



Prolonged Usage of Herbicides Reduces Heterotrophic Aerobic Bacteria and Fungi Population and Alters Soil Physicochemical Parameters

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The study aimed to examine the effect of commonly used herbicides on soil microbiota (bacteria and fungi) as well as its physicochemical properties. Topsoil (0 to 5cm depth) samples were collected from different plots at Ayeye, Iwaro Oka Akoko, Ondo State, Nigeria with known prior herbicide treatments. The plots had different history of glyphosate, paraquat and both combination application. The soil samples were serially diluted and cultured on nutrient agar and potato dextrose agar for bacteria and fungi, respectively. The physicochemical properties of the soil samples showed that all the soil samples had pH values which ranged from 7.4 to 9.4. The moisture contents ranged from 2.6 to 15 with soil sample without prior usage of herbicide having the highest moisture content. The average total bacterial counts ranged from 4.0×10^8 to 2.5×10^9 cfu/g while the average total fungal counts ranged from 3.0×10^3 to 3.1×10^4 cfu/g with the untreated soil containing the highest loads. Soil samples with prolonged use of herbicides generally contained lower soil moisture contents, organic matter contents, as well as lower microbial loads. It could be concluded from the study that the pattern of change in the bacteria and fungi population vary as a result of differences in the composition of herbicides and year of usage.

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1. INTRODUCTION

One of the major challenges that demoralizes farming business around the world is the invasion by various types of weed which reduce both the quality and quantity of crop production. This is primarily due to the favourable environmental conditions such as high moisture resulting from frequent rainfall, suitable sunlight and high soil fertility [1]. This necessitated the use of herbicides to either prevent or at least reduces the competition of weed with the crops. The use of herbicides in agriculture has contributed significantly to increase in both food and cash crop production all over the world. The use of herbicides in controlling the weeds has led to the flooding of the agrochemical market with different kind of herbicides [2]. Conceivably, the effectiveness of these herbicides in controlling the target weeds has resulted in the continuous usage of these chemicals by farmers.

Many herbicides have been used as pre-emergent and post-emergent weed killers in the world. In Nigeria, herbicides are used to control weeds in agricultural systems [3]. But soil microorganisms are seriously affected by the application of these herbicides commonly used in contemporary agricultural practices to achieve optimum crop yield [4,5]. The sensitivity of soil microorganisms to herbicides will interfere with the vital metabolic activities and geochemical activities of the microorganisms in nature, which in turns affect the availability of nutrients in the soil [4].

The over application of herbicides inhibits some of the non-target natural processes (such as soil organic material degradation, soil organic matter decomposition and soil nutrient cycling) and reduces the efficiency of the non-target organisms [6]. However, some soil organisms use these herbicides as carbon energy source for their metabolic activities. Several studies have shown that the use of herbicides reduces soil microbial populations which eventually affects the decomposition of celluloses and recalcitrant compounds like lignin in ecosystem [7]. Consequently, the variations in the populations of these microorganisms will affect the regeneration of nutrients to support plant growth and increase crop yield.

The occurrence of herbicide residues in soil could directly influence soil microbes. However, at field recommended rates, herbicides are perceived to have little or no major or long-term effect on soil microbial populations. A study by Sebiomo et al. [2] indicates that some soil microorganisms can degrade the herbicide while some others were adversely affected depending on the application rates and the chemical composition of herbicide used. Hence, effects of herbicides on microbial growth depend on the chemicals (type and concentration), microbial species and environmental conditions [8].

The effect of herbicides on soil microbial community has been covered in some researches and studies [7,9,10,11,4]. The consequential effects of herbicides on soil microorganisms can be related to both qualitative and quantitative changes of soil microbial populations. The reduction of heterotrophic aerobic bacterial and fungal populations was observed in paraquat and glyphosate treated soils [12]. This reduction in microbial (bacteria and fungi) population may be related to the fact that the application of herbicides has affected the presence and existence of bacterial and fungal populations [13]. However, positive relationship with soil microbes may still exist with some organophosphates as noted in study by Sebiomo et al. [2].

The increasing reliance of crop cultivation on herbicides has led to concern about their ecotoxicological behaviour in the environment [7,9]. Soil health and microbial diversity have become vital problems for the sustainable agriculture. Loss of microbial biodiversity can affect the functional stability of the soil microbial community and soil health. Largely, there are some negative effects of herbicides on the microbial population level or species composition [7,2]. Several herbicides are used in weed management programs in agriculture to control weeds. Among these, paraquat and glyphosate are more common. Determining the impact of prolonged usage of herbicides on soil microbial growth and population is of considerable interest. The assessment of unforeseen consequences on microbial communities especially soil heterotrophic bacteria and fungi due to prolonged use of herbicide is important to provide deeper insight for herbicide risk management in soils. The

objective of this research is to isolate and identify aerobic heterotrophic bacteria and fungi in soil polluted with different herbicides.

