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Unregulated Use of Glyphosate Herbicide Has Effect on the Abundance of the Microbiota and Nutrient in the Soil

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Abstract There is a growing usage of herbicides in agriculture to improve productivity, however, there is limited studies on the effect of herbicides to the soil microbiota and nutrients. This study assessed the effect of glyphosate herbicide on the abundance and activity of soil microbiota on the agricultural soil at Kauzeni village, Morogoro, Tanzania. Two groups of soil samples; 15 from soil with herbicides, and another 15 without herbicide application were assessed. Abundance of bacteria and fungi were determined using culture-based method, while soil organic matter and Nitrogen were determined using muffle furnace and Kjeldahl methods, respectively. Bacterial load in soil with glyphosate was found to be $(1.5 \pm 0.4) \times 10^5$ CFU/mL and without herbicide (2.2 ± 0.4) x10^5 CFU/mL. The fungi load in soil with herbicides was $(1.2 \pm 0.5) \times 10^5$ CFU/mL and without $(1.8 \pm 0.5) \times 10^5$ CFU/ml. The organic matter in soil with herbicide was (12 ± 7) % and without herbicide (28.3 ± 17) %, while Nitrogen in soil with herbicide was (7.0 ± 3.8) % and without (17 ± 6.6) %. ANOVA revealed significant difference in soil microbiota of soil with and without herbicides, P-value = 0.0002 and P-value = 0.0064 for bacterial and fungi, respectively. Soil organic matter and nitrogen contents were also different, p-value = 0.0023 and p-value = 0.00002 for the organic matter and nitrogen content, respectively. Glyphosate affects the abundance of soil microbiota and hence nutrient levels in the soil. There is a need to study the effect of herbicides to different types of soil and provide guideline of its use for a comprehensive management of the soil.

Keywords: *Glyphosate, Soil, Bacteria, Fungi, Organic matter, Nitrogen, Herbicide, Microbiota*

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1. Introduction

Herbicides are the most effective method of controlling weeds in agricultural activities. This method has proved to be an effective; however, it has an influence on the abundance, diversity and activity of soil microbiota, which can lead to the ecological imbalance and, hence soil infertility. The effect of continuous use of herbicide depends on the quantity and time interval of the application [1]. Some studies have shown that the effects of herbicides on microbial ecology depend on chemical type, concentrations, microbial species and environmental conditions [2,3].

The balance of the soil microbiota is vital to the functioning of the ecosystem of the soil, such as the production of nutrients and the formation of organic matter [4]. Various mechanisms are used by soil microbiota to enhance nutrient uptake, improve soil fertility and increase crop yields, such as nitrogen fixation, potassium and phosphorus solubilization, guarding plants from abiotic and biotic stresses and detoxification of belowground pollutant [5,6]. Decomposition of plants and animals in soil to produce nutrients used by plant for growth is another benefit of soil microbiota. Studies show that, microbial activity including bacteria, fungi, *Azotobacter* and phosphate solubilizing fungal population are highest when no herbicides applied in the soil [2], while in rhizospheric soil, a negative effect is reported [7], thus affecting the activity of this important nitrogen-fixing bacteria species.

Researchers have identified the persistence of herbicide residues in soil as a major threat to soil microbiota that play critical role in supporting a number of ecosystem services [3]. Glyphosate-based herbicides, which are commonly regarded as less harmful, have shown toxicity to a range of aquatic organisms and adversely affect the soil and intestinal microbiota [8]. Intensive use of glyphosate over the last 20 years led to the appearance of at least 48 glyphosate-resistant weed species worldwide [9]. Despite the occurrence of widespread resistance in weeds to other herbicides, industry is rather developing transgenic crops with additional herbicide resistance gene

[10,11]. Agricultural management based on broad-spectrum herbicides further decreases diversity and abundance of wild plants and affects arthropod fauna and other farmland animals relying on those plants [12,13,14].

While herbicide doses optimization targets the performance on killing weeds [15,16], there is lack of studies considering the effect on the soil microbiota and soil organic matter. Analysis of a range of physico-chemical parameters is a common approach to assess soil quality for agricultural production, however, inclusion of biological parameters such as the abundance, diversity and functional services of soil microbiota is critical for the ecological systems balance.

