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BIODIVERSITY AND CHANGES IN SOIL MICROBES IN A MAIZE FIELD TREATED WITH SOLE AND HERBICIDES MIXTURES IN SOUTHWEST, NIGERIA

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Abstract
The diversity and population of soil microbes play significant role in the health state of arable field. This research was designed to investigate the effect of diuron and S-metolachlor on soil microbial population and species diversity in a maize-based cropping system. Soil samples were taken at 0 – 15 cm depth using a soil auger five times at two weekly intervals, starting from the time before the herbicide was applied and after herbicide spraying and soil microbial population and species composition were determined. Variable mixtures of the two herbicides, Diuron (2.5 l/ha) and S-metolachlor (4 l/ha) constitute the 5 treatments and a control. Standard methods were used in isolation, enumeration and identification of microorganisms. Data on microbial population were analysed using Analysis of Variance with means separated by Duncan Multiple Range Test (DMRT) at 5% probability level while bacterial and fungal species composition were displayed as percentage occurrence on bar charts. The population growth of bacteria was enhanced by 96.8% and 107.4% respectively under the two herbicides when compared with the result obtained before herbicide was sprayed, Diuron and S-metolachlor at the recommended rate (100%) at 2 WAS with reference to the control. At the plots that received a quarter of Diuron concentration mixed with 75% S-metolachlor, bacterial population increased by 50% while the rate of increase reduced to 16.3% at 4 WAS. Further reduction in microbial population was observed at 6 WAS from 31% to 21% at 8 WAS. From 2 WAS to 6 WAS, there was no significant different in the fungi population among all the treatments and control. The most common species of bacteria that occurred and identified on the plots include, Proteus vulgaricus (38.92%), Pseudomonas telluria (50.04%) and Bacillus stearothermophilus (50.04%). Penicillium dangeardi while fungal species encountered were Aspergillus restrictus (44.48%), Penicillium roqueforti (38.92%). The two herbicides affected the bacteria and fungi in terms of species composition and population variation.

Keywords: Diuron, S-metolachlor, Bacillus stearothermophilus, Penicillium roqueforti Pseudomonas telluria

Introduction
S-metolachlor is relatively non-persistent (DT50=21 days, field, aerobic) and moderately mobile (KOC=226 L kg−1) in soil, but very persistent in water (Shaner et al., 2006; Anonymous (PPDB), 2009). Both the racemic mixture and the S-enantiomer have the potential to cause adverse effects on microorganisms (Moorman et al., 2001; Papaefthimiou et al., 2004) and on higher terrestrial and aquatic organisms (PPDB 2009;Papaefthimiou et al., 2004; Liu et al., 2006; Xuet al., 2010). Freshwater macrophytes and microalgae are however the most vulnerable ones (Pérezet al., 2011;PPDB, 2009;Junghans et al., 2003).

Manufacturers have adopted flooding the agrochemical market with all kinds of herbicides that are meant for the elimination of different kinds of weeds at different stages of their growth (Sebiomoet al., 2011). Perhaps, the efficacy of these herbicides in controlling the target weeds has resulted in the application of these chemicals by most farmers. The soil serves as the repository for all agricultural contaminants which function as a major habitat for most microbial communities such as soil bacteria, fungi and actinomycetes whose activities influences the soil fertility and soil health (Zain et al., 2013), through organic material degradation, organic matter decomposition and nutrient cycling (De-Lorenzo et al., 2001; Hutsch, 2001). Nonetheless, application of these chemicals often inhibit some of these natural processes, and decreases the performance of the non-target organisms (Subhani et al., 2000). However, some soil organisms use these herbicides in the process of degradation as carbon energy source for their metabolic activities. The research was designed to investigate the effect of two herbicides, diuron and S-metolachlor on soil microbial population and species diversity.

Materials and Methods

The experiment was carried out at the Teaching and Research farm of the Faculty of Agricultural Sciences, Ladoke Akintola University of Technology, Ogbomoso (4o101 E and 8o101 N), Oyo State, Nigeria during the 2014 cropping season. Annual rainfall can be as high as 1286 mm/annum with average precipitation of 100 mm/annum extending from March to November, warm and moist conditions start from April to October and temperature ranges from 28°C to 33°C. Humidity is as high as 74% all year round except in January when dry wind blows from the north. Soil prevalent in the area is well drained and of a sandy loamy texture.

The land was manually prepared and then divided into 6 plots sizes of 5 m x 5 m, replicated three times. The experiment was laid out in a Randomized Complete Block Design (RCBD) with 5 treatments, each block separated by 3 m spacing.

Soil samples were taken at 0 – 15 cm depth using a soil auger (located three spots per plot), five times at two weekly intervals, starting from the first sample taken before spraying and after herbicide spraying. The Initial samples were mixed together and sub sampled. The initial sample was used along with the other samples for soil microbial population and species composition analyses.

Variable mixtures of two the herbicides, Diuron and S-metolachlor constitute the treatments.

- T1 - 100% of Diuron (D) (2.5 l/ha)
- T2 - 100% of S-metolachlor (S) (4 l/ha)
- T3 - 25% S-metolachlor (1 l/ha) + 75% of Diuron (1.875 l/ha)
- T4 - 50% of S-metolachlor (2 l/ha) + 50% of Diuron (1.25 l/ha)
- T5 - 75% of Diuron (1 875/ha) + 75% of S-metolachlor (3 l/ha)
- T6 - Control

Spraying was done with knapsack sprayer with spray volume of 200 l/ha, the control plot was not sprayed.

Isolation, Enumeration and Identification of Microorganisms

The isolation of the associated microorganisms was carried out by standard microbiological techniques. Ten (10 g) grams of the soil samples were thoroughly mixed with 90 ml of sterile distilled water. The mixtures were serially diluted and 0.1 ml of 103 and 105 dilutions was used for bacterial and fungal isolation respectively. Pure cultures obtained were characterized and identified using Barnett and Hunter (1972) and Onions et al. (1995). The microbial density of the soil samples was done by carrying out the serial dilution fold and each sample was plated from the dilution fold on plate count agar in duplicates. They were then incubated at 35oC for 24 hours before the plates were counted. Another 20 g of the soil was collected using sterile sampling bottle. Analysis of the sample was performed in the laboratory. Standard inocula were prepared from the stock culture taking one looful of the isolate. The plates were incubated at room temperature for 24 hours. Isolates were identified on the basis of their characteristics by the identification schemes as prescribed by Raul et al. (2001).

