



Greener Journal of Soil Science and Plant Nutrition

ISSN: 2384-6348

Submitted: 27/10/2016

Accepted: 31/10/2016

Published: 23/11/2016

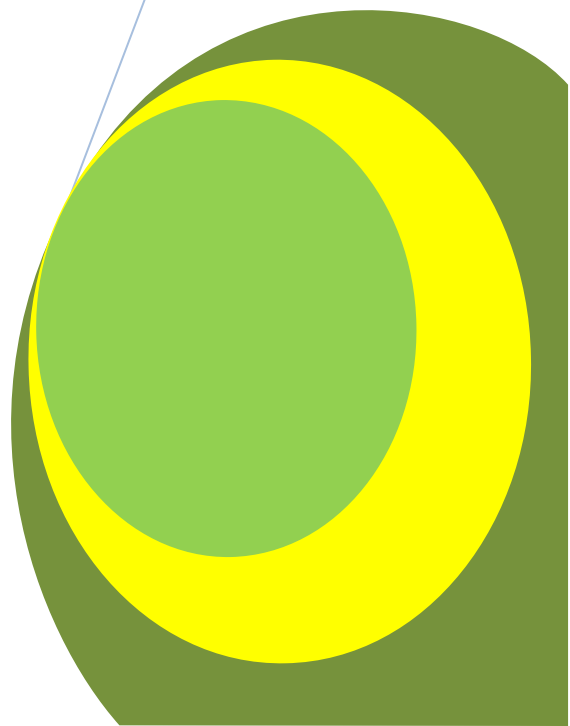
Subject Area of Article: Soil Microbiology

DOI: <http://doi.org/10.15580/GJSSPN.2016.1.102716175>

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Research Article (DOI: <http://doi.org/10.15580/GJSSPN.2016.1.102716175>)

The Effect of the Application of Different Rates of Herbicides on Soil Microorganisms

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ABSTRACT

Background: The use of herbicide in weed control has grown significantly in Nigeria in recent years. Most of the application are indiscriminately carried out by illiterate farmers, and therefore pose a significant threat to the environment and on crop yield. This study assessed the effects of the use of glyphosate for land preparation, and different concentrations of primextra dual gold (atrazine and metolachlor), for weed control on the type and population of soil micro-organisms.

Methods: The study was carried out in Port Harcourt in two phases, using a randomized complete block design with three replicates. Soil samples were collected at the depths of 0-15cm and 15-30cm, and analyzed 4WAP and 12WAP. Serial dilution technique was used for the enumeration of the bacteria and fungi in the soil samples, using the spread plate method, and the appropriate media and incubation conditions.

Results: The use of the herbicides resulted in changes in the soil, and in the type and number of soil micro-organisms. There were reductions in the organic matter and exchangeable minerals content of the soil, and in the total count of the micro-organisms in the second phase of the study. The mean bacteria count in the first phase of the study were 5.75×10^8 CFU and 1.13×10^8 CFU at the depths of 0 – 15cm and 15 – 30cm respectively, compared to 2.88×10^4 CFU and 6.32×10^4 CFU in the second phase of the study. The reductions were more in the plots in which the herbicides had been applied above the recommended dose.

Conclusion: The application of the herbicides resulted in changes in the soil, and type and total number of soil micro-organisms, especially when they are applied at above the recommended rate. Efforts are therefore needed to educate farmers on the proper dosing of the herbicides.

Keywords: Herbicides, Glyphosate, atrazine, metolachlor, bacterial count, soil fungal count, Nigeria.

1. INTRODUCTION

The use of herbicides in weed control has grown significantly in Nigeria in recent years (Iyagba, 2013; Agahiu et al 2012; Agahiu et al 2011). This increase has been linked to the ease of application, the effectiveness of the herbicides in controlling the common weeds in Nigerian farms (Agahiu et al 2012; Agahiu et al 2011); and the growing difficulty in hiring labour to carry out the traditional method of manually cutting off the weeds (Tijani, 2006). The use of the herbicides is also actively promoted for its tendency to increase crop, especially in Nigeria, where there is growing food insecurity (Eme et al, 2014; Ojo and Adebayo, 2012; Iyagba, 2013; Agahiu et al, 2012).

