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Full Length Research Paper

Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity

A. Sebiomo[1]*, V. W. Ogundero[2] and S. A. Bankole[2]

[1]Department of Biological Sciences, Tai Solarin University of Education, Ijagun Ijebu-Ode, Ogun State, Nigeria.
[2]Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

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The effect of four herbicides (atrazine, primeextra, paraquat and glyphosate) on soil microbial population, soil organic matter and dehydrogenase activity was assessed over a period of six weeks. Soil samples from cassava farms were treated with herbicides at company recommended rates. Soil dehydrogenase activity was measured at four-day sampling intervals up to the 20th day. Bacterial, fungal and actinomycetes populations decreased upon treatment with herbicides when compared to the control. There was significant reduction in percentage organic matter after the herbicides were applied to soils. Soil organic matter then increased after continuous application from the second to the sixth week of treatment. Herbicide treatment resulted in a significant drop in dehydrogenase activity when compared to the control soil samples. Obtained results indicated that soils treated with primeextra had the lowest dehydrogenase activity of 16.09 µg ($g^{-1}min^{-1}$) after the sixth week of treatment, while soils treated with glyphosate had the highest dehydrogenase activity of 20.16 µg ($g^{-1}min^{-1}$) when compared to other herbicides used for treatment. Dehydrogenase activity increased from the second to the sixth week of treatment. This study indicated significant response of soil microbial activity to herbicide treatment and increased adaptation of the microbial community to the stress caused by increase in concentration of the herbicides over weeks of treatment.

Key words: Herbicides, soil organic matter, dehydrogenase activity, treatment.

INTRODUCTION

The increased use of pesticides in agricultural soils causes the contamination of the soil with toxic chemicals. When pesticides are applied, the possibilities exist that these chemicals may exert certain effects on non-target organisms, including soil microorganisms (Wardle and Parkinson, 1990; Simon-Sylvestre and Fournier, 1979). The microbial biomass plays an important role in the soil ecosystem where they fulfill a crucial role in nutrient cycling and decomposition (De-Lorenzo et al., 2001).

During the past four decades, a large number of herbicides have been introduced as pre and post-emergent weed killers in many countries of the world. In Nigeria, herbicides have since effectively been used to control weeds in agricultural systems (Adenikinju and Folarin, 1976). As farmers continue to realize the usefulness of herbicides, larger quantities are applied to the soil. But the fate of these compounds in the soils is becoming increasingly important since they could be leached, in which case groundwater is contaminated or immobile, and persist on the top soil (Ayansina et al., 2003). These herbicides could then accumulate to toxic levels in the soil and become harmful to microorganisms, plant, wild life and man (Amakiri, 1982). There is an increasing concern that herbicides not only affect the target organisms (weeds) but also the microbial communities present in soils, and these non-target effects may reduce the performance of important soil functions. These critical soil functions include organic matter degradation, the nitrogen cycle and methane oxidation (Hutsch, 2001).

Both glyphosate and paraquat have been reported to cause activation in soil urease and invertase soil enzymes (Sannino and Gianfreda, 2001), while diquat and

*Corresponding author. E-mail: rev20032002@yahoo.com. Tel: +2348077675121 or +2347039334401.

Abbreviations: NA, Nutrient agar; **PDA,** potato dextrose agar; **INT,** iodonitrotetrazolium chloride; **DH,** dehydrogenase; **INTF,** iodonitrotetrazolium formazan.

paraquat increased fungal populations (Mewatankarn and Sivasithamparam, 1987). A degradative microbial population that has adapted to the introduced compounds may exist in many contaminated locations, therefore, it is necessary to search for various microorganisms which would be able to reduce water or soil pollution.

All the transformations of nutrients occurring in soil are stimulated by the enzymes that condition their conversion into forms available to plants and microorganisms. Enzymes are frequently referred to as markers of soil environment purity (Aon and Colaneri, 2001). Microbial activity measurements appear as good indicators of the degree of pollution of contaminated soils (Nordgren et al., 1988; Aoyama and Nagumo, 1995; Insam et al., 1996; Kuperman and Margret, 1997). Dehydrogenase is thought to be an indicator of overall microbial activity, because it occurs intercellularlly in all living microbial cells and is linked with microbial oxydoreduction processes (Quilchano and Maranon, 2002; Stepniewska and Wolinska, 2005). It is a specific kind of enzyme which plays significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors. Soil dehydrogenase activity is considered to be a valuable parameter for assessing the side effects of herbicides treatments on the soil microbial biomass.