Data Analysis

Data on microbial population were analysed using Analysis of Variance with means separated by Duncan Multiple Range Test (DMRT) at 5% probability level while bacterial and fungal species composition were displayed as percentage occurrence on bar charts.

Results and Discussion

Results

Population growth of bacteria was enhanced by 96.8% and 107.4% under the two herbicides, Diuron and S-metolachlor at the recommended rate (100%) at 2 WAS when compared with the control plot though the increase was not significantly different (Table 1). At 4 WAS, the bacterial population reduced by a factor of 66.5% under 100% S-metolachlor plot while the reduction observed under Diuron was 34.4% but not as much as the other herbicide mixtures at 4 WAS. The population in the control increased in manifold at 4 WAS. Reduction in the bacterial population persisted till 8 WAS (11.1%) under S-metolachlor recommended rate. There was a sudden increase in bacterial population under Diuron (100%) plot at 6 WAS (Table 1) but sharp reduction afterwards (14.8%) under the same treatment. At the plots that received a quarter of Diuron concentration mixed with 75% S-metolachlor, bacterial population increased by 50% while the rate of increase reduced to 16.3% at 4 WAS, further reduction set in at 6 WAS by 31% to 21% at 8 WAS respectively. There was reduction in bacterial population up to 4WAS but increased at 6WAS by 65.6% and then later crashed by 1.1% under equal amount of the two herbicides (50:50). Enhanced bacterial population at 4 WAS by 166% was recorded at higher Diuron (75%) in the mixture; there was a slight increase which was followed by 31.1% and 20.6% reduction at 6 WAS and 8 WAS respectively.

Table 1: Population (x104CFUg-1) and Percentage Change in Population of Bacteria Extracted from the Soil

Treatment	2 WAS	%	4 WAS	%	6 WAS	%	8 WAS	%
Initial Soil	620.0cd	-	620.0cd	-	620.0cd	-	620.0cd	-
Control	626.7cd	-	1433.3ab	-	203.3c	-	270.0b	-
Diuron (D) (100%)	1233.3abc	**96.8**	940.0b	**-34.4**	716.7a	**252.5**	230.0b	**-14.8**
S-metolachlor(S) (100%)	1300.0ab	**107.4**	480.0c	**-66.5**	176.2c	**-13.1**	240.0b	**-11.1**
25% D + 75% S	940.0bcd	**50.0**	1666.7a	**16.3**	140.0c	**-31.1**	213.3b	**-21.0**
50% D + 50% S	480.0d	**-23.4**	1126.7abc	**-21.4**	216.7c	**65.6**	267.6b	**-1.1**
75% D + 25% S	1666.7a	**166.0**	150.0ab	**4.7**	140.0c	**-31.1**	263.3b	**-2.6**

Values in the table with the same alphabet along the column were not significantly different
WAS = Weeks after spraying
% = Percentage change in population

Initial bacterial abundance in the soil was shown in Figure 1a. The species diversity was summarized for all the plots at each sampling period and depicted on bar charts to show percentage occurrence.

At two weeks after spraying (2 WAS) bacteria species extracted from the soil on all the plots occurred at variable percentages. Eight of them appeared on the plots more than 25% with the highest as Proteus vulgaricus having 38.92%. Others were *Salibacillus spp.* and *Spirilum spp.* 33.36%; 27.8% for each of *Bacillus subtilis*, *C. sporogenes*, Pseudomonas fluorescens, *P. telluria* and *Bacillus stearothermophilus* (Figure 1b). Eight bacteria spp. had the lowest occurrence of 5.5%. Bacterial extraction revealed almost constant number of bacteria species of 33 but the composition of the species varied with some new ones encountered while some that were present on the plots by 2 WAS disappeared by 4 WAS. Only five species had over 25% occurrence, P. telluria, 50.04%; B. stearothermophilus, 44.48%; Proteus vulgaricus, 38.92; B. subtilis and P.flurescens, 27.8% (Figure 1c).

Pseudomonastelluria and *B. stearothermo-philus* increased drastically all over the plots from 2 WAS to 4 WAS. The lowest occurred species were eleven with 5.56%.

B. stearothermophilus and *P. vulgaricus*, 50.04%; *P. telluria* and *B. subtilis*, 38.92%; P. fluoroscens, 33.3% and Salibacillus spp. were recorded at 6 WAS. Only four species out of 24 had the lowest percentage occurrence of 5.56% (Figure 1d).Only 23 species of bacteria were extracted on all the plots at 8WAS.

Bacillus stearothermophilus had 50.04%; *P. telluria* and *P. vulgaricus*, 33.36%; *P. fluorescens*, *B. subtilis* and *Salibacillus spp.*, 27.8%. Four out of the species encountered 8 WAS had the least percentage occurrence of 5.56% (Figure 1e).