Most of the application of the herbicides in Nigeria are however carried out by illiterate farmers who apply the herbicides indiscriminately, mostly because they had not received any training on the judicious application of the herbicides (Agahiu et al 2012; Agahiu et al 2011; Tijani 2006). This poses a significant threat to the environment, crop yield and on human health, as studies have indicated that such indiscriminate use of herbicides can leach into the environment, threatening non-target organisms (Ize-iyamu et al, 2007); harm soil micro-organisms, thus affecting

soil fertility (Ayansina & Oso, 2006); and can accumulate in harvested crops, possibly causing direct harm to humans, upon the consumption of the harvested crops. (Gushit et al, 2013; Myers et al 2016). There is therefore a need to carry out an elaborate study on the use of the herbicides in Nigeria, especially as the quoted studies and many other previous studies did not fully replicate the current field situation in Nigeria. Ayansina and Oso (2006) carried out a study on the effects of the different concentrations of the herbicides on soil microflora, but the study was carried out in-vitro, in laboratory condition, and not in the farming conditions in which the herbicides are commonly used in Nigeria (Ayansina and Oso, 2006); while Partoazar et al (2011) carried out a study on the effects on soil microbial activities of the application of glyphosate, a herbicide that is commonly used in land preparation for farming purposes, but they did not assess the effects of the further use of commonly used selective herbicides for weed control, in farmland previously prepared with glyphosate.

This study assessed the effects on soil micro-organisms of the sequential use of the pre-emergent herbicide, glyphosate that was used for land preparation, and common post-emergent herbicides, atrazine and metolachlor that were used for weed control. It thus assessed the possible effects of the herbicides in different scenarios, including the use of the herbicides in land preparation and weed control, and the effects of the different concentrations of the herbicides. It is therefore hoped that the study would provide greater insight into the effects of the herbicides on the soil micro-organisms. The study was carried out in maize (*Zea mays* L), because it is a short duration crop, and one of the crops the herbicides are commonly used to cultivate in Nigeria.

2. MATERIALS AND METHODS

2.1. Experimental site and location

The field studies were carried out in a plot of land at the University of Port-Harcourt during the 2013 and 2014 farming seasons, with the experiment laid out in a randomized complete block design with three replicates, and plot size of 2m x 2m. The experimental farm is well drained plot of land, with sandy loam soil, located at a latitude of 4.9027° N, and a longitude of 6.9205° E. It is in the rainforest ecological zone of the Niger delta region, and like most other communities in southern Nigeria has two seasons, the rainy season and the dry season, with an average annual rainfall of about 3,000 mm (118.1 in).

2.2. Land preparation and application of herbicides

The experimental site was fallowed for two years, and was prepared for the experiment by the application of "Round-Up", a proprietary glyphosate herbicide, bought from the Port Harcourt office of the Rivers State Agricultural Development Programme (ADP). This non-till methods was adopted for the study, because it has been shown to reduce leaching and erosion (Ding et al, 2002), which is vital in containing the experimental herbicides within the experimental plots.

Glyphosate [N-(phosphonomethyl) glycine] is the most widely used herbicide in the world, preferred for its low toxicity to mammals, and its low environmental impact (Franz et al, 1997). It was used in preparing the experimental plot for the study, not only for its low toxicity to mammals and the environment, but also because of its broad spectrum, non-selective weed killing capacity, and its proven effectiveness in non-till agriculture (Franz et al, 1997). The glyphosate was applied to the experimental plot at the manufacturer's recommended rate; and resulted in the complete burning off of the weeds. The experimental site was then left for two weeks, to allow the burnt grasses to completely dry, before the maize seeds are planted in rows.

The maize variety used for the study, the swan-1-yellow was obtained from the Green River Project of the Nigerian Agip Oil Company, and is widely cultivated in the farming communities of the Niger delta region. The seeds were sown using the recommended seed rate of 25kg/hectare, at a planting distance of 75cm between the rows, while an intra-row spacing distance of 25cm was maintained on flat. Extra plants were thinned out at the early growth stage, to maintain one plant per hole after two weeks of planting.