This study was designed to investigate the effect of four types of herbicides on microbial population, soil organic matter and soil microbial activity, using soil dehydrogenase as an indicator of soil microbial activity.

MATERIALS AND METHODS

Soil sampling

Top soil (up to 5 cm depth) samples were collected from cassava farm in Ijebu-Ode (Ogun State, Nigeria), with no prior pesticide treatment. The soil samples were sieved through a 2.0 mm mesh size to remove stones and plant debris.

Herbicides

The herbicides used in this study were obtained from a local agricultural dealer store in Ibadan. The herbicides used were: Atrylone 80WP, trademark of Insis Limited (atrazine); primextra, a product of Syngenta (a combination of atrazine and metolachlor); Glysate, Nantong Ji Angshan Agrochemicals (glyphosate); and Gramoxone, a product of Syngenta (paraquat).

Soil treatments

The treatments were carried out for a period of 6 weeks at company recommended rates of 4l/h (at 350 ml in 15 L sprayer) for paraquat, glyphosate and primeextra, while recommended rate of 3 kg/h (atrazine powder) was used for atrazine treatment (soil treatments were carried out in triplicates).

Microbial enumeration

Nutrient agar (NA) was used for the enumeration of total heterotrophic bacteria by the pour plate method. Incubation was done at 30°C for 24 - 48h. Potato dextrose agar (PDA) was used for enumeration and isolation of fungi. Incubation was at 25°C for 48 h. Bacterial and actinomycetes isolates were characterized based on cultural characteristics, staining reactions and biochemical reactions. Identification was thereafter made with reference to Bergey's manual of systemic bacteriology (1984). Starch Casein Agar was used for the enumeration of total actinomycetes counts. Fungal isolates were characterized as described by Barnett and Hunter (1972).

Determination of organic matter in soil

The percentage organic matter was determined by the method described by FAO (1974). Soil samples were collected and ground to pass through 0.5 mm sieve. One gram of each soil sample was weighed into 250 ml Erlenmeyer flasks and 10 ml of $K_2Cr_2O_7$ solution was dissolved into each flask and swirled gently to disperse soil. Twenty milliliters of concentrated H_2SO_4 was rapidly added using automatic pipette and swirled gently until the soil and reagents were mixed, then the mixture was swirled more vigorously for one minute, the flasks were then rotated and allowed to stand in a sheet of asbestos for about 30 min. One hundred milliliters of distilled water was added to each flask, then 3 - 4 drops of indicator (ferroin) was added and filterated with 0.5 N ferrous sulphate solution to the end point, from greenish or dark green to red (maroon colour) and in reflected light against a white background. The organic matter was calculated according to the following formula:

$$\% \text{ Organic matter} = \frac{(\text{me } K_2SO_4 - \text{me } FeSO_4) \times 0.003 \times 100 \times f \times 1.729}{\text{Weight of air-dried soil}}$$

Where, correlation factor "f" = 1.33, me = normality of solution × milliliter of solution used and 1.729 = conversion.

Soil dehydrogenase activity

Soil microbial activity was estimated by soil dehydrogenase activity (DH). Soil DH-activity was measured at four-day sampling intervals up to the 20th day. Measurements were made according to the iodonitrotetrazolium chloride (INT) method (von Merci and Schinner, 1991). Sub samples of 5 g (air-dried basis) were weighed into test tubes and mixed with a 10 ml aqueous solution of INT (10 mgl^{-1}). Test tubes were sealed and incubated in the dark at 40 ± 0.5°C for 2 h and slightly shaken. Developed iodonitrotetrazolium formazan (INTF) was extracted by keeping the test tubes in the dark for 1 h, shaking vigorously every 20 min and finally, filtering the solution. Soil moisture content was maintained at 60% water holding capacity by weighing and correcting for any weight loss, using sterile ultra pure water. The INTF was measured spectrophotometrically at 464 nm, after extraction with 10 ml of N, N-dimethylformamide/ethanol solution. Soil DH-activity and all soil treatments were done in triplicates. Dehydrogenase activity was determined using the following equation:

$$A = \frac{C_f V 10^6 W}{M_f m t}$$

Where, A = Soil dehydrogenase activity, C_f = concentration of iodonitrotetrazolium formazan (mg ml^{-1}), V = volume of added solutions, M_f = molar mass of INTF, m = soil mass, W = dampness coefficient and t = time.