2. MATERIALS AND METHODS

2.1 Soil Sampling

Topsoil (0 to 5 cm depth) samples were collected from different plots at Ayepe, Iwaro-Oka in Akoko South West Local Government Area of Ondo State with known prior herbicides treatments and control (soil without prior herbicide's treatment) in a sterile polyethylene bag and transported to Microbiology Laboratory of Adekunle Ajasin University, Akungba Akoko for further analysis. The pH of the surrounding soil measured confirmed the previous information that soil pH of location was near neutral (pH 7.4) before the application of herbicides.

Sample A: Soil without history of herbicide usage; Sample B: Paraquat and glyphosate simultaneously used for less than 1 year; Sample C: Paraquat has been used as herbicide constantly for 4 years; Sample D: Glyphosate has been used as herbicides constantly for the past 5 years; Sample E: Paraquat herbicide constantly used for 8 years.

2.2 Microbiological Analyses

The media used for this study are nutrient agar (NA) and Potato dextrose agar (PDA) (Hi-media, Nigeria). The media were prepared according to manufacturers' specifications. Chloramphenicol was incorporated into the medium (PDA only) and both media were sterilized in an autoclave for 121°C for 15 minutes, cooled down to 45°C on the work bench before utilization [14].

Bacteria and fungi were isolated using the method of Adegbehingbe et al. [14] with modifications. A gram of soil sample was weighed into a sterile test tube containing 9 ml of sterile normal saline and shaken properly and 1 ml of the mixture was pipetted aseptically into a test tube containing 9 ml of sterile normal saline to make six ten-fold (10^{-6}) serial dilutions. NA and PDA which has been sterilized by autoclave cooled at about 45°C were poured aseptically into sterile Petri dishes and allowed to set, respectively. An aliquot of 0.1 ml from the 10^{-6} and 10^{-3} dilution was aseptically pipetted into sterile plate and spread uniformly on the plate using sterilized glass spreader, respectively for

NA and PDA. The NA plates were incubated in an incubator at 37°C for 24 to 48 hours, while the PDA plates were incubated in an incubator at 25°C for 72 hours. Bacterial and fungal growth observed after 24 and 72 hours, respectively were counted and the results normalized using appropriate dilution factor and counts were expressed in colony-forming unit per gram of soil sample (cfu/g). The representative colonies were sub-cultured on the appropriate plates while pure cultures were obtained by repeated streaking of fresh colonies and picking of fungi mycelia or spores on appropriate media. The isolates were identified based on their morphological examination and biochemical characterizations [14,15,16]. Their respective pure cultures were maintained on agar slants containing NA and PDA at refrigeration temperature 4°C for further use [1,17].

2.3 Physicochemical Analyses

2.3.1 Determination of soil pH

The pH of the soil samples was determined using the pH meter (Mettler Toledo, UK). 2 g of the soil sample was measured and dissolved in 10 ml of sterile distilled water. The soil solution was agitated for 30 minutes and further allowed to stand for 30 minutes. The pH was measured by dipping the electrode connected to the meter into a buffer solution (pH 4 and pH 7, respectively) to standardize the pH meter, this was followed by cleaning the electrode with sterile distilled water and with a tissue paper, the electrode was then inserted into the prepared sample and the reading was taken in triplicates [18].

2.3.2 Determination of soil moisture content

The moisture content of the sample was determined using the oven-drying method. A clean, empty Petri dish was weighed, and 10 g of the soil sample was added. The evaporating dish and its content were put in the thermosetting oven at 105°C until a constant weight was obtained [19,18].

2.3.3 Determination of ash in soil

An empty crucible was weighed and later weighted with oven dried soil sample in it. The crucible and its content were placed in the furnace for 5 hours at 550°C after which it was removed and allowed to cool in the desiccators and then weighed [19].

2.4 Statistical Analysis

All statistical analyses were performed using the program R 3.4.0 (<http://www.rproject.org/>). The bacterial and fungal populations and physicochemical parameters in this study were analysed separately using a One-way analysis of variance (ANOVA) (Statistical Procedures for Agricultural Research package (agricolae). Tukey HSD multiple post-hoc tests was used to assess the significance of the differences among the means ($p = 0.05$).

3. RESULTS AND DISCUSSION

The results from this study showed that soil without history of herbicide application had the highest number of both bacterial and fungal counts (2.5×10^9 and 3.1×10^4 cfu/g, respectively) and are significantly different ($p = 0.05$) from the microbial counts from other soil with different history of herbicides treatment (Fig. 1). Soil with

both glyphosate and paraquat application for one year had the lowest bacterial counts. However, there was no significant difference ($p = 0.05$) among the number of bacteria isolated from soil samples treated with glyphosate (5 years), paraquat (4 years) and paraquat (8 years). While the fungal count decreased with increase in the year of herbicide application (i.e., 1 to 8 years) and there was no significant difference ($p = 0.05$) between the fungal counts in soil samples treated with glyphosate for five years and paraquat for eight years as both had the lowest fungal counts ($p = 0.05$).