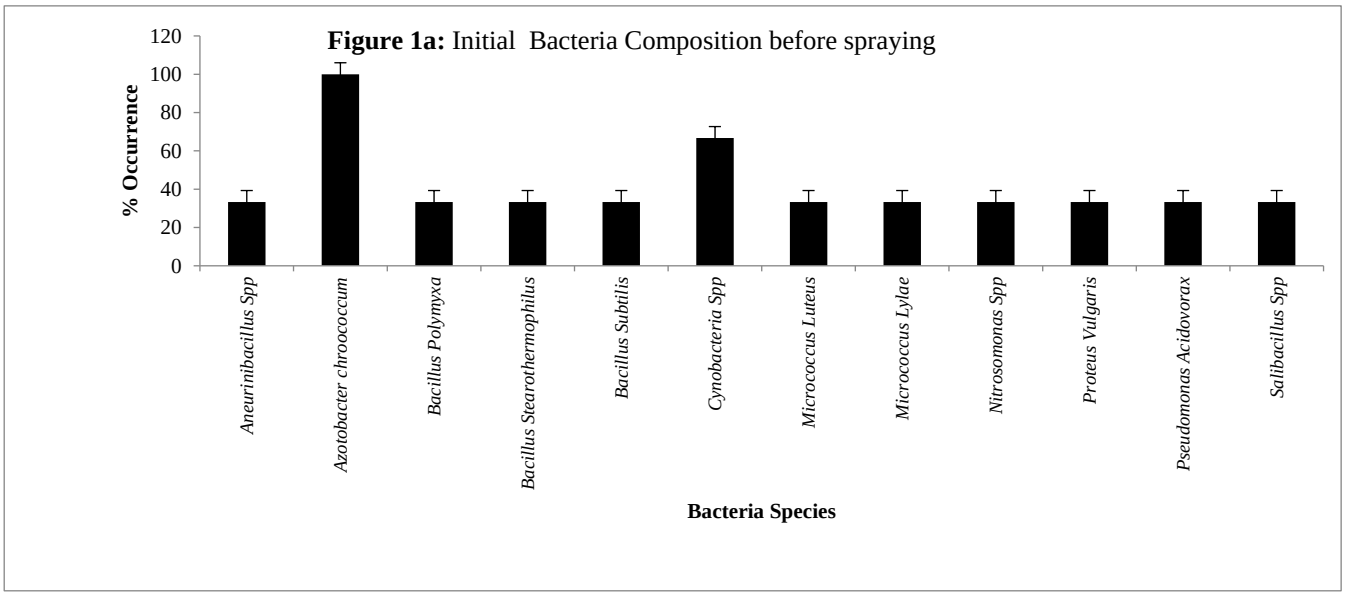


Figure 1a: Initial Bacteria Composition before spraying

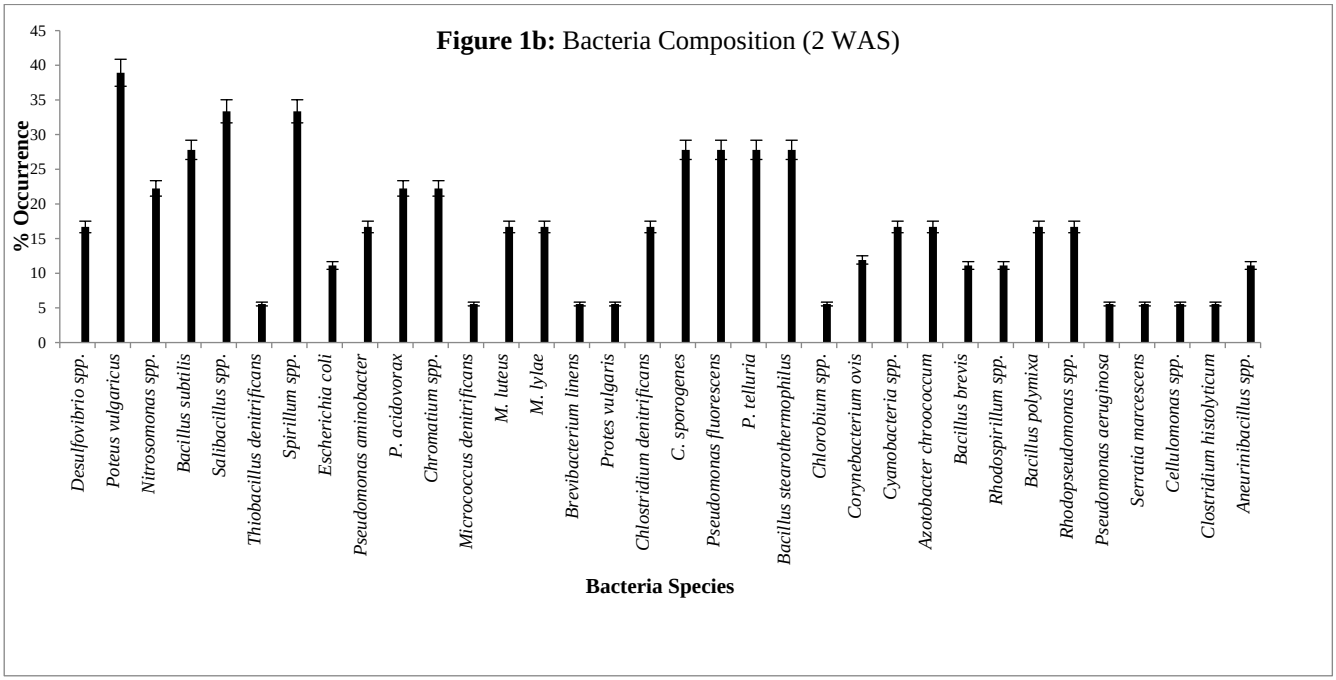


Figure 1b: Bacteria Composition (2 WAS)