Primextra dual gold, a proprietary herbicide containing 290g/l of metolachlor and 370g/l of atrazine, manufactured by Syngenta Nigeria Limited, and purchased from a local dealer in Port Harcourt was then applied to the experimental plot, immediately after planting the maize. Atrazine {2-chloro-4-(ethylamino)-6-isopropylamino-1,3,5-triazine} is one of the most widely used 5-triazine herbicide, used either singly, or in combination with other herbicides, as a pre-emergence herbicide in the control of broadleaf and grassy weeds (Munier-Lamy et al 2002; Hamid et al, 2011); while metolachlor [2-chloro-N-(ethy-6-methy(phenyl)-N-(2- methoxyl-1-methylethyl acetamide)] on the other hand is commonly used in combination with other herbicides, because it is a very selective herbicide, renowned for its activity against grassy weeds (Cao et al, 2008).

The Primextra dual gold was applied to the experimental plots at five different rates, corresponding to a quarter of the recommended dose, half of the recommended dose, the recommended dose, 1.5 times the recommended dose, and twice the recommended dose. The information label of the Primextra dual gold used for the study showed that the product contains 290g/l of metolachlor and 370g/l of atrazine, with a recommended dose for maize farm of 3.2 liters/hectare, which is equivalent to 2.11 kg ai/ha. Therefore, the herbicide was applied at 0.59 kg ai/ha, 1.06 kg ai/ha, 2.11 kg ai/ha, 3.17 kg ai/ha and 4.22 kg ai/ha to correspond to a quarter of the recommended dose, half of the recommended dose, the recommended dose, 1.5 times the recommended dose, and 2 times the recommended dose.

The control plot did not receive any herbicide application, before and after the maize seeds were planted. The plot was manually cleared using the weeding hand hoe.

2.3. Identification and enumeration of the soil microorganisms

Soil samples were collected diagonally from the experimental plots, for the identification and enumeration of the soil microorganisms, at two different depths of 0-15cm and 15-30cm, before land preparation, and then four and twelve weeks after the application of the herbicides.

Serial dilution technique was used for enumeration of the bacteria and fungi contained in the soil samples, using the spread plate method. The mineral salt medium was used in screening the bacteria and fungi from the soil samples, with antibiotics added into the fungal medium, to inhibit the growth of bacteria. Appropriate volumes of these media were then dispensed into Erlenmeyer flasks, plugged, and then sterilized, before being poured into sterile glass Petri dishes, to solidify. 0.1ml of a 10^{-6} dilution of each of the media was then inoculated into the appropriate nutrient agar and incubated appropriately. The media for heterotrophic bacteria were inoculated in a nutrient agar, and incubated at 30°C for 24 - 48hrs; Potato Dextrose Agar (PDA) was used for the fungi, and incubated at 25°C for 3 - 5days; while the mineral salt agar (MSA) was used for the degrader bacteria and fungi. After the incubation, the bacteria and fungi in the plates were identified with the aid of the appropriate biochemical tests, counted and expressed as colony forming units per gram (cfu/g).

2.4. Data Analysis

The data collected during the study were put in a database, and then analyzed using SPSS and Microsoft Excel, after checking for consistency and completeness. Summary measures were calculated for each outcome of interest, while the test of significance was conducted using the relevant statistical test, at 95% confidence interval, with P-value of 0.05 or less considered statistically significant.

3. RESULTS

The physio-chemical characteristics of the soil of the experimental plots are presented Table 1. The soils are sandy loam in texture, very acidic and have a good content of organic matter. The electrical conductivity of the soil, at both depths, in the first phase were higher than the conductivity in the second phase of the study. The soil in the first phase of the study was also more acidic, contained more organic matter, more silt, and more exchangeable minerals than the soil at the second phase of the study.

The total heterotrophic bacteria in the soil of the experimental plots at the depth 0 - 15cm in both phases of the study are shown in Table 2. The bacteria counts in first phase were significantly higher than the count in the second phase of the study (p -value < 0.001). The mean bacteria count in the first phase of the study were 5.75×10^8 and 1.13×10^8 at the depths of 0 - 15cm and 15 - 30cm respectively, compared to 2.88×10^4 and 6.32×10^4 in the second phase of the study. In each phase of the experiment, the bacterial count in the soil of the various application doses of the herbicides were comparable to the baseline level and control, up to the manufacturer recommended dose (2.11ai/ha); and then significantly decreased in the soil in which the herbicides were applied at above the manufacturer recommended dose. In the first phase of the study, the bacterial counts were 6.7×10^8 in the plot in which a quarter of the recommended dose of the herbicide was applied, was 6.1×10^8 in the control plot, and 4.1×10^8 in the plot in which twice the recommended dose of the herbicide was applied.