Since the soil is vital for life on Earth and its biodiversity, being a nonrenewable and a threatened resource; preserving its quality in terms of biodiversity and nutrients is crucial to maintain a sustainable agricultural productivity. In the Tanzania context, there is a gap in knowledge of a long-term effect of herbicides application on the soil microbiota and nutrients levels to the agricultural land in Tanzania. A need to bridge this gap necessitated to undertake a pilot study to assess the effect of unregulated application of the Glyphosate herbicides to soil bacterial and fungal abundance, as well as soil organic matter (SOM) and nitrogen levels to a selected agricultural land in Morogoro region, Tanzania.

2. Materials and Methods

2.1. Research Ethical Approval

This study was approved by research committee of the Department of Science and Laboratory Technology at Dar es salaam Institute of Technology with reference Ref No. LabTech/2021/11-1.

2.2. Geographical Location of Study Site

The study was done at Kauzeni village located 6◦53’48” S 37◦35’09” E near Kauzeni Primary School in Morogoro region, Tanzania where agricultural activities is intensively practiced (Figure 1).

Figure 1. Study Site at Kauzeni village in Morogoro, Tanzania located at 6◦53’48” S 37◦35’09” E. The site is used extensively for Agricultural activities. Maize and tomato are the major food crops grown

2.3. Sample Collection

We collected thirty (30) soil samples, that is fifteen (15) from the farm with glyphosate herbicide, and fifteen (15) samples in the farm without glyphosate herbicides. The samples were labelled and then transported to the microbiology laboratory in the department of science and laboratory technology at Dar es Salaam Institute of Technology for analysis.

2.4. Microorganisms’ Enumeration

18g of Mueller Hinton agar and 17.5g of Saboraud dextrose agar were measured using analytical balance and put into a separate conical flask, then distilled water was added to make a total volume of 500mL, the suspension was heated with constant stirring in order to make the media dissolve completely, the dissolved media was then autoclaved at 121˚C for 15 minutes. When the process of sterilization was complete, the conical flask was removed from the autoclave and cooled to 40˚C, and the media was poured into sterile petri dishes under sterile conditions in laminar flow chamber.

10g of soil sample was measured and put into a conical flask followed by addition of 20mL of water and shaken for 30 minutes, then, the prepared nutrient agar and petri dishes was transferred into autoclave for sterilization, 25mL of sterilized nutrient agar was put into the petri dishes, then left for 15minutes to cool and solidify, then, the soil sample was poured into the petri dishes containing the nutrient agar by streak method, the petri dishes were incubated for 24 hours at 37 ˚C.

2.5. Estimation of the Microbes in Soil

Serial dilution was carried out to come-up with colonies that can be counted after the incubation. The plates with 30 to 300 colonies were chosen for counting. The number of microbes per mL of soil sample was calculated by dividing the number of colonies by the dilution factor.

The Colony Forming Unit per mL (CFU/mL) was calculated using the formula below;

CFU/mL = (number of colonies × dilution factor)/ volume of culture plate.

2.6. Determination of Organic Matter

A sieved (2mm) 5g of soil sample was measured using analytical balance and put into crucible, the dried empty crucible was measured and recorded as M1, the crucible with soil samples was placed in a drying oven set at 105◦C and dried for 4 hours. The crucible was removed and placed in desiccator with silica gel to cool, and then the weight of crucible with contents was measured using analytical balance and recorded as M2, the crucible with its contents was placed into the muffle furnace set at 400◦C for 4 hours, then the crucible was removed and cooled in desiccator and then weighed and recorded as M3.

The percentage of organic matter (%OM) was given by: %OM = (M2 – M3)/ (M2 – M1) ×100. Where M1 = Weight of crucible, M2 = Weight of crucible and Soil sample, and W3 = Weight of crucible and digested soil sample.