The pH of the soil samples generally ranged from near-neutral to moderately alkaline with glyphosate usage for 5 years had the highest pH value (9.4) and was significantly different from other soil samples and the soil with glyphosate treatment for one year had the least pH (7.4), while the soil without history of herbicide application had a pH of 7.9 (Fig. 2).

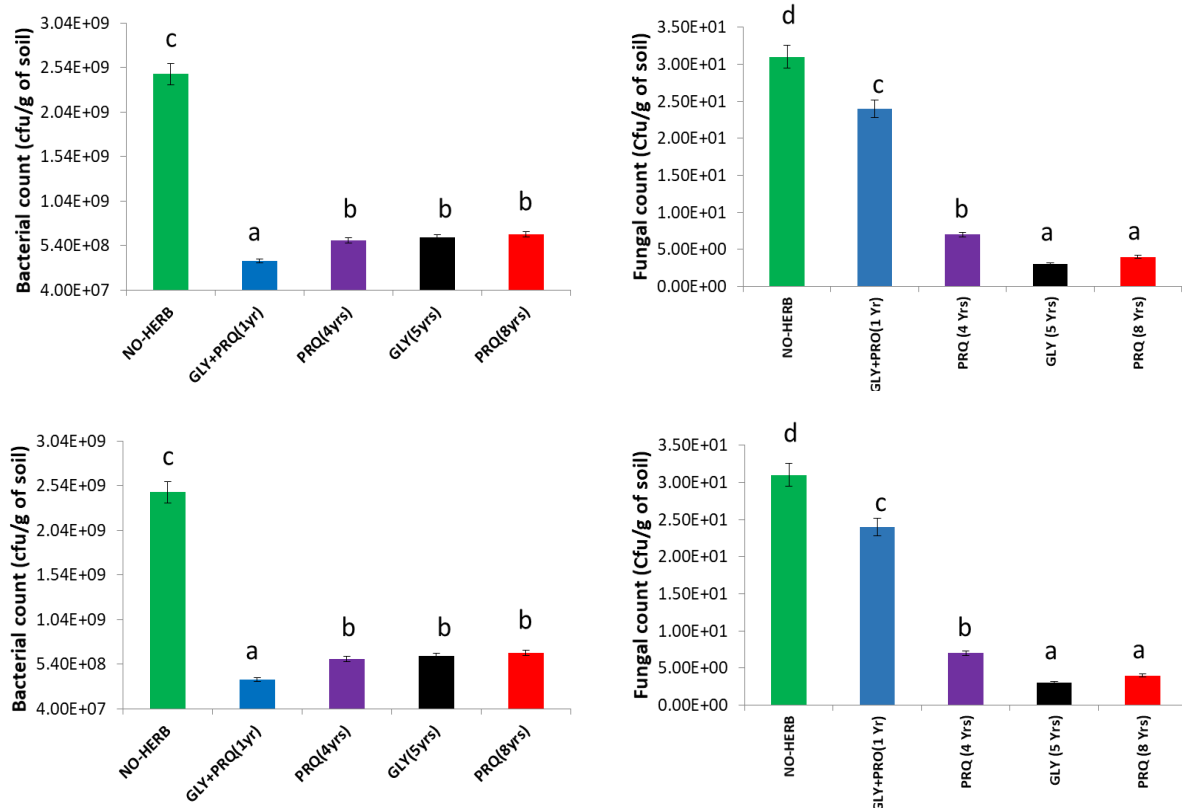


Fig. 1. The bacterial and fungal counts on the soil polluted with different herbicides, Data represents mean of the triplicate samples and error bars are the standard error of the mean
 NO- HERB = Soil without history of herbicide, GLY = Soil that received glyphosate, PRQ = Soil that received paraquat, yr (s) = periods of herbicides application in year ($p = 0.05$)

The moisture content was significantly higher in soil sample without history of herbicides application compared to the soils with different history of herbicides application ($p = 0.05$). However, the moisture content of the soil decreased with the increase in the duration of herbicides application and the soil in which paraquat was applied for eight years had the lowest moisture content (Fig. 2). Similarly, the organic matter content in soil without history of herbicides application was significantly higher than the soils with history of herbicides application ($p = 0.05$) (Fig. 2).