The total fungal count of the soil of the experimental plots, four weeks and 12 weeks after planting are shown in Table 3. The fungal count 4WAP was higher than the count 12WAP, although the difference is not statistically significant (p -value = 0.51). The mean fungal counts 4WAP were 2.68×10^4 and 2.38×10^4 in the first and second phases of the study, compared to 2.12×10^4 and 1.67×10^4 12 weeks after planting. The fungal count in the plot treated with herbicides also followed the same trend as the bacterial count. The counts for the plots treated with herbicides at or below the recommended dose were comparable to those of the control, while the counts in plots in which the herbicides were applied at above the recommended dose were significantly lower.

Seven species of bacteria, and four species of fungi were isolated at baseline, whereas six bacterial species and three species of fungi were isolated at the end of the two phases of the study. The bacterial species that were isolated at baseline include *Bacillus* sp, *Aeromonas* sp, *Micrococcus* sp, *Pseudomonas* sp, *Alcaligenes* sp, *Acinetobacter* sp and *Acromonas* sp; whereas the *proteus* sp, and *actinetobacter* sp were not isolated at the end of the study. The four species of fungi that were isolated at baseline include: *Penicillin* sp; *Aspergillus* sp, *Cladosporium* sp and *Fusarium* sp, whereas *Penicillin* sp; and *Fusarium* sp were not isolated at the end of the study.

DISCUSSION

The study showed that the population of the micro-organisms in the soil treated with various concentrations of glyphosate, atrazine and metolachlor are comparable to those in the soil not treated with the herbicides, up to the recommended doses of the herbicides. This finding is different with the findings of a laboratory-based study carried out in Nigeria (Ayansina & Oso, 2006) which noted a steady decrease in the population of the microorganisms with increasing dose of the herbicides. This disparity can be explained by several reasons, including the fact that the herbicides have been found to be less toxic in field conditions, than in the laboratory (Sprankle et al, 1975; Busse et al, 2001). Busse et al (2001) found that glyphosate was very toxic to bacteria and fungi, when cultured in soil-free media, as shown by the culturable populations, growth rate and metabolic diversity of the surviving organisms; but was found to be less toxic to the microorganisms when the glyphosate was added directly to soil, up to the concentrations that are up to 100-fold greater than the concentration found to be toxic when the herbicide was directly added to a soil-free media.

This difference in the toxicity of glyphosate has been attributed to the fact that the glyphosate tends to get adsorbed onto soil, especially to loam soil, where it is often rapidly degraded by soil microorganisms (Franz et al., 1997), thus becoming more inactive and less antimicrobial (Sprankle et al., 1975b). The soil of the experimental plots is sandy loam, has a high organic matter content and was not tilled. These characteristics have been found to have a strong adsorptive effect on the herbicides that were applied to the experimental plots, and to promote their degradation (Ding et al, 2002; Ayansina & Oso, 2006). Ding et al (2002) found that tilling practices significantly affects the sorption and desorption behaviour of metolachlor; while (Ayansina & Oso, 2006) noted that increasing the organic matter content of the soil that have been treated with herbicides significantly increases the rate of degradation of the herbicides.

The populations of the microorganisms in the treated plots were found to be comparable to the populations at baseline, and in some cases higher than the populations recorded in the control plot that did not receive any application of herbicide. The population of soil bacteria at a soil depth of 15 – 30cm at baseline in the first phase of the study was 2.2×10^8 , which is higher than the population of 1.2×10^8 found in the control plot, and comparable to the population in some of the treated plots. These findings are similar to those of several other studies that indicate the capacity of glyphosate to increase the population of the soil micro-organisms, especially those of heterotrophic bacteria and fungi (Busse et al, 2001; Partoazar et al, 2011; Araújo et al, 2003). (Partoazar et al (2011) had in a field study conducted in Iran shown that the application of glyphosate in an agricultural land significantly increased the activity and population of the soil microorganisms, as shown by the increased level of dehydrogenase activity and soil respiration. The ability of the glyphosate to increase the population of soil microorganism has been linked to its use as a source of nutrient for the micro-organisms. (Rueppel et al, 1977; Busse et al, 2001; Krzysko-Lupicka et al., 1997).