Table 1. Effect of herbicide treatment on actinomycetes count (ANOVA).

Factors	P-value
Herbicide treatment	0.201
Weeks of herbicide treatment	0.007
Herbicide × weeks of treatment	0.354

Table 2. Effect of herbicide treatment on bacterial count (ANOVA).

Factors	P-value
Herbicide treatment	<0.001
Weeks of herbicide treatment	<0.001
Herbicide × weeks of treatment	<0.001

Statistical analysis

Data generated from this study was expressed in bar charts, line graphs and subjected to analysis of variance (ANOVA) and correlation coefficient analysis.

RESULTS

Presented in Table 2 is the effect of herbicide treatment on bacterial count. Herbicide treatment had high significant effect on bacterial count ($P < 0.001$). The interaction between the herbicides and the weeks of herbicide treatment also resulted to high significant effect on bacterial count ($P < 0.001$). The weeks of herbicide treatment followed similar trend as stated above ($P < 0.001$). In Figure 1, soil bacterial populations in the cont-rol samples were found to be significantly higher than those of herbicide treated soils. The bacterial populations for all soil samples increased from 2nd to the 6th week of treatment (including control samples). After the second and fourth weeks of treatment, soils treated with glyphosate had the highest bacterial populations of 3.9×10^4 and 5.67×10^4 cfu/g of all treated soils. The glyphosate treated soil also after the 6th week of treatment had the highest bacterial population of 5.9×10^4 cfu/g, while soil samples treated with paraquat had the lowest bacterial populations of 2.63×10^4, 3.67×10^4 and 4.57×10^4 cfu/g after the second, fourth and sixth weeks of treatment, respectively (Figure 1).

Analysis of variance results presented in Table 1 shows that only the weeks of herbicide treatment had significant effect on actinomycetes count ($P < 0.007$), meanwhile herbicide treatment and the interaction between herbicide and weeks of herbicide treatment had little effect on actinomycetes population ($P = 0.201$ and $P = 0.354$). Figure 2 shows the effect of herbicide treated soils on actinomycetes populations. Similar to Figure 1, the control soil samples had the highest actinomycetes populations of 8.53×10^3, 5.23×10^4 and 8.47×10^4 cfu/g after the second, fourth and sixth weeks of treatment, respectively. Soil samples treated with primeextra had the highest actinomycetes population of 2.67×10^4 cfu/g after two weeks of treatment, while paraquat treated soils had the highest actinomycetes population of 3.93×10^4 cfu/g after six weeks of treatment (Figure 2).

In Figure 3, the fungal populations fluctuated between the second and sitxh weeks, while the control samples had the highest fungal populations of 1.9×10^4 and 1.6×10^4 cfu/g on the second and sixth week, respectively. The soils treated with primeextra had the highest fungal population of 1.8×10^4 cfu/g. Paraquat treated soils had the lowest fungal population of 8.7×10^3 cfu/g after six weeks of treatment. The ANOVA results presented in Table 3 followed similar trends with the bacterial counts presented in Table 2.

The herbicide treatments had significant effect on percentage organic matter of the soil ($P < 0.001$) (Table 4 and Figure 4). Weeks of herbicide treatment and the interaction between herbicides and weeks of treatment had similar effect ($P < 0.001$) (Table 4). The percentage organic matter of the soils treated with herbicides reduced significantly when compared with the control. Soil samples treated with glyphosate had the lowest percentage organic matter contents of 1.25, 2.35 and 3.17 of all herbicide treated soils after the second, fourth and sixth week of treatment, respectively (Figure 3). Paraquat treated soils had the highest percentage organic matter contents of 4.68, 5.03 and 5.20 after the second, fourth and sixth week of treatment, respectively. Meanwhile, percentage organic matter of herbicide treated soil samples as well as those of control samples increased from the second to the sixth week of treatment. The results obtained in correlation coefficient analysis between soil organic matter and actinomycetes counts shows positive correlation (correlation coeficient value = 0.376). There was also positive correlation between soil organic matter and fungi count (correlation coeficient value = 0.462). Meanwhile, the corrrelation coefficient analysis between soil organic matter and bacterial counts showed the strongest positive correlation (correlation coeficient value = 0.672).