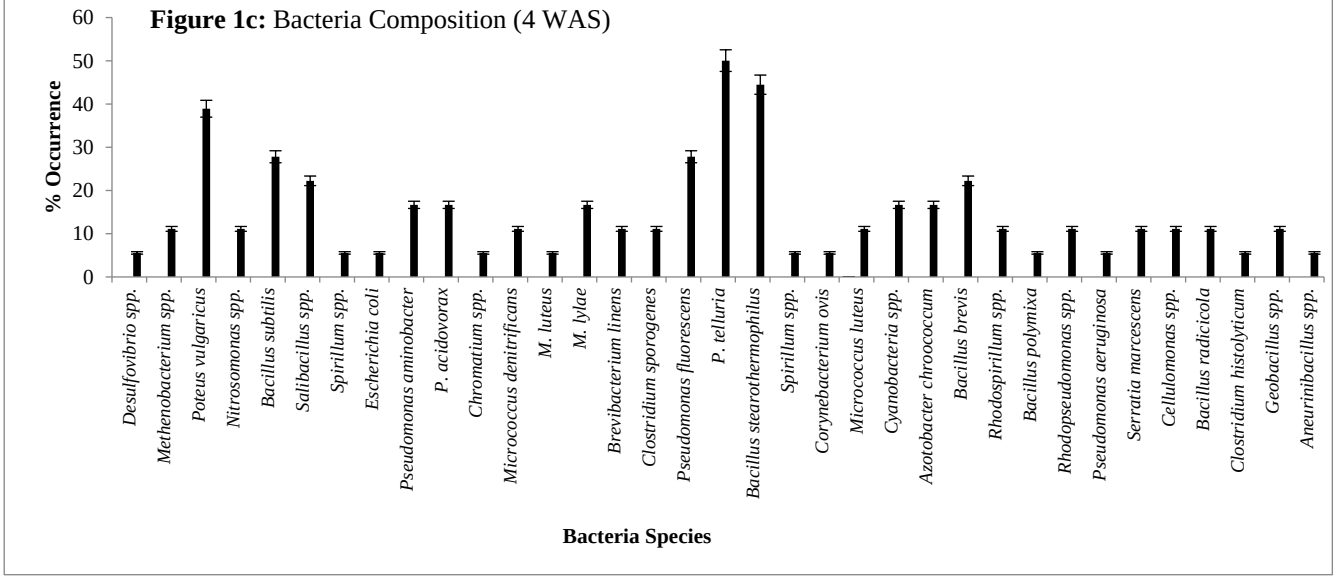


Figure 1c: Bacteria Composition (4 WAS)

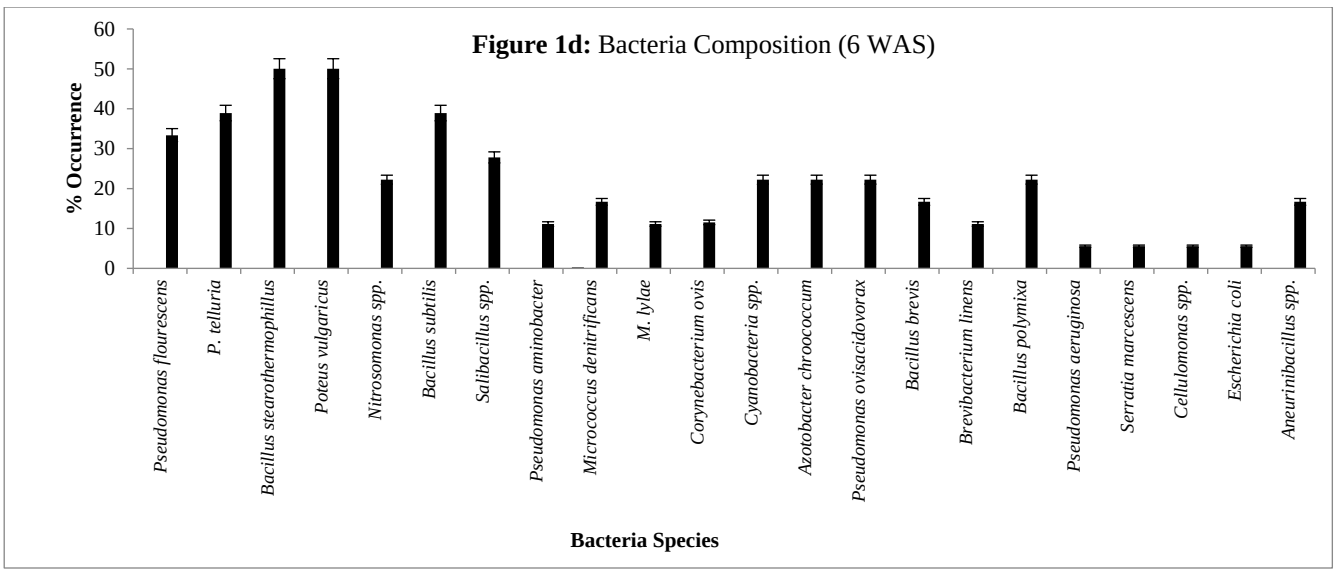


Figure 1d: Bacteria Composition (6 WAS)

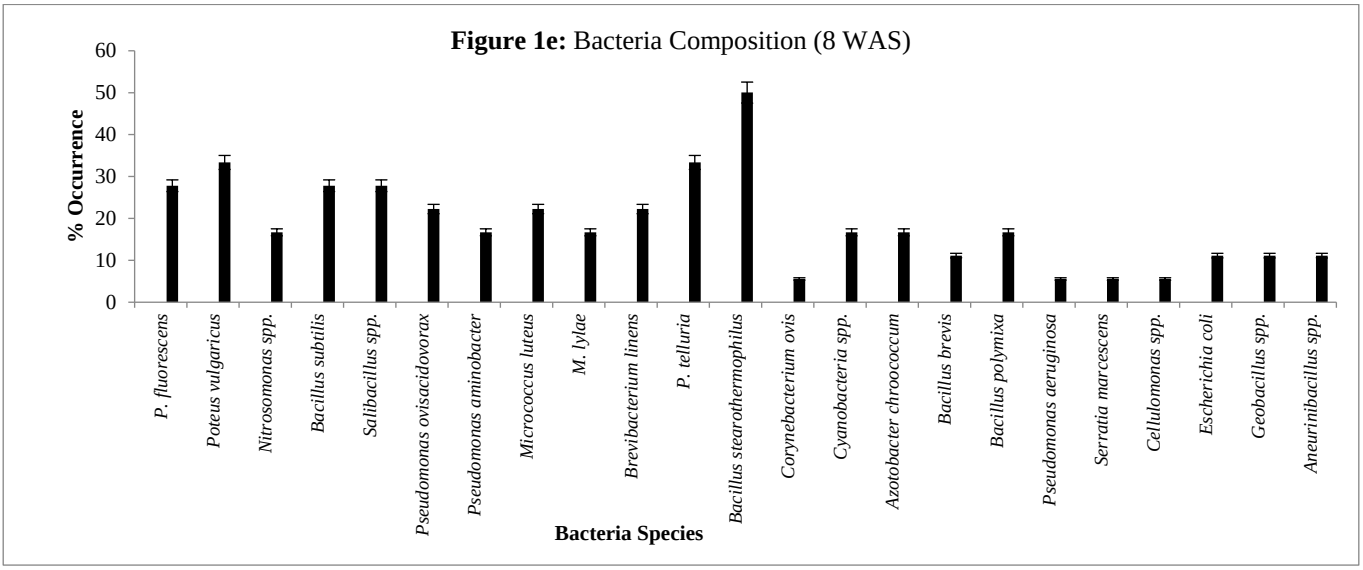


Figure 1: (a-e): Bacteria Diversity Before and 2, 4, 6, 8 Weeks after Spraying (WAS) of Herbicides.