The study also showed that the populations of the soil micro-organisms were higher in the first phase of the study than in the second phase that was carried out one year later, in the same experimental plots. The mean bacteria count at the soil depth of 0 – 15cm, four weeks after the application of the herbicides was 2.63×10^5 in the first phase of the study, compared to 5.18×10^4 in the second phase of the study. This can be attributed to the lingering effects of the herbicides in the experimental plots (Myers et al, 2016), and the physio-chemical changes caused to the soil by the use of the herbicides (Ayansina and Oso, 2006; Liu et al., 1991; Sprankle, et al 1975; Krzysko-Lupicka et al., 1997). Studies conducted by Myers et al (2016) have shown that herbicides persist in the soil longer than previously reported, and so the effects of the herbicides applied during the first phase of the study can easily extend to the second phase of the study.

The differences in the populations of the microorganisms in the two phases of the study can also be due to the changes in the physio-chemical properties of the soils. There was a significant decrease in the organic matter and phosphorus content of the soil in the second phase of the study. The organic matter content of soil decreased from 3.2% at the beginning of the first phase of the study to 2.15% at the beginning of the second phase of the study, while the phosphorus content decreased from 48ppm in the first phase to 29ppm in the second. This is consistent with the findings of Ayansina and Oso (2006) that recorded similar percentage of reduction. Soil microorganisms have been found to thrive in soils with high content of organic matter (Ayansina & Oso, 2008); while high phosphorus

content has been found to assist glyphosate to increase the population of bacteria and fungi in the soil, by serving as a source of phosphorus for some bacteria (Liu *et al.*, 1991; Sprankle, *et al* 1975; Krzysko-Lupicka *et al.*, 1997).

The study also shows that the herbicides have disproportionate effects on the type and total population of the micro-organisms in the soil. The application of the herbicides resulted in the death of some of the microorganisms such as *proteus sp.*, *actinetobacter sp.*, *penicillin sp.*; and *fusarium sp.* These findings are consistent with those of other studies (Ayansina & Oso, 2006; Ayansina & Oso, 2008), and are reflective of the effects of the herbicides on both the type and population of the micro-organisms. Ayansina and Oso (2006) had noted that the application of atrazine and metolachlor resulted in the death of *Flavobacterium sp.*, *Proteus sp.*, and *Actinomycetes* species in the soil; and the proliferation of *Bacillus* and *Pseudomonas* species in the treated soil. The study also found that the application of the herbicides also resulted in the death of *A. flavus*; *A. ochracuis*, *Rhizoctonia sp.*; *Fusarium sp.*, and *Penicillium sp* in the soil; and the proliferation of *A. niger* and *Trichoderma sp* in the herbicide treated soils. Also, a meta-analysis carried out by Zhao *et al* (2012) showed that whereas the application of herbicides can increase the overall count of nematodes in the soil, it however causes a reduction in the population of fungivores and predators, and an increase in the population of bacterivores, plant parasites and omnivores. This means that the use of the overdose of the herbicides can cause the sterility of the soil, and significant reduction in the fertility of the soil. This therefore emphasizes the importance of not exceeding the recommended doses of the herbicides.

The populations of the micro-organisms were noted to be higher at four weeks after the application of the herbicides, compared to the population 12 weeks after the application of the herbicides. The mean bacterial count at 4WAP at the soil depth of 0 – 15cm in the first phase of the study was 5.75×10^8 compared to 2.63×10^5 12WAP; while the mean bacterial count at 4WAP at the soil depth of 15 – 30cm in the first phase of the study was 1.233×10^8 compared to 5.13×10^5 12WAP. These findings can be attributed to the waning of the growth boosting effects of glyphosate (Busse *et al*, 2001; Araújo *et al*, 2003) that was used as a pre-emergent herbicide in preparing the experimental plots; and the lingering anti-microbial effects of atrazine and metolachlor that were applied after the glyphosate, as post-emergent herbicides (Bouchard *et al*, 1982; USEPA, 1980). Metolachlor has been found to be moderately persistent in loamy soil, taking up to 10.1 weeks to halve the initial herbicide concentration at the temperature of 23 degrees Celsius (Bouchard *et al*, 1982). It has also been found to be more stable in acidic, water-logged soil, with high organic content, as found in the experimental plots of this study, with a half-life at 20 degrees C of more than 200 days in highly acid waters (USEPA, 1980).