The number of bacteria isolated from soil samples varied with the type of herbicides and the duration of herbicides application. A total of sixteen bacteria were isolated from all the samples in which five were Gram negative; *Enterobacter hafniae*, *Enterobacter aerogenes*, *Alcaligenes faecalis*, *Klebsiella pneumoniae* and *Enterobacter agglomerans*, while eleven others were Gram positive; *Bacillus subtilis*, *Agromyces* spp., *Staphylococcus aureus*, *Micrococcus luteus*, *Corynebacterium minutissimum*, *C. paurometabolum*, *C. jeikelum*, *C. bovis*, *C. mycetoides*, *C. cystitidis*, *Staphylococcus saccharolyticus*, *S. lugdunensis*, *S. anaerobius*, *S. epidermidis* and *S. hyicus* (Table 1). The soil

without history of herbicide application was dominated by different species of *Staphylococcus*. The soil in which both glyphosate and paraquat were applied for one year was dominated by *Corynebacterium* species and *Alcaligenes faecalis*. The soil sample in which paraquat was applied for four years was dominated by *Corynebacterium* species while *Corynebacterium* and *Enterobacter* were dominated in soil treated with glyphosate for five years. The soil with paraquat application for eight years was dominated by different species of *Staphylococcus*, *Corynebacterium*, *Klebsiella* and *Enterobacter* (Table 1).

A total of nineteen different fungi were isolated from all the soil samples. This include *Rhodotorula minuta*, which was prominent in all the soil samples. *Rhodotorula minuta*, *Candida tropicalis*, *Walleimia sebi*, *Cadophora fastigiata*, *Aspergillus flavus*, *A. candidus*, *Botrytis cinerea*, *Cladosporium macrocarpum*, *Oidiodendron griseum* and *Chrysonilia sitophila* were prominent in soil samples without history of herbicide application. *Rhodotorula minuta*, *Walleimia sebi*, *Saccharomyces cerevisiae*, *Aspergillus acidus*, *Zygosaccharomyces bailii*, and *Ulocladium alternariae* were prominent in soil which both paraquat and glyphosate are applied for one

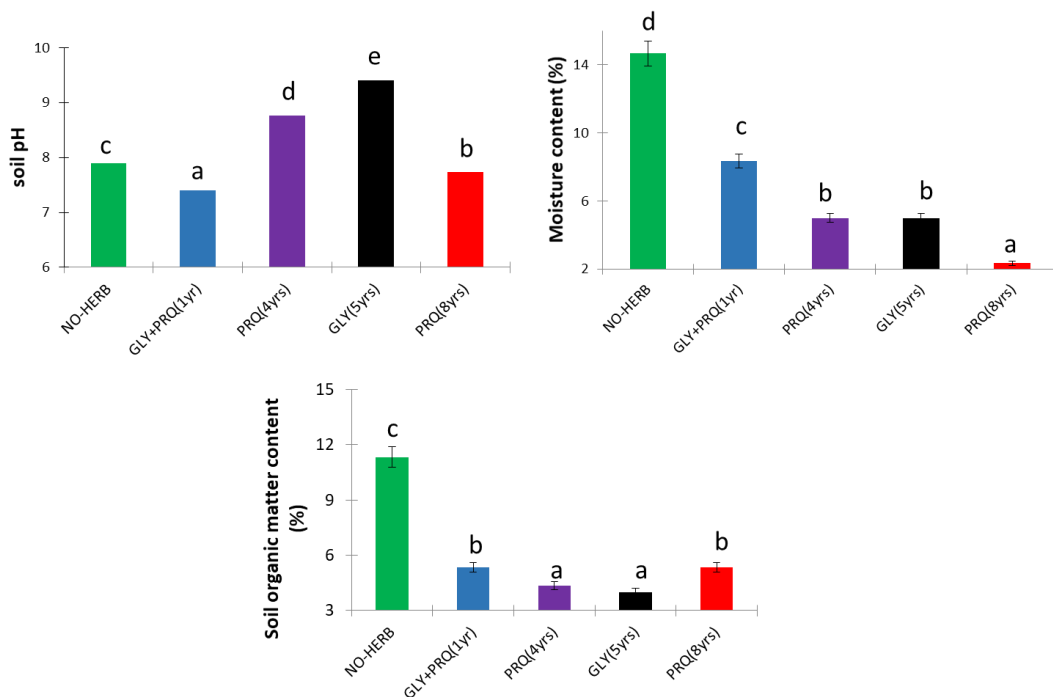


Fig. 2. The physicochemical parameters of the soil polluted with different herbicides
 Data represents mean of the triplicate samples and error bars are the standard error of the mean,
 NO- HERB = Soil without history of herbicide, GLY = Soil that received glyphosate,
 PRQ = Soil that received paraquat, yrs = periods of herbicides application in year ($p = 0.05$)

year. *Rhodotorula minuta*, *Acremonium strictum*, and *Pichia membranifaciens* were prominent in soil with paraquat application for four years. *Rhodotorula minuta*, *Candida tropicalis*, and *Penicillium chrysogenum* were prominent in soil which glyphosate was applied for five years while *Rhodotorula minuta*, *Saccharomyces cerevisiae* and *Ulocladium alternariae* were prominent in soil which paraquat was applied for eight years (Fig. 3 and Table 2).