All the herbicides used for treatment in this study resulted in significant ($P < 0.001$) (Table 5) reductions in soil dehydrogenase activity when compared to the control. Soils treated with primeextra had the lowest set of soil dehydrogenase activities of 9.02, 12.55 and 16.09 µg ($g^{-1}min^{-1}$) after the second, fourth and sixth week of treatment, respectively (Figures 5, 6 and 7). Meanwhile, soils treated with glyphosate had the highest set of soil dehydrogenase activities after the second and sixth week of treatment (Figures 5 and 7) when compared to other herbicide treatments, while soils treated with atrazine had the highest dehydrogenase activity of 14.32 µg ($g^{-1}min^{-1}$) after the fourth week of treatment. In Figure 7, after the sixth week of treatment, glyphosate treated soils had the

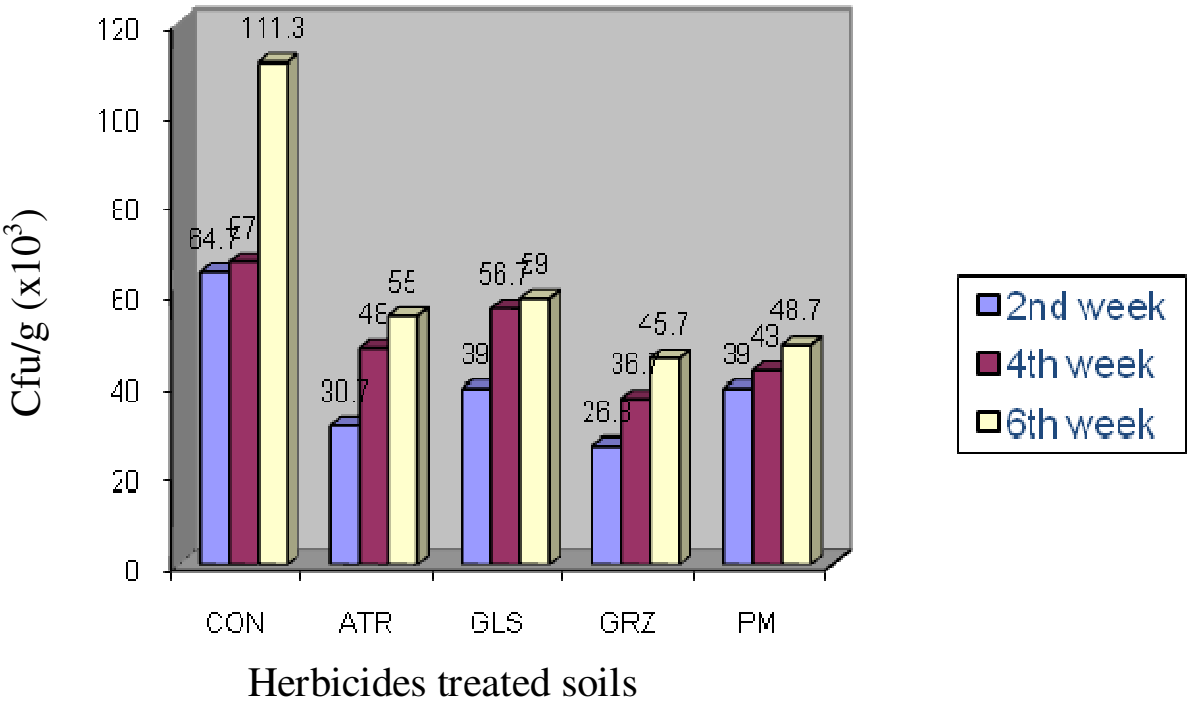

Figure 1. Effect of herbicides on soil bacterial populations. CON = Control; ATR = atrazine treated soil; GLS= glyphosate treated soils; GRZ = paraquat treated soils; PM = primeextra treated soils. For herbicide treatment, weeks of herbicide treatment and herbicide x week of treatment, P-value <0.001.