2.7. Determination of Nitrogen Content

Determination of Nitrogen content in soil was done as reported by [17]. Briefly, the 0.7g of Copper sulphate was measured and dissolved in distilled water to make a total volume of 70mL; Then 60g of potassium sulphate was measured then transferred into measuring cylinder, then distilled water was added to make a total volume of 400mL; and then, the suspensions were heated to dissolve completely. Then, 1g of soil sample was measured and placed in Kjeldahl flask, then 10mL of copper (ii) sulphate, 20mL of potassium sulphate and 30mL of Sulphuric acid was added, then heated gently for 30 minutes, The flask was removed from the heater and cooled, Followed by addition of 50mL of water and was transferred to the distilling flask, 25mL of HCl was placed in the receiving conical flask so that was excess of at least 5ml of the acid, followed by addition of 2 - 3drops of methyl red indicator, Enough water was added to cover the end of the condenser outlet tubes, tap water was run through the condenser, 30mL of 35% NaOH was added in the distilling flask in such a way that the contents do not mix. The contents were heated to distil the ammonia for about 30 – 40 minutes, the receiving flask was removed and the outlet rinse the outlet tube into the receiving flask with small amount of distilled water. The excess acid in distillate was titrated with 0.1M NaOH, the blank was determined on reagents using the same standard acid in a receiving conical flask.

Calculation of nitrogen was given by:

%N = 1.401[(V1M1 – V2 M2) – (V3 M1 – V4M2)/W × df; Where; V1 – volume of acid in receiving flask of samples, V2 – volume of NaOH used in titration, V3 – volume of acid in receiving flask for blank, V4 – volume of NaOH used in titrating blank, M1 – molarity of acid, M2 – molarity of NaOH, W - Weight of sample taken, df – dilution factor of sample (if 1g is taken for estimation, the dilution factor is 100), 1000mL of 0.1M HCl or H_2SO_4 corresponds to 1.401g of N.

3. Results

3.1. Study Site Information

The agriculture officer at Kauzeni village provided details of the agriculture practices exhibited by farmers, including information on soils where glyphosate herbicides are used, usage rates and doses, and also areas where the glyphosate herbicide was not used. Details of the sampling site and pesticide application is shown in Table 1.

Table 1. Description of sampling sites

Sample Site	Herbicide	Dosage	Crops	Application Interval
Site A: Herbicide Applied	Glyphosate	200 - 400 mL in 16 – 20 Lt	Maize Tomato	3 – 4 Months
Site B: No Herbicide Applied	-	-	Maize Tomato	-

3.2. Bacterial and Fungal Load in the Soil

Bacterial load in soil with and without glyphosate herbicide is shown in Table 1.

The average bacterial load in soil with herbicide was (1.5 ± 0.4) $\times 10^5$ CFU/mL, while in soil without herbicide was (2.2 ± 0.4) x 105 CFU/mL. The Fungal load in soil with and without herbicide is shown in Table 2.

Table 2. Bacterial load in soil with glyphosate herbicide and soil without glyphosate

Sample ID	Herbicide Applied ($x10^4$ CFU/mL)	Sample ID	No Herbicide Not Applied ($\times 10^4$ CFU/mL)
SH1	1.7	SNH1	2.0
SH2	1.8	SNH2	2.1
SH3	1.6	SNH3	2.4
SH4	2.4	SNH4	2.6
SH5	1.2	SNH5	2.1
SH6	2.0	SNH6	1.4
SH7	1.0	SNH7	2.6
SH8	1.7	SNH8	2.7
SH9	1.0	SNH9	1.8
SH10	1.3	SNH10	1.6
SH11	1.1	SNH11	2.3
SH12	1.5	SNH12	2.9
SH13	1.3	SNH13	2.0
SH14	2.2	SNH14	2.5
SH15	0.9	SNH15	1.9
Mean	15.13		21.9
Stdev	4.5		4.2