Generally, the occurrence of bacterial isolates from the soil samples varied with different herbicide and the duration of herbicide usage (Table 3). The percentage of occurrence the bacterial isolates reveal that soil which paraquat was applied for eight years had the highest percentage of bacterial occurrence compared to other herbicide treatment and control (soil without history of herbicide application). Additionally, the occurrence of fungal isolates also varied with different herbicide and the duration of herbicide usage (Table 3). The percentage of occurrence the fungal isolates reveal that soil without history of herbicides application had the highest percentage of fungal occurrence compared to the herbicide treated.

The observed decrease in bacterial and fungal count in the soils in which herbicides are applied compared to the control (soil sample without history of herbicide) corroborates the reports of Ayansina and Oso (2006), Sapundzhieva et al. [20], Zain et al. [8], Adomako [21], Al-Ani et al. [22] and Meena et al. [23], who reported reductions in bacterial and fungal populations in soil treated with herbicides at recommended rates in their independent studies. Mayeetreyee et al. [12]; Adomako, [21], Al-Ani et al. [22] and Meena et al. [23] also observed the reductions of heterotrophic aerobic bacterial and fungal populations in soils where paraquat and glyphosate were used. The reduction in the microbial populations could be attributed to the susceptibility of the microbes to the herbicides and the products of soil-herbicide interactions, which are both bactericidal and fungicidal. The results obtained from the present studies contradicts findings by Sebiomo et al. [2] who observed increases in microbial populations after six weeks of herbicide application. The reason for the differences in the results of present study and the previous studies by Sebiomo et al. [2] could be attributed to the differences in concentration, site and duration of herbicide application [22]. However, before degradation, herbicides have toxic effects on microorganisms,

reducing their abundance, activity and consequently, the diversity of their communities. The toxic effects of herbicides are normally most severe immediately after application [19,23]. Later, microorganisms take part in a degradation process, and then the degraded organic herbicides provide carbon rich substrates for the microbial population in the soil [24,25,23]. But where there is continuous application of herbicides as the one in the present study, soil microorganisms that survived the previous herbicide application might not have fully recovered before the subsequent herbicide application. The results of the interaction of such microorganisms with subsequent herbicides could be fatal especially if more than one type of herbicides is used on the same soil.

The changes in pH of the soil treated with different herbicides could be attributed to the interaction between different soil component and herbicides compared to the soil without history of herbicide application [26]. It is acknowledged that soil is highly heterogenous in nature, the proximity of the plots from which the samples were taken with the previous information on the plots indicates that the initial pH of the plots was near-neutral. The increase soil pH coupled with the toxicity of the herbicides could led to decreased in microbial population [27].

The reduction in the organic matter content in soils polluted with herbicides generally indicated that the herbicides are toxic to microbial life in the soil. Also, the activities of soil fauna such as the earthworm and other group of microorganisms that brings about the formation of organic matter in the soil are inhibited by herbicides [28]. This observation was supported by the observed death of earthworm and other soil inhabiting macroorganisms in soils where herbicides are recently applied compared to the soil without herbicide. This observation is supported by Arora and Sahni [28] who observed similar incidence on soils where herbicides are applied. The observed reduction in the percentage organic matter content of the soils treated with herbicides corroborates the study of Meena et al. [23] who observed a decrease in soil organic matter content after herbicides application but contrary to the report by Sebiomo et al. [2] that continuous application of herbicides increases the soil organic matter. The resulted increased in soil organic matter by Sebiomo et al. [2] could be attributed to the differences in the experimental set ups as their experiment was carried out in the laboratory for

Table 1. The biochemical and morphological features of heterotrophic aerobic bacteria isolated from soil polluted with herbicides and control