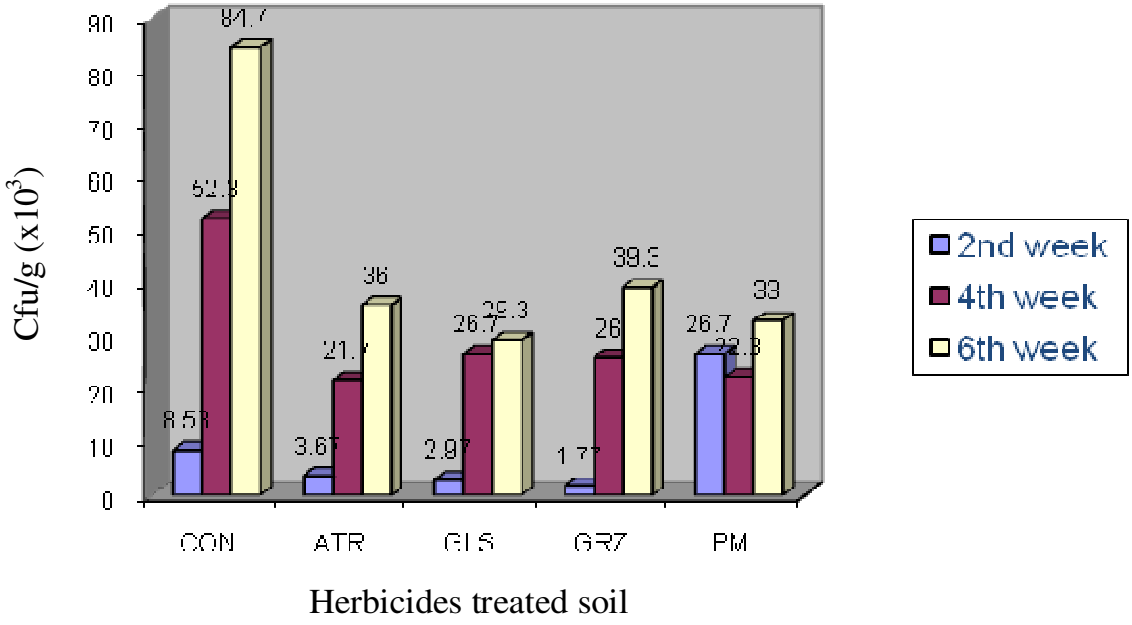

Figure 2. Effect of herbicides on actinomycetes populations. P-values for herbicide treatment, weeks of herbicide treatment and herbicide x week of treatment were 0.201, 0.007 and 0.354, respectively.

highest dehydrogenase activity of 20.16 µg ($g^{-1}min^{-1}$), while soils treated with primeextra had the lowest dehydrogenase activity of 16.09 µg ($g^{-1}min^{-1}$) after the sixth week of treatment when compared to other herbicide treatments. In Table 5, weeks of herbicide treatment had significant ($P < 0.002$) effect on soil dehydrogenase activity. All soil dehydrogenase activities increased from the second to the sixth week.