Thus, there was conspicuous reduction in species composition on the plots from 4 WAS to 6 WAS. Initial fungal abundance in the soil was shown in Figure 2a with 14 species recorded.

Table 2 shows the population and percentage change in population of fungi extracted from the soil. From 2 WAS to 6 WAS, there was no significant different in the fungi population among all the treatments and control. However, the population of fungi at 8 WAS decreased in all the treatments except the initial population and control. The same trends observed in population changes for bacteria in the treated soil was also observed for fungal population, the increase and decrease was in haphazard manner from 2 WAS to 6 WAS.

Table 2: Population ($x10^4 CFUg^{-1}$) and Percentage Change in Population of Fungi Extracted from the Soil.

Treatment	2 WAS	%	4 WAS	%	6 WAS	%	8 WAS	%
Initial Soil	186.7a	-	186.7a	-	186.7a	-	186.7a	-
Control	1900.0a	-	2033.3a	-	1833.3a	-	2366.7a	-
Diuron (D) (100%)	2400.0a	**26.3**	1500.0a	**-26.2**	2633.3a	**43.6**	1666.7b	**-29.6**
S-metolachlor (S) (100%)	8133.0a	**328.1**	1333.3a	**-34.4**	1766.3a	**-3.7**	1700.0ab	**-28.2**
25% D + 75% S	1933.0a	**1.7**	2333.3a	**14.8**	1633.3a	**-10.9**	1033.38b	**-56.3**
50% D + 50% S	1833.0a	**-3.5**	1933.3a	**-4.9**	2133.3a	**16.4**	2033.3a	**-14.1**
75% D + 25% S	8133.3a	**328.1**	2100.0a	**3.3**	1633.3a	**-10.9**	1833.3a	**-22.5**

Values in the table with the same alphabet along the column were not significantly different
WAS = Weeks after spraying
% = Percentage change in population

Species composition of fungi was very high with 39 different species encountered 2WAS. Eight of the species had percentage occurrence higher than 25%. These were Penicillium dangeardi and Aspergillus restrictus, 44.48%; P. roqueforti, *A. oryzae*, 38.92%; *Talaromyces flavus*, *Chlorophyceae spp., A. niger* and *Waksmania spp.* had 27.8% occurrence. Lowest percentage, 5.56% was recorded by eight fungal species (Figure 2b).

Two weeks after spraying, the species composition dropped drastically to 22 fungal species with only P. dangeardi having the highest, 22.24% occurrence. Ten of the species occurred with the lowest percentage of 5.56% (Figure 2c).

A slight increment in fungal species composition and occurrence were observed at 6WAS with seven fungal species occurred more than 25%. *Penicillium dangeardi*, 55.6%; *Talaromyces flavus*,

A. restrictus, *P. roqueforti*, and *Waksmania spp.*, 38.92%; and *Neosaterya fischen* having 27.8% occurrence. Four species had the least percentage of 5.56% (Figure 2d).

About the same species composition (28 species) was recorded at 8WAS with only five of them occurring more than 25% on all the plots. These were *Penicillium dangeardi*, 50.04%; *P. roqueforti*, 38.92%; *A. nidulans* and *Fusarium nivale*, 33.36%, and *Rhizopus oligosporus* having 27.8% occurrence (Figure 2e).

In contrast to the species composition of bacteria earlier mentioned, fungal species composition crashed after two weeks of spray but later gradually increased up to eight weeks but not to the level of attaining the initial number encountered.

Discussion

Herbicides can enhance or inhibit the activities of soil microbial organisms depending on the type of herbicides and the microbe species involved. According to Wardle and Rahman (1992) the increase suggests the capacity of the organisms to degrade some aspect of the herbicide and utilize it as a carbon source to support their growth and multiplication. Also, the increase in bacterial population after the herbicide treatment has been reported by Sebiomo et al., (2011), who also recorded an increased bacterial population in the first and second week of herbicide treatment.

The 50% equal concentrations of the two herbicides reduced the population of bacteria below the control and the initial sample at 2WAS, 4WAS and 8WAS suggesting that the synergistic effects of the mixture was inhibitory to the microbial activities. The same trend was observed with fungal population. Anderson et al. (2000) reported that there was a sharp decrease in bacterial population which might be due to the fact that the rise in bacterial population was lethal. Zain et al. (2013) also reported such free-fall decrease in microbial population. The general reduction in population of both fungi and bacteria at 8WAS could be due to shortage of the herbicides in the soil and subsequent reduction in available carbon source for energy utilization. Certain bacterial and fungal species appear and disappear along the tenure of the experiment. The numbers of the microbes vary from 2WAS to 8WAS so also was the species diversity. This could be due to the food requirement based on the applied herbicides. Some will prefer the sprayed active ingredients while some will dwell on the breakdown products for their energy source. Furthermore, the active ingredients of Diuron and S-metolachlor may be inhibitory to some species while the breakdown products may be inimical to the microbes. Michael and Stephen (2016) reported that some fungi found in the baseline determination were not seen after the herbicide treatment and vice versa. Some bacteria and fungi species were able to survive; even some at higher population, from the beginning to the termination of the experiment at 8WAS e. g. Pseudomonas aeruginosa, Bacillus stearothermophilus, Bacillus subtilis and *Nitrosomonas spp.* Fungi species include *Aspergillus glaucus*, *A. nidulans*, *A. restrictus* and *Penicillium roqueforte*.