The study also found that the population of the microorganisms at a soil depth of 0 – 15cm were significantly higher than those at a deeper soil depth of 15 – 30cm. The mean bacteria count at the depth of 0 – 15cm in the first phase of the study was 5.75×10^8 compared to 1.233×10^8 at a depth of 15 – 30cm; while the mean bacteria count at the depth of 0 – 15cm in the second phase of the study was 6.32×10^4 compared to 2.88×10^4 in the second phase of the study. The higher population of the microorganisms at the soil depth of 0 – 15cm, compared to 15 – 30cm can be explained by the higher organic content of the soil at that depth that support the growth of the microorganisms (Ayansina & Oso, 2008).

CONCLUSION

The application of herbicides resulted in changes in the soil and the total number of soil micro-organisms, especially when they are applied at above the recommended rate. Efforts are therefore needed to educate farmers on the proper dosing of the herbicides.

ACKNOWLEDGEMENT

We wish to thank the staff of the herbarium of the department of Plant Science and Biotechnology of the University of Port Harcourt demonstration farm, for their assistance during the field studies.

Table 1: Physico-chemical characteristics of the soil

Characteristics	Phase 1 0 – 15cm	Phase 2	Phase 1 15 – 30cm	Phase 2
Sand (%)	58	60	54	56
Clay (%)	13	13	17	17
Electrical conductivity 1:2:5 (mS/Cm/25oC)	0.32	0.13	0.23	0.07
Soil reaction 1:2:5 (PH)	4.6	3.9	4.7	4.1
Organic Matter (%)	3.2	2.2	2.5	1.4
Total nitrogen (%)	0.16	0.11	0.13	0.08
Available phosphorous (ppm)	48	29	34	22
Exchangeable calcium (ppm)	167	152	172	109
Exchangeable magnesium (ppm)	34	22	27	13
Exchangeable potassium (ppm)	85	47	71	35
Exchangeable sodium (ppm)	48	40	48	2

Table 2: Total heterotrophic bacteria count at four weeks after planting (4WAP)

TREATMENT	2013 (0 – 15cm)	2014	2013 (15 – 30cm)	2014
0.53ai/ha	6.7×10^8	9.6×10^4	1.7×10^8	3.7×10^4
1.06ai/ha	6.4×10^8	6.1×10^4	1.4×10^8	3.1×10^4
2.11ai/ha	5.9×10^8	5.9×10^4	1.2×10^8	2.9×10^4
3.17ai/ha	5.3×10^8	5.6×10^4	0.9×10^8	2.3×10^4
4.22ai/ha	4.1×10^8	4.7×10^4	0.4×10^8	1.9×10^4
Weed free	6.1×10^8	6.0×10^4	1.2×10^8	3.4×10^4
Baseline	5.9×10^8		2.2×10^8	
p=0.05	< 0.01	< 0.01	< 0.01	< 0.01

Table 3: Comparison of the effects of the herbicides on total fungal count at 4 and 12 weeks after planting

TREATMENT	4 WAP 0 – 15cm	12 WAP	4 WAP 15 – 30cm	12 WAP
0.53ai/ha	4.2×10^4	3.1×10^4	3.3×10^4	2.4×10^4
1.06ai/ha	3.3×10^4	2.5×10^4	2.9×10^4	2.1×10^4
2.11ai/ha	2.7×10^4	2.1×10^4	2.5×10^4	1.5×10^4
3.17ai/ha	2.1×10^4	1.4×10^4	1.8×10^4	1.1×10^4
4.22ai/ha	1.4×10^4	1.1×10^4	1.3×10^4	0.7×10^4
Weed free	2.4×10^4	2.5×10^4	2.5×10^4	2.2×10^4
Baseline	5.2×10^4		3.9×10^4	
P-value	< 0.01	< 0.01	< 0.01	< 0.01

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Cite this Article: Best-Ordinioha JC, Ataga AE, Ochekwu EB (2016). The Effect of the Application of Different Rates of Herbicides on Soil Microorganisms. *Greener Journal of Soil Science and Plant Nutrition*, 3(1):025-031, <http://doi.org/10.15580/GJSSPN.2016.1.102716175>