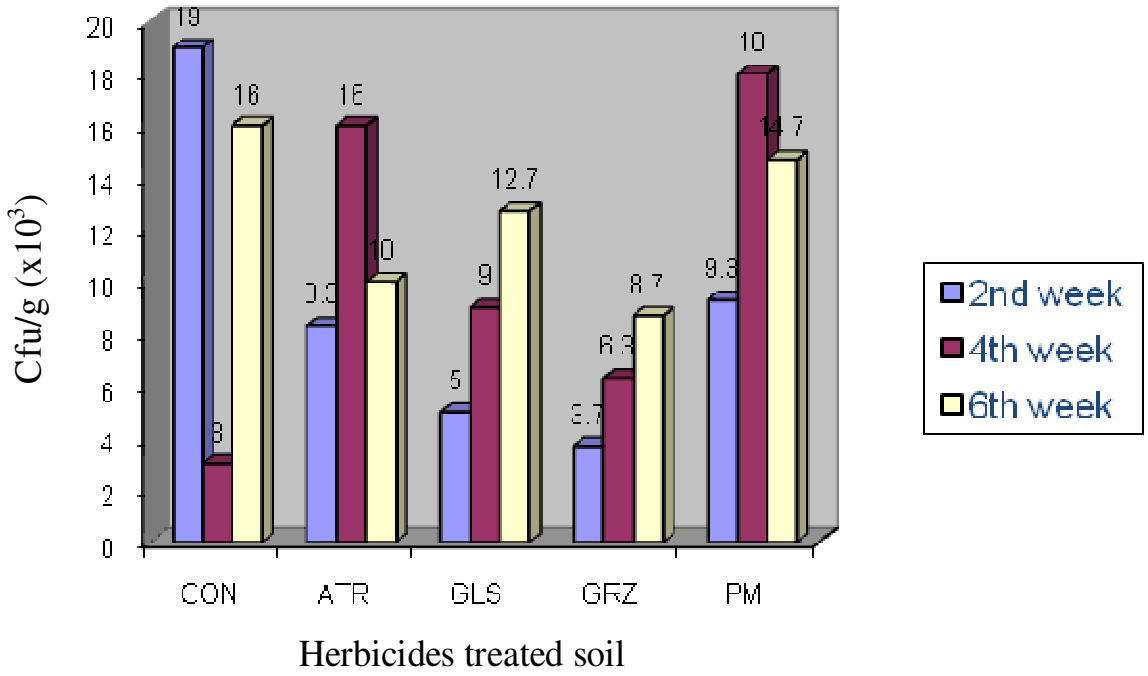

Figure 3. Effect of herbicides on soil fungal populations. For herbicide treatment, weeks of herbicide treatment and herbicide x week of treatment, P-value <0.001.

Table 3. Effect of herbicide treatment on fungal count (ANOVA).

Factors	P-value
Herbicide treatment	<0.001
Weeks of herbicide treatment	<0.001
Herbicide × weeks of treatment	<0.001

Table 4. Effect of herbicide treatment on soil organic matter (ANOVA).

Factors	P-value
Herbicide treatment	<0.001
Weeks of herbicide treatment	<0.001
Herbicide × weeks of treatment	<0.001

The days of incubation had significant effect (P 0.001) (Table 5) on soil dehydrogenase activities. The results in Figures 5, 6 and 7 shows that as the days of incubation increased, the soil dehydrogenase activities also increased concomitantly to the 20th day. The results of the control samples showed higher increases than those of the herbicide treated soils which dropped upon treatment with herbicides. After the 20th day of incubation, glyphosate treated soils had the highest dehydrogenase activities of 11.49 and 20.16 µg ($g^{-1}min^{-1}$) at the second and sixth week, respectively, while prime-extra treated soils had the lowest after 20th day of incubation with values of 9.02, 12.55 and 16.09 µg ($g^{-1}min^{-1}$) after the second, fourth and sixth week, respectively.

DISCUSSION

In this study, the observed trends in microbial population were similar to observations made by Korpraditskul et al. (1988). Ayansina and Oso (2006) discovered that higher concentrations of herbicides treatments resulted in much lower microbial counts when compared to soils treated with recommended doses. Experiments have shown that microbes may use herbicides as a source of carbon (Radosevich et al., 1995). This may consequently explain the increase in microbial populations obtained in this study from the second to the sixth week of application of the herbicides. Some studies report increased populations of actinomycetes and fungi after treatment with glyphosate (Araujo et al., 2003), increased soil microbial biomass (Hanley et al., 2002) or no long-term change in microbial populations (Busse et al., 2001)

There was significant reduction in percentage organic matter after the herbicides were applied to soils, although organic matter increased after continuous application from the second to the sixth week of treatment. Ayansina and Oso (2006) reported that soil treatment with atrazine resulted in significant changes in percentage organic matter measurements. Ali (1990) had shown that the fate of pesticides in soils is greatly affected by the presence of organic matter in the soil by aiding their disappearance.