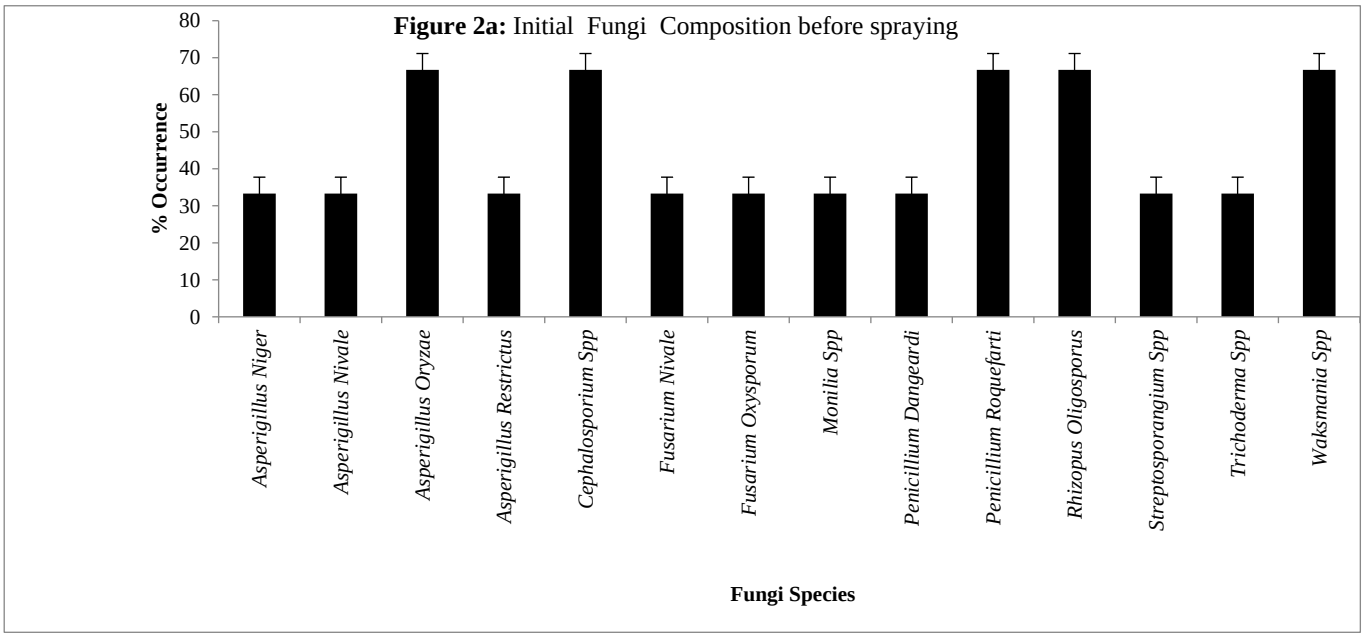


Figure 2a: Initial Fungi Composition before spraying

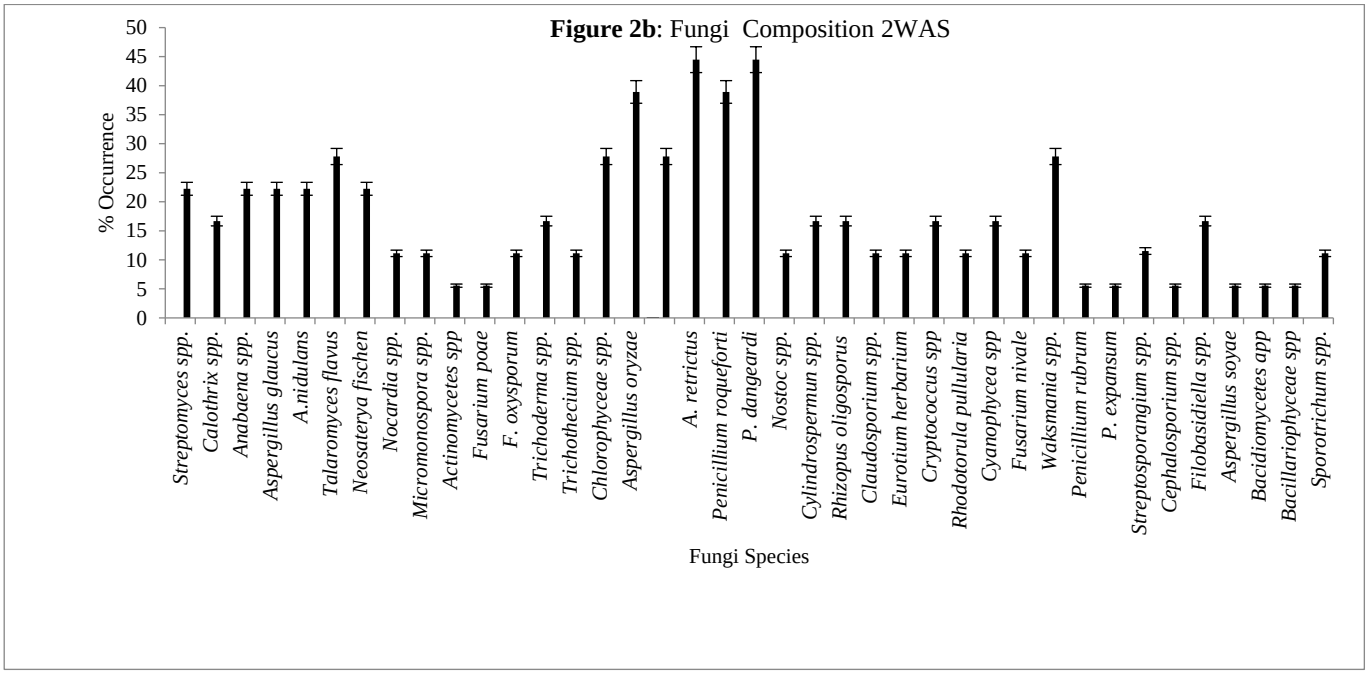


Figure 2b: Fungi Composition 2WAS

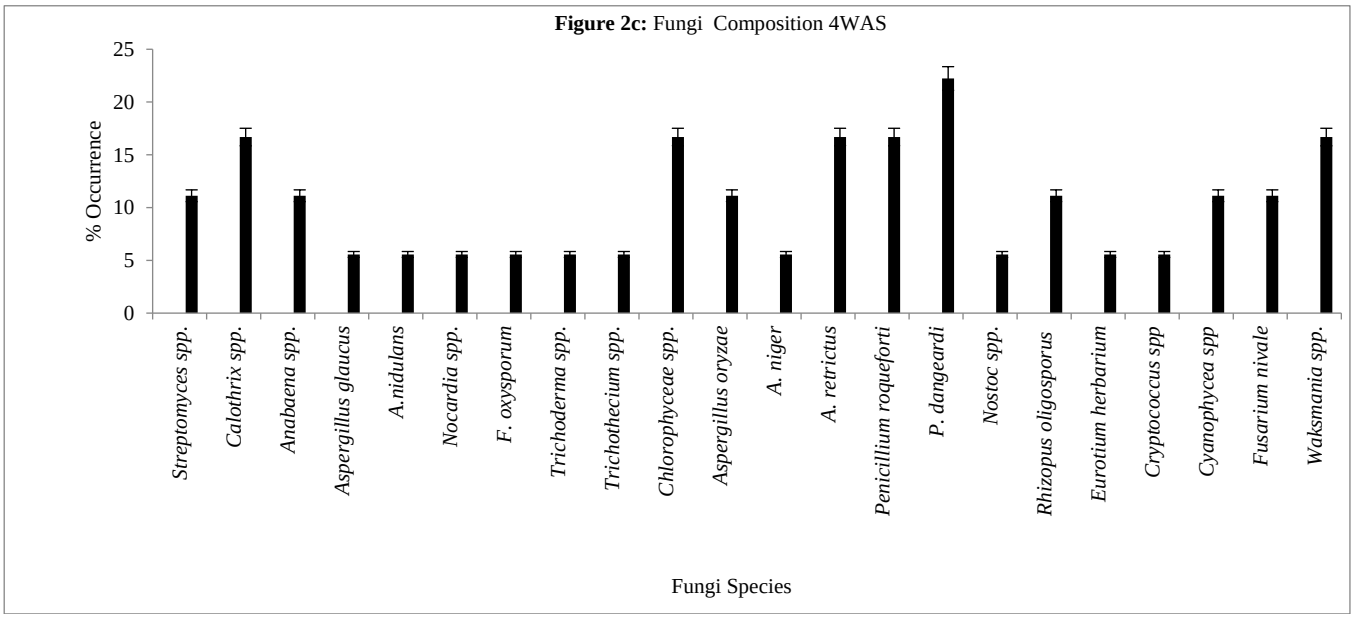


Figure 2c: Fungi Composition 4WAS

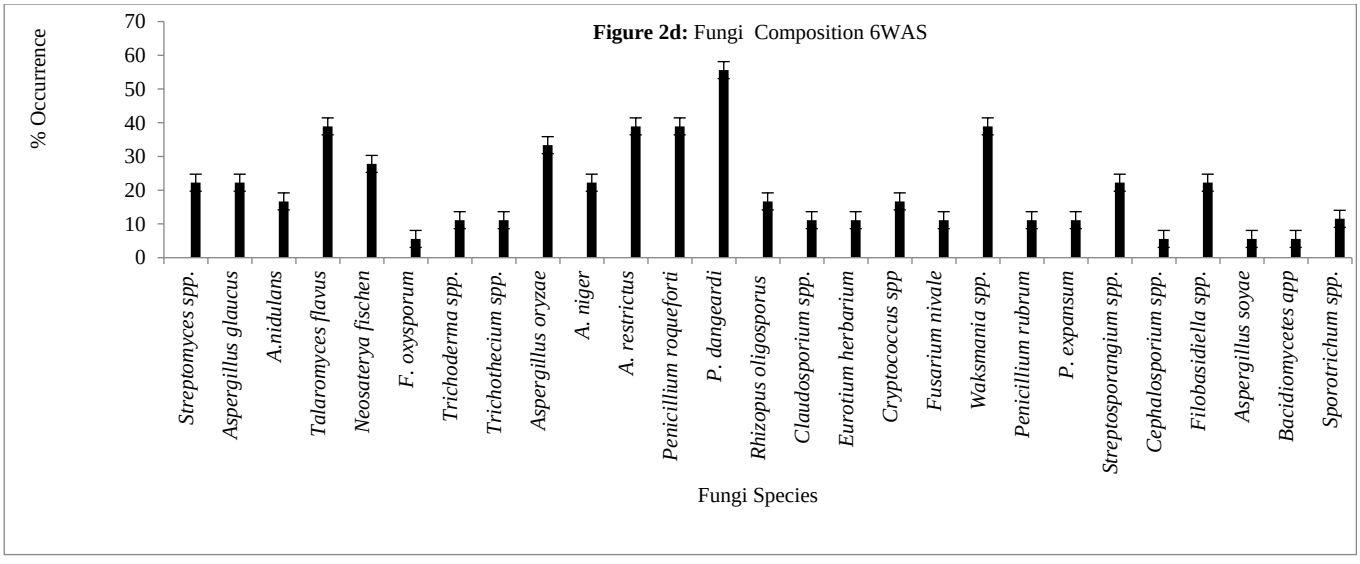


Figure 2d: Fungi Composition 6WAS

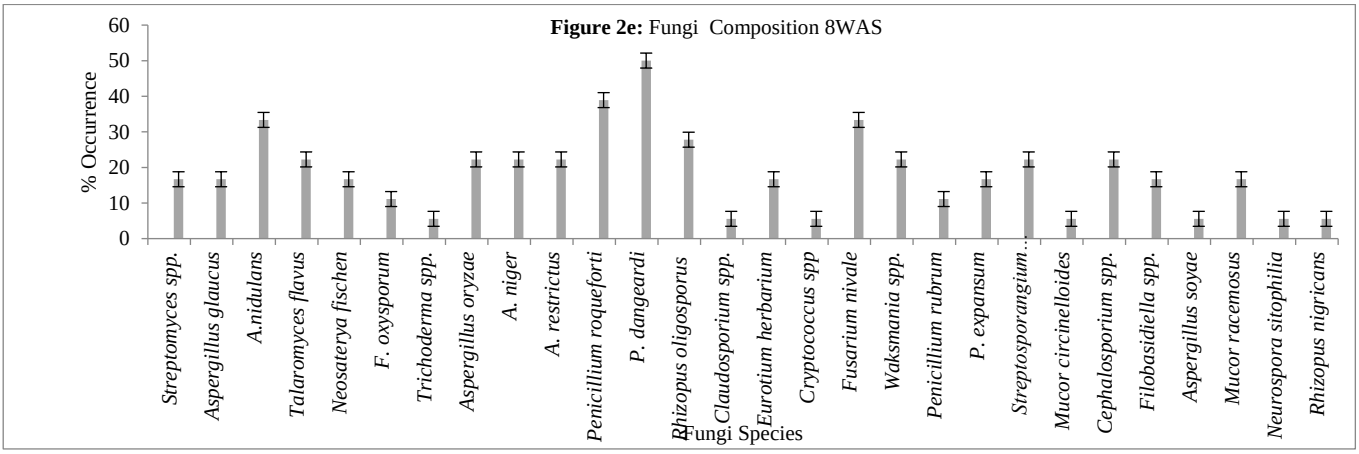


Figure 2: (a-e): Fungi Diversity Before and 2,4,6, 8 Weeks After Spraying (WAS) of Herbicides.

Conclusion

Appreciable change in both population and species diversity of the bacteria and fungi were observed in this study. The two herbicides affected the bacteria and fungi almost the same way in terms of species composition and population variation. Some bacteria and fungi that were recorded previously may not be encountered subsequently while new ones appear.

References

Anderson L., Diwan B. A., Fear N. T., & Roman E. (2000). Critical windows of exposure for children's health: cancer in human epidemiological studies and neoplasms in experimental models. Environmental Health Perspect, 108, 573–594

Barnett H. L. and Hunter B. B. (1972). Illustrate General of imperfecti Fungi, 3rd edition, burgess publishing company. Minneapolis. Pp. 331.

De-Lorenzo M. E., Scott G. I. & Ross P. E. (2001). Toxicity of pesticides to aquatic microorganisms: a review. Environmental Toxicology Chem. 20, 84-98.

Hutsch B. W. (2001). Methane oxidation in non-flooded soils as affected by crop production. Invited paper. Eur. J. Agronomy, 14, 237-260.

