Lactobacillus acidophilus* (AY1)	+	1.32	Moderate	+	-	Gamma
9.	*Lactobacillus plantarum* (EH1)	+	1.44	Moderate	+	-	Gamma
10.	*Lactobacillus* sp. (AX1)	+	1.77	High	+	-	Gamma
11.	*Pediococcus* sp. (AX8)	+	-	-	-	-	Gamma
12.	*Pseudomonas aeruginosa* (AX2)	+	1.53	High	-	-	Beta
13.	*Staphylococcus aureus* (AD1)	+	0.47	Low	-	-	Beta
14.	*Streptococcus bovis* (AX7)	+	0.73	Low	-	-	Gamma
15.	*Enterococcus faecium* (AX6)	+	0.46	Low	-	-	Gamma
16.	*Streptococcus* sp. (EN1)	+	0.78	Low	-	-	Beta

Key: + = positive result, - = negative result

Data Analysis

A two-factor analysis of variance without repeated measures was conducted to test the effects of time and treatment concentrations on colony counts, and to determine if there was an interaction between these variables. The analysis revealed a significant difference in colony counts across different time points and among different treatment concentrations ($p < 0.001$). The results in this study indicated that bacterial populations changed significantly over weeks of the experiment and that different herbicide concentrations

resulted in significantly different bacterial populations. However, no significant interaction was found between time and treatment concentration ($p = 0.724$), suggesting that the effect of herbicide concentration on bacterial count was consistent across the different weeks. The analysis of soil bacterial populations revealed a consistent trend of decreasing bacterial counts across all tested herbicides, indicating a general toxic effect on soil bacteria. However, while the overall bacterial counts were significantly affected by herbicide treatments, the two-factor ANOVA showed no significant difference in total bacterial counts among the different herbicide treatments ($p > 0.01$), suggesting that the total abundance of bacteria was similarly affected by the herbicides. Notably, the concentration of herbicides played a significant role, as higher concentrations generally led to a substantial reduction in bacterial populations ($p < 0.001$), highlighting a dose-dependent effect. The correlation coefficient ranges from -1 to 1. A value of 1 indicates a perfect positive correlation, -1 indicates a perfect negative correlation, and 0 indicates no correlation. The correlation coefficient between colony counts and dehydrogenase activity is approximately 0.464, indicating a weak positive correlation.

Discussion

The results of the findings of this study showed that selected herbicides had a negative impact on soil bacterial community and DHA. The reduction in bacterial counts across all herbicide treatments indicated a negative effect of these herbicides on soil bacteria. This finding aligns with previous research demonstrating the adverse effects of herbicides on non-target microorganisms (Sebiomo *et al.,* 2011; Wang *et al.*, 2016). Soil, as a complex living ecosystem, relies heavily on the metabolic functions of its microbial community for processes like nutrient cycling and organic matter degradation. Consequently, any external stressor, such as herbicide application, that disrupts this community structure or function can have profound implications for long term soil health and fertility. The findings presented herein detail the extent of this impact, providing critical context for sustainable agricultural practices. Inherent physicochemical properties of the soil, particularly pH, organic matter content, and texture showed consistency in values obtained across all treated soil samples. The high organic carbon content strongly correlated with greater microbial biomass and enzyme activity, making soil organic matter a critical component of soil health (Sebiomo *et al.,* 2011; Cui *et al.*, 2019; Pertile *et al.,* 2020). Figures 1, 2, 3, and 4 confirmed the effects of the selected herbicides on the soil bacterial community over the observation period. This highlighted the magnitude of the initial impact and the comparative toxicity profile of the five tested herbicides. Herbicides act as selective agents, and the rapid reduction in viable cell counts (CFU) can be attributed to acute cell membrane damage and interference with enzymatic pathways.

Aerococcus, Bacillus, Enterobacter, Enterococcus, Streptococcus, Lactobacillus, Klebsiella Pediococcus, Pseudomonas, and *Staphylococcus* species were among the bacterial genera isolated and identified. Similar observation was made in the reports of Adeoyo (2019) and Qingwei *et al.* (2023). The reduction in bacterial counts across all herbicide treatments indicated a general toxic effect of these herbicides on soil bacteria, which aligns with previous research demonstrating the adverse effects of herbicides on non-target microorganisms (Sebiomo *et al.,* 2011; Wang *et al.,* 2016; He *et al.,* 2023). The observed dose-dependent effect, where higher herbicide concentrations resulted in lower bacterial counts, further supports the idea of a direct toxic effect on bacterial cells. There was no significant difference between herbicides and variations in the degree of inhibition among different herbicides may be due to differences in their chemical structures and modes of action, which can influence their interactions with bacterial cells (Gao *et al.,* 2020).

Dehydrogenase is perhaps the most critical indicator of overall soil microbial health, as it is an intracellular enzyme found in virtually all living microbial cells and is fundamentally linked to the biological oxidation of soil organic matter (Wolińska *et al.,* 2015). Dehydrogenases are essential for microbial respiration, and their activity is often used as an indicator of soil microbial biomass and activity (Liu *et al.,* 2017). The observed decrease in DHA suggested that the herbicides disrupted the physiological processes of soil microorganisms, which could have implications for nutrient cycling and soil fertility (Shi *et al.,* 2019). Differences in how herbicides affected DHA might be related to their varying effects on bacterial populations or direct inhibitory effects on the enzyme itself (Chen *et al.,* 2020). The study also revealed that the herbicides affected bacteria with beneficial potentials for the soil. Several identified bacteria are known to exhibit plant PGPR traits, such as IAA production and phosphate solubilization. The reduction in the population of these bacteria due to herbicide application could have negative consequences for plant growth and soil health. The number of these beneficial organisms was reduced in the treated soil and the reducing effect of the herbicide on colony counts subsequently led to depletion of PGPR present in the soil (Almario *et al.,* 2013; Peng *et al.,* 2019; Filimon *et al.,* 2021).

CONCLUSION

This study contributes data on the impact of these herbicides on a particular soil type and location. This adds valuable context to the existing knowledge, as herbicides can influence soil DHA and affect microbial communities. This study demonstrated that herbicides application exhibited a clear dose-dependent effect where higher concentrations lead to greater inhibition. It was noted that while overall bacterial counts decreased, specific beneficial genera such as *Bacillus* spp., *Lactobacillus* spp., and *Pseudomonas* spp., were affected. These findings underscore the detrimental effects of the tested herbicides on key components of soil health, emphasizing the need for careful consideration of their use in agricultural practice. Also, the results of this research will help to contribute to the enlightenment of farmers over the impact of excessive use of herbicides and understanding the impact of specific herbicides on soil health. Also, there is the need to pave way for sustainable agricultural practices such as the use of bio-fertilizers and bio-herbicide production. Finally, future research should focus on long-term field studies to assess the cumulative and residual effects of these herbicides and other commonly used herbicides on soil microbial community structure and functional resilience under varying environmental conditions.

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