KEY: SH – Soil with glyphosate herbicide, SNH- Soil without glyphosate herbicide

Table 1. Fungal load in soil with and without glyphosate herbicide

Sample ID	Herbicide Applied ($x10^4$ CFU/mL)	Sample ID	No Herbicide Applied ($\times 10^4$ CFU/mL)
SH1	6	SNH1	13
SH2	5	SNH2	11
SH3	8	SNH3	10
SH4	11	SNH4	26
SH5	14	SNH5	18
SH6	14	SNH6	19
SH7	13	SNH7	16
SH8	21	SNH8	25
SH9	10	SNH9	22
SH10	8	SNH10	12
SH11	17	SNH11	24
SH12	23	SNH12	27
SH13	10	SNH13	14
SH14	18	SNH14	21
SH15	9	SNH15	18
Mean	12.4	Mean	18,4
Stdev	5.3	Stdev	5.6

SH – Soil with glyphosate herbicide applied, SNH- Soil without glyphosate herbicide

The average fungal load in soil with herbicides was found to be (1.2 ± 0.5) $\times 10^5$ CFU/mL and soil without herbicide was (1.8 ± 0.5) $\times 10^5$ CFU/mL. Bacterial and fungal load profile in soil with and without herbicide is shown in Figure 2. Soil without herbicides had higher bacterial and fungal load than soil where herbicides was applied.

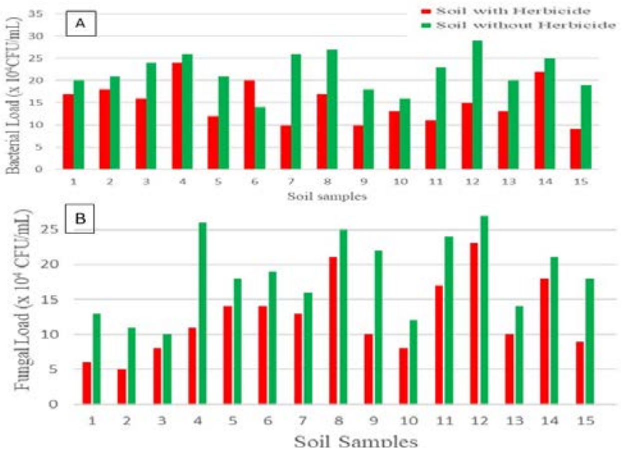

Figure 2. Bacterial and Fungal load in soil with and without herbicides. Part [A] show bacterial load in soil without herbicide (green) and soil with herbicide (red), and part [B] is the Fungal load in soil without herbicide (green) and soil with herbicide (red)

Statistical comparison of bacterial load between soil with and without herbicide was done using the analysis of variance (ANOVA). Results shows a significant difference in both bacterial and fungal load for the soil with and without glyphosate herbicide, p-value = 0.000236 and p-value = 0.0064 for the Bacterial and Fungal, respectively.

3.3. Soil Organic Matter and Nitrogen Content

Organic matter and Nitrogen content in soil with and without glyphosate herbicide is shown in the Table 3 and Table 4, respectively.

Table 4. Organic matter content in soil with and without glyphosate herbicide

Sample ID	Herbicide Applied (% Organic Matter)	Sample ID	No Herbicide Applied (% Organic matter)
SH1	11.9	SNH1	24.4
SH2	4.8	SNH2	35.0
SH3	7.5	SNH3	56.4
SH4	22.5	SNH4	51.4
SH5	5.1	SNH5	11.1
SH6	4.8	SNH6	17.5
SH7	7.3	SNH7	21.2
SH8	25.0	SNH8	12.2
SH9	12.8	SNH9	38.2
SH10	9.8	SNH10	61.9
SH11	14.3	SNH11	32.5
SH12	10.0	SNH12	13.9
SH13	25.6	SNH13	18.9
SH14	11.6	SNH14	10.8
SH15	4.8	SNH15	14.9
Mean	12	Mean	28.1
Stdev	7.2	Stdev	17.1

Key: SH – Soil with glyphosate herbicide applied; SNH- Soil without glyphosate herbicide

Figure 3 shows bar plot for the organic and nitrogen content in soil with and without herbicides. The average organic matter in soil with herbicide was found to be (12 ± 7) %, while in soil without herbicide was (28.3 ± 17) %. The average Nitrogen content in soil with herbicides was (7.0 ± 3.8) %, while soil without herbicide had (17.0 ± 6.6) %.