Herbicides & Isol	G.S	Shape	Cat.	Coag.	ID.	Cit.	Ur.	Gluc.	Gal.	Dex.	Fruc.	Man.	Meth.	Pro. Bac.
No-HERB A	+	Cocci	+	+	-	+	-	+g	-	+	+	-	-	<i>Staphylococcus anaerobicus</i>
B	+	Cocci	+	-	-	+	+	+g	+g	+	+g	-	-	<i>Staphylococcus epidermidis</i>
C	+	Cocci	+	+	-	+	+	+	+g	+g	+	-g	-	<i>Staphylococcus hyicus</i>
D	+	Cocci	+	-	-	+	-	+g	+g	+	+	-g	-	<i>Staphylococcus lugdunensis</i>
GLY+PRQ(1yr) A	+	Rod	+	+	-	+	-	+g	-	+g	+g	-g	-	<i>Corynebacterium mycetoides</i>
B	+	Rod	+	+	-	+	-	+g	+g	-g	-g	-g	-	<i>Corynebacterium jelkeium</i>
C	+	Rod	+	+	-	+	-	-	-g	-g	-g	-g	-	<i>Corynebacterium paurometabolum</i>
D	-	Rod	+	+	-	+	-	-g	+g	+g	-g	-g	-	<i>Alcaligenes faecalis</i>
PRQ(4yrs) A	+	Rod	-	-	-	+	-	+g	-g	-g	+g	-	-	<i>Corynebacterium bovis</i>
B	+	Rod	-	+	-	+	-	+	+	+	+g	-g	-	<i>Corynebacterium mycetoides</i>
GLY(5yrs) A	+	Rod	+	-	-	+	-	+g	-	+	+g	+g	-	<i>Corynebacterium minutissimum</i>
B	+	Rod	+	+	-	+	-	-g	-	-g	-g	-	-	<i>Corynebacterium paurometabolum</i>
C	-	Rod	+	-	-	+	-	-g	-	+	-g	-	-	<i>Enterobacter hafniae</i>
D	-	Rod	+	+	-	+	-	-g	+g	+g	+g	-	-	<i>Enterobacter aerogenes</i>
E	+	Rod	+	-	-	+	-	+	+	-	-g	-g	-	<i>Corynebacterium jelkeium</i>
PRQ(8yrs) A	-	Rod	-	-	-	+	+	+	-	+	+g	-g	-	<i>Klebsiella pneumoniae</i>
B	+	Rod	-	+	-	+	+	+g	-	-g	+	-	-	<i>Corynebacterium cystitidis</i>
C	+	Cocci	+	-	-	+	-	+	-g	+g	+	-g	-	<i>Staphylococcus saccharolyticus</i>
D	+	Cocci	+	-	-	+	+	+	-	-g	+	-g	-	<i>Staphylococcus lugdunensis</i>
E	-	Rod	+	+	-	+	-	+	-g	+	+	+	-	<i>Enterobacter agglomerans</i>
F	+	Rod	+	+	-	+	-	-	-	-	-g	-g	-	<i>Corynebacterium paurometabolum</i>

- = negative, + = positive, g = gas. NO-HERB = Soil without history of herbicides, GLY = Glyphosate, PRQ = Paraquat. Isol = Isolate, G.S = Gram's stain, Cat. = catalase, Coag. = Coagulase, ID. = Indole, Cit. = Citrate, Ur. = Urease, Gluc. = Glucose, Gal. = Galactose, Dex. = Dextrose, Fruc. = Fructose, Man. = Manitol, Meth. = MethylRed and Pro.Bac. = Probable Bacteria

Table 2. Morphological and microscopic features of heterotrophic aerobic fungi isolated from soil polluted with herbicides and control

Isolate	Rate	Colour	Hyphae	Conidium Formed	Probable fungi	Sample
1	Moderate	Creamy	Pseudophypha formed	Ovid shaped produce literally mycelium.	<i>Rhodotorula minuta</i>	All sample
2	Slow	Brownish to black	Upright phialide	Conidiophores arising from long broad thick-walled end of the phialide	<i>Cadophora fastigiata</i>	No-herb
3	Slow	Lime green cream	Septate	Conidia heads are radiate and conidiophores are rough	<i>Aspergillus flavus</i>	No-herb
4	Fast	Brown		Brown and branched conidiophores	<i>Botrytis cinerea</i>	No-herb
5	Slow	Pale cream	Aseptate	Large white vesiculate conidiophores	<i>Aspergillus candidus</i>	No-herb
6	Slow	Initially pale yellow, then orange	Branch pattern	Produces abundant spores. Arthrospores are formed when the older hyphae break up	<i>Cladosporium macrocarpum</i>	No-herb
7	Slow	Yellow	Septate and hyaline	Conidiophore smooth walled	<i>Eurotium herbariorum</i>	No-herb
8	Moderate	Greyish	Erect, pigmented	Smooth walled	<i>Oidiodendron griseum</i>	No-herb
9	Moderate	Pink	Septate	Hyaline conidia	<i>Chrysonilia sitophila</i>	No-herb
10	Moderate	Creamy	Pseudohypha formed	Ovid shape produced laterally mycelium	<i>Candida tropicalis</i>	No-herb and GLY(5yrs)
11	Moderate	Blackish brown	Septate	conidiophore cylindrical and smooth	<i>Wallemia sebi</i>	No-herb and GLY+PRQ(1yr)
12	Moderate	Greyish to blackbrown	Upright phialide	Conidiophores smooth, long and coarse vesicles.	<i>Aspergillus acidius</i>	GLY+PRQ(1yr)
13	Moderate	Cream	Pseudohyphae formed	Conidiophore smooth and round	<i>Zygosaccharomyces bailii</i>	GLY+PRQ(1yr)
14	Moderate	Creamy	Pseudohyphae formed	Ovid shape produced laterally mycelium	<i>Saccharomyces cerevisiae</i>	GLY+PRQ(1yr) and PRQ(8yrs)
15	Moderately rapidly	Brown	Septate brown	Conidiophores are simple, branched, smooth, strongly geniculate and bear the conidia	<i>Ulocladium alternariae</i>	GLY+PRQ(1yr) and PRQ(8yrs)
16	Moderate	Cream			<i>Pichia membranifaciens</i>	PRQ(4yrs)
17	Moderate	Pale pink	Upright phialide which forms basipetal chains of dry conidia	Conidium grow as wet clusters or dry chains	<i>Acremonium strictum</i>	PRQ(4yrs)
18	Moderate	Yellowish orange	Fibrous hyphae	Pyriiform conidia	<i>Epicoccum nigrum</i>	PRQ(4yrs)
19	Moderate	Shade of green	Aerial hyphae	Conidial in loose columns	<i>Penicillium chrysogenum</i>	GLY(5yrs)