Junghans M., Backhaus T., Faust M., Scholze M. & Grimme L. H. (2003).Predictability of combined effects of eight chloroacetanilide herbicides on algal reproduction. Pest Manag. Sci. 59:1101–1110.

Liu H., Ye W., Zhan, X & Liu W. (2006). A comparative study of rac- and S-metolachlor toxicity to Daphnia magna. Ecotoxicol Environ Saf. 63:451–455.

Michael Opoku Adomako and Stephen Akyeampong (2016). Effect of Some Commonly Used Herbicides on Soil Microbial Population. Journal of Environment and Earth Science ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online) 6 (1): 30-38.

Moorman T. B., Cowan J. K., Arthur E. L., Coats J. R. (2001). Organic amendments to enhance herbicide biodegradation in contaminated soils. Biol Fertil Soils.33:541–545.

Onions A. H. S., Allsopp D., and Eggins H. O. W. (1995). Smith introduction to industrial mycology 8th edition. The pitman press, Bath Pp. 65-92.

Papaefthimiou C., Cabral M. G., Mixailidou C., Viegas C. A. & Sá-correia I. (2004). Comparison of two screening bioassays, based on the frog sciatic nerve and yeast cells, for the assessment of herbicide toxicity. Environ Toxicol Chem. 23:1211–1218.

Pérez J., Domingues I., Soares A.M.V.M. & Loureiro S. (2011). Growth rate of Pseudokirchneriella subcapitata exposed to herbicides found in surface waters in the Alqueva reservoir (Portugal): a bottom-up approach using binary mixtures. Ecotoxicology.20:1167–1175.