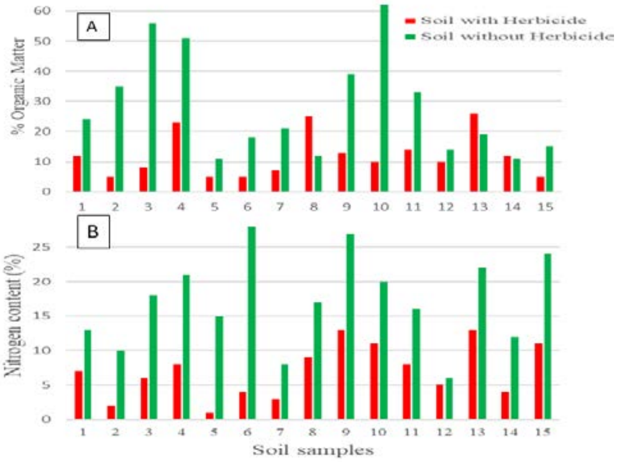

Figure 3. Organic matter and Nitrogen content in soil with and without Glyphosate herbicide. Part [A] show percentage organic matter in soil with herbicide (red) and without (green). Part [B] show Percentage of Nitrogen in Soil with herbicide (red) and without herbicide (green)

Statistical comparison for the soil organic matter and Nitrogen content in soil with and without herbicide was done using the analysis of variance (ANOVA). Results show a significant difference in both organic matter and Nitrogen content for the soil with and without glyphosate herbicide, p-value = 0.0023 and p-value = 0.000022, for the Organic matter and Nitrogen content, respectively.

Table 5. Nitrogen content in load in soil with and without glyphosate herbicide

SAMPLE ID	Soil with Herbicide (% Nitrogen)	Sample ID	Soil without Herbicide (%Nitrogen)
SH1	7.0	SNH1	12.6
SH2	2.1	SNH2	9.8
SH3	5.6	SNH3	18.2
SH4	8.4	SNH4	21.0
SH5	1.4	SNH5	15.4
SH6	3.5	SNH6	28.0
SH7	2.8	SNH7	7.7
SH8	9.1	SNH8	16.8
SH9	12.6	SNH9	26.6
SH10	11.2	SNH10	19.6
SH11	8.4	SNH11	16.1
SH12	4.9	SNH12	6.3
SH13	13.3	SNH13	21.7
SH14	4.2	SNH14	11.9
SH15	10.5	SNH15	23.8
Mean	7	Mean	17.1
Stdev	3.8	Stdev	6.6

Key: SH – Soil with glyphosate herbicide applied, SNH- Soil without glyphosate herbicide

4. Discussion

This study revealed a decrease in the microbiota abundance as well as the soil organic matter and nitrogen levels in the soil as a result of the application of Glyphosate herbicide. To our knowledge, this is the first study assessing the impact of herbicides application on agricultural soil in Tanzania. The indiscriminate use of herbicides to increase agricultural productivity to ensure food security is on increase everywhere. The consequences of this practice to the soil ecosystems and services are hardly taken into consideration, especially in low and middle-income countries like Tanzania where modern agricultural practices using chemicals are relatively new.

While in an agricultural context, soil quality is judged as the ability to support field production; the decrease in the abundance of soil bacteria and fungi that are the major decomposers of plant and animal waste to release nutrient in soil renders it sterile and hence of poor quality in the long run. Bacteria and fungi are part of the biological properties of soil and their activity are interconnected with soil physical and chemical properties such as aeration, soil organic matter, pH, and greatly contribute to Carbon and nutrient cycling [4]. Plant roots has role in the growth and functions of soil microorganisms such as plant growth-promoting bacteria, Mycorrhizal fungi, Endophytic bacteria, Actinomycetes, nematodes, protozoans which has impacts on plant health and growth [18]. The significant decrease in the abundance of bacterial and fungal in soil with herbicide, and consequently a low levels of soil organic matter and nitrogen underscores the importance of soil microbiota to plant nutrients. Microbiota plays role in soil health-regulating dynamics of soil organic matter (SOM) and plant nutrients availability in agroecosystems [19,20]. It is now apparent that, agricultural land where chemical fertilizers and herbicides are commonly used, the soil becomes absolute not productive if chemical fertilizer is not applied.