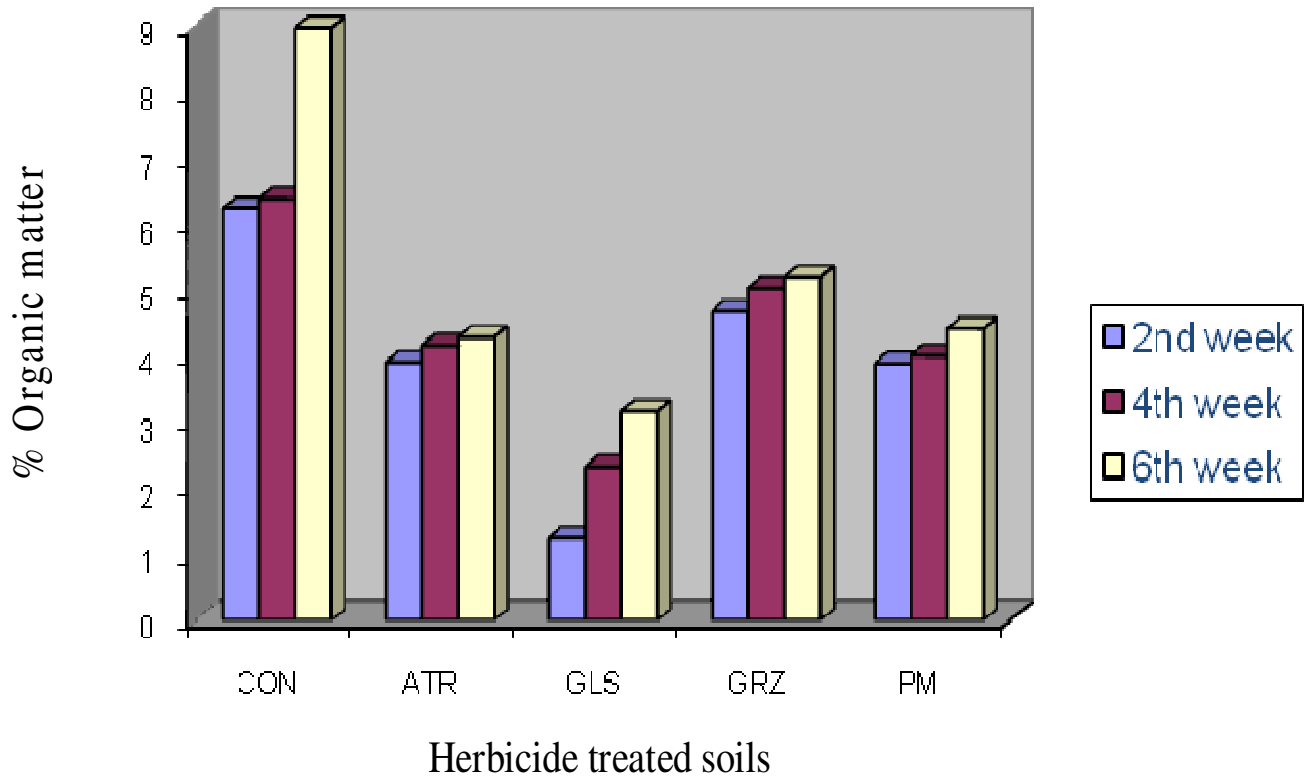

Figure 4. Percentage organic matter content of soil samples. For herbicide treatment, weeks of herbicide treatment and herbicide x week of treatment, P-value <0.001.

Table 5. Effect of herbicide treatment on soil dehydrogenase activity (ANOVA).

Factors	P-value
Herbicide treatment	<0.001
Weeks of herbicide treatment	<0.002
Days of incubation	<0.001

The application of herbicides to the soils led to a significant drop in dehydrogenase activity with respect to untreated control soil samples. Obtained results indicated that soils treated with primeextra had the lowest dehydrogenase activity, while soils treated with glyphosate had the highest dehydrogenase activity when compared to other herbicides used in this study. In literature, opposite effects on several soil enzymes are reported (Gianfreda et al., 1994, 2005; Quilchano and Maranon 2002). Glyphosate was found to inhibit dehydrogenase activities in sandy loam soil (Dzantor and Felsot, 1991). No effects on soil dehydrogenase activity were detected by Lethbridge et al. (1981) and Nakamura et al. (1990). Reduced enzymatic activities were also found by Perucci and Scarponi (1990) and Dzantor and Felsot (1991) in studies on the interference of atrazine with phosphatase, dehydrogenase and esterase activity of soil.

In this study, dehydrogenase activity increased from the second to the sixth week of treatment. This might be due to increase in microbial populations with the capability of utilizing the herbicides as carbon source. Under laboratory conditions, a normal dose of glyphosate inhibited dehydrogenase activity by 5 - 10% (3 weeks after herbicide application) (Nada and Mitar, 2002). A tenfold dose of glyphosate affected negatively, the activity of this oxide-reducing enzyme by 5% (11 weeks after herbicide application) (Schuster and Schroder, 1990).