No-herb = Soil without history of herbicides, GLY = Glyphosate, PRQ = Paraquat, yrs = years

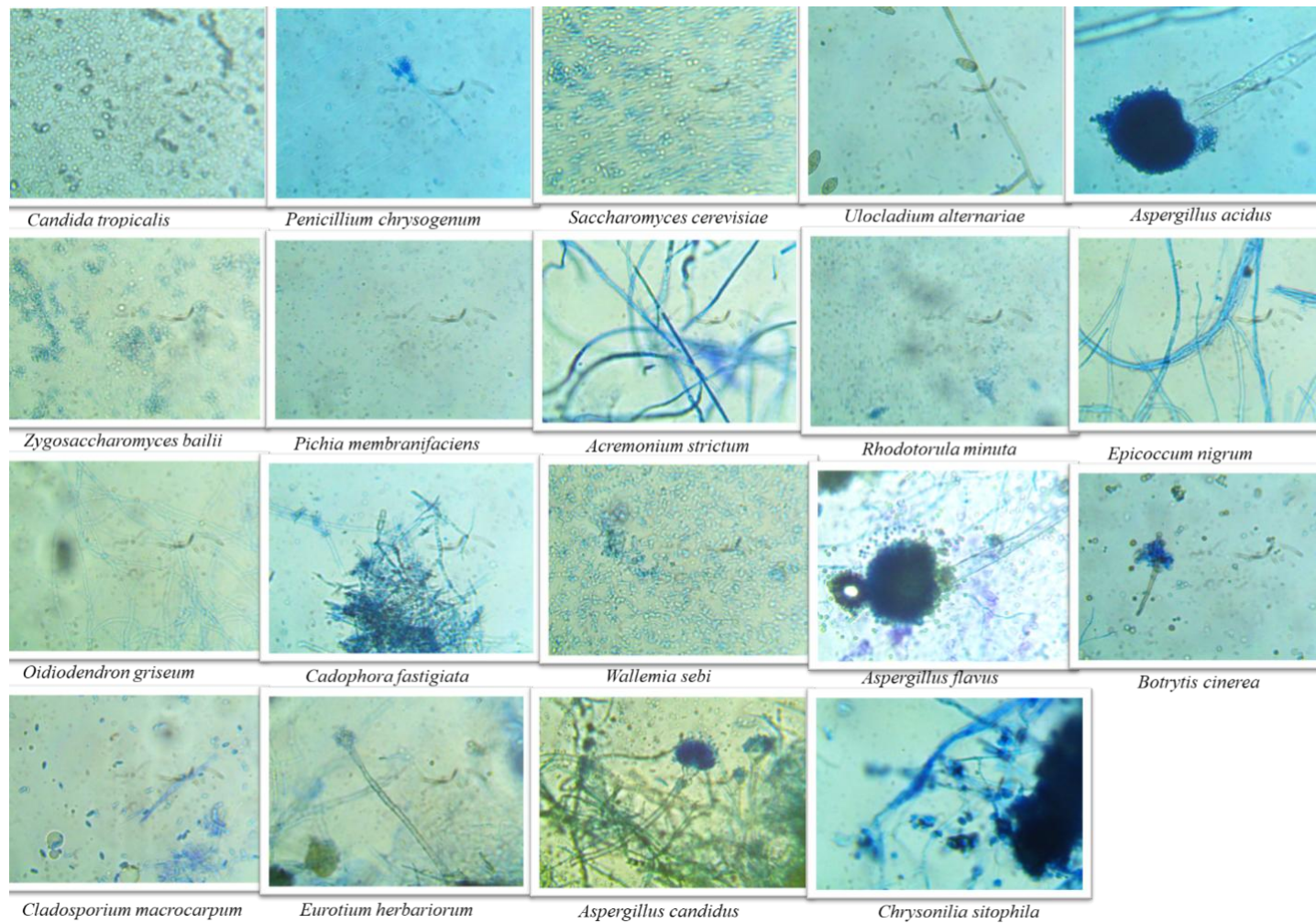


Fig. 3. The photomicrographs of the isolated heterotrophic aerobic fungi from herbicide polluted soil samples