Example of chemical effect in agriculture is reported by Macit [21]; where improvement of degraded soil microbiota was possible by the application of phosphorus biofertilizer enriched with beneficial bacterial strains. Potential reason for poor soil productivity is attributed to by the lack of soil microbial decomposers that might have disappeared following a prolonged application non selective concentrated chemicals to the soil. Higher diversity microbiota in soil is essential for the management of soil health because it has plant growth-promoting or biocontrol effects that is advantageous for the host plant and alter plant physiology and nutrition. Some soil microbiota has antagonistic activity and ability to defend plants from soil-borne diseases [22]. The use of herbicides to kills weeds in agricultural practices is undeniably also killing untargeted plant associated microbes, and hence decline in the abundance and diversity of microbiota in soil that are crucial for nutrient cycling.

Severe loss of soil organic matter degrades soil functionality, its capacity to provide essential soil ecosystem services and soil health [23]. Soil organic matter content affects crop yield through its role in enhancing and sustaining soil quality [24] and soil health [23]. It is a critical indicator of soil health through positive impact on soil properties and processes (Doran and Zeiss 2000). We found 16% decrease in soil organic matter i.e. from (28.3 ± 17) % to (12 ± 7) % as a result of glyphosate application. Despite the fact that the recommended level of soil organic matter for agricultural ranges from 2 -10% (Loveland 2003), a decrease by a factor of 16% due to herbicides application warrantees' further action in the management of soil heath. Likewise, a significant drop in the nitrogen levels by more than 10% (17 ± 6.6) % to (7.0 ± 3.8) % in soil with herbicide implies that, the rate of organic matter decomposition and the nitrogen cycle were affected and low levels of organic matter content and nitrogen as the main indicators of soil fertility.

Soil is a non-renewable and threatened resource worth protection to maintain a range of ecosystem services critical to ecological balances and agricultural production [4]. Traditionally, crop performance has been used as an indicator for soil quality and fertility [25], where only physico-chemical parameters were assessed; today, it is becoming evident that soil quality must be evaluated by combining parameters that includes both the physico-chemical and the biological. Soil microbiota is also reported in suppressing soil-borne plant pathogens in organic farming [22], a decline in the abundance and diversity as a results herbicide's application is likely to increase susceptibility of plants to soil pathogens, and hence poor productivity in the long run.

Despite an increased awareness of the importance of organically produced food; farmers in developing countries still tend to apply inorganic chemical fertilizers, herbicides and toxic chemical pesticides beyond the recommended doses [26]. The dosage levels of 200 – 400mLof glyphosate concentrate in 16 - 20 Litres of water reported at Kauzeni farm is on the higher side, and there are no bases as to how they have arrived to it. There is an urgent need to establish dosage levels of various chemicals used in agriculture taking into consideration of the physical, chemical and biological properties of the soil of a given location. This study has demonstrated that the uncontrolled application of glyphosate herbicide has an adverse effect on the abundance and activity of soil microbiota.

This study has shown that uncontrolled application of glyphosate herbicide on agricultural soil has a negative impact on the abundance and activity of soil microbiota, consequently lowering the soil nitrogen and organic matter levels. We recommend to establish a comprehensive soil microbiota profile of different agricultural soil using molecular approach, and then a standardized herbicide dosage for different agricultural. This approach will safeguard the soil microorganisms and ensure a sustainable ecosystem service, ultimately a sustainable agricultural productivity.

5. Conclusion

The study has shown that uncontrolled application of glyphosate herbicide on agricultural soil has a negative impact on the abundance and activity of soil microbiota, consequently lowering the soil nitrogen and organic matter levels.

Establishing a comprehensive healthy soil microbiota of different agricultural soil using molecular approach, and establishing a standardized herbicide dosage for different soil microbiota profiles is strongly recommended for a sustainable agricultural productivity.

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Declaration of Conflict of Interest

Authors declares that there is no conflict of interest to disclose.

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