PPDB (2009). The Pesticide Properties Database (PPDB) developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, funded by UK national sources and the EU-funded FOOTPRINT project (FP6-SSP-022704). Available: http://sitem.herts.ac.uk/aeru/footprint/en/index.htm. Accessed 20 April 2012.

Raul J. C. and Jaime S. C. (2001). Applied and environmental microbiology California Polytechnique. Pp. 779-786.

Sebiomo A., Ogundero V. W. &Bankole S. A. (2011).Effects of four herbicides on microbial population, soil organic matter and dehydrogenase activity. African Journal Biotechnology, 10(5), 770-778

Shaner D. L., Brunk G., Belles D., Westra P. & Nissen S. (2006). Soil dissipation and biological activity of metolachlor and S-metolachlor in five soils. Pest Management Science. 62:617–623.

Subhani A., El-ghamry A. M., Huang C., & Xu J. (2000).Effect of Pesticides (Herbicides) on Soil Microbial Biomass – A Review. Pakistan Journal of Biological Sciences, 3(5), 705-709

Wardle D. A. & Rahman A. (1992). Influence of pasture grass and legume swards on seedling emergence and growth of Carduus nutans L. and Cirsium vulgare L. Weed Resource, 32(2): 119-128. Water Environment Research, 66, 611-623.

Xu D., Wen Y.& Wang K. (2010).Effect of chiral differences of metolachlor and its (S)-isomer on their toxicity to earthworms. Ecotoxicol Environ Saf. 73:1925–1931.

Zain M. M. M., Rosli B. M., Kamaruzaman S., NurMasirah M. & Yahya A. (2013). Effects of selected herbicides on soil microbial populations in oil palm plantation of Malaysia: A microcosm experiment. African Journal of Microbiology Research, 7(5), pp. 367-374