Table 3. Occurrence of bacterial and fungal isolates from herbicide polluted soil samples

Sample Source/ Bacteria Isolate	<i>Corynebacterium minutissimum</i>	<i>Corynebacterium paurometabolum</i>	<i>Enterobacter hafniae</i>	<i>Enterobacter aerogenes</i>	<i>Corynebacterium jeikeium</i>	<i>Corynebacterium bovis</i>	<i>Corynebacterium mycetoides</i>	<i>Alcaligenes faecalis</i>	<i>Klebsiella pneumoniae</i>	<i>Corynebacterium cystitidis</i>	<i>Staphylococcus saccharolyticus</i>	<i>Staphylococcus lugdunensis</i>	<i>Enterobacter agglomerans</i>	<i>Staphylococcus anaerobius</i>	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus hyicus</i>	Percentage of occurrence (%)
No- herb	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	19
PRQ+GLY (1 yr)	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	19
PRQ (4 yrs)	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	9.5
GLY (5 yrs)	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	23.8
PRQ (8 yrs)	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-	28.6

Sample source /Fungi isolated	<i>Rhodotorula minuta</i>	<i>Candida tropicalis</i>	<i>Wailemia sebi</i>	<i>Cadophora fastigiata</i>	<i>Aspergillus flavus</i>	<i>Botrytis cinerea</i>	<i>Aspergillus candidus</i>	<i>Cladosporium macrocarpum</i>	<i>Eurotium herbariorum</i>	<i>Oidiodendron griseum</i>	<i>Chrysomyia sitophila</i>	<i>Saccharomyces cerevisiae</i>	<i>Aspergillus acidus</i>	<i>Zygosaccharomyces bailii</i>	<i>Ulocladium alternariae</i>	<i>Epicoccum nigrum</i>	<i>Acremonium strictum</i>	<i>Pichia membranifaciens</i>	<i>Penicillium chrysogenum</i>	Percentage of occurrence (%)
No-herb	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	41
GLY+PRQ (1yr)	+	-	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	22
PRQ (4yrs)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	15
GLY (5yrs)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	11
PRQ (8yrs)	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	11

- = absent, + = present, No-herb = Soil without history of herbicides, GLY = Glyphosate, PRQ = Paraquat, yrs = years

twenty days compared to this study that deal with what happened on the field. The decreased in the organic matter content in soil treated with herbicides could be attributed to lack of vegetation cover on soil from which samples were taken which exposed the top soil to different kind of erosion and absence of macroorganisms and soil fauna that could help in the processes of aeration (earthworm) and mineralization process in the soil due to herbicide application [28].

The general decreases in the soil moisture contents could be attributed to the time of the season (November) in which the soil samples were collected due to the reduced rainfall and precipitation (if any) during this period coupled with the onset of harmattan season. The decreases in moisture contents in soil where herbicides were used compared to the control could be attributed to lack of vegetation cover (grasses) which exposed the topsoil directly to sunlight thus accelerated the rate at which it loses moisture into the atmosphere through evapotranspiration. Also, the exposure of topsoil to direct sunlight exposes the photosensitive microorganisms to the harmful effect of the ultraviolet radiation which led to the death of some microorganisms reducing the population of soil microorganisms. However, soil without herbicides is covered with grasses (mulch) which helps to retain the soil moisture. The presence of grass cover and straw subsequently increases the soil organic matter content as the presence of moisture increases the activities of microorganisms that degrade the dead plant material in the soil without history of herbicides application [28,29,30].

The composition of bacteria species isolated on the soils treated with different herbicides varied based on the type of herbicides used and duration of application compared to the control. This shift in heterotrophic aerobic bacteria and fungi population composition in soil indicates that different herbicide exhibits different selective toxicity on soil aerobic heterotrophic bacteria and fungi [31,23]. These results are also supported by Zabaloy [5] who observed that herbicides altered the soil microbial population structure. Additionally, Araújo [32] discovered that application of glyphosate on soil causes changes in the activity of soil microbes. The occurrence of *Rhodoturula minuta* in all soil samples indicates that the fungus could resist the toxicity effect of glyphosate and paraquat or

degrade it (herbicides) and use it as a source of carbon and energy [25,24].

4. CONCLUSION

The results of the present study indicate that the prolonged usage of herbicides in agricultural soils pose a danger to non-target soil inhabitants like heterotrophic microorganisms (bacteria and fungi) by reducing their population and changes the microbial species structure. Also, the prolonged usage of herbicides has been implicated in altering the soil pH, reducing the moisture content and soil organic matter content. Hence, the use of herbicides as a weed controller in modern agriculture at recommended rate should be cautioned. Also, it is advised that the agrochemicals industries should find alternative herbicides formulations that are less toxic to the non-target organisms in the soil.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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