Dehydrogenase activity increased from the 4th to the 20th day of incubation in the present study. Moreno et al. (2007) reported that an increase in metabolic activity with atrazine concentration and with incubation time can be deduced from their work. Similar results were presented by Rossel et al. (1997).

This study has shown that there exists positive correlation between microbial population and soil organic matter and that the variation in soil microbial activity represents the capacity of microorganisms to respond to inputs of herbicides. Microbial activity increased as an adaptation to the stress caused by increase in concentration of the herbicides over weeks of treatment. The results obtained demonstrate a potential capacity for adaptation of the microorganisms in soils when large amounts of herbicides are added. Dehydrogenase activity

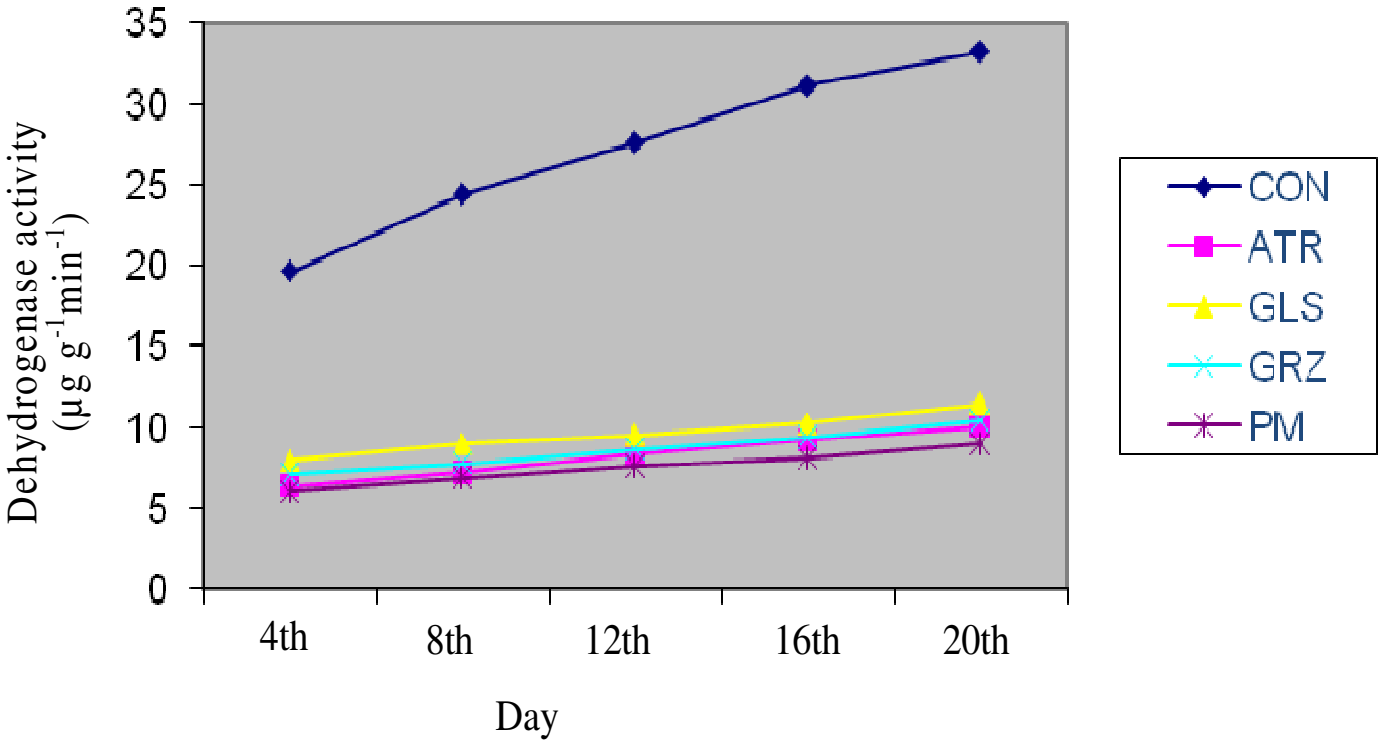

Figure 5. Effect of herbicide treatment on soil dehydrogenase activity after two weeks of treatment. For herbicide treatment, weeks of herbicide treatment and days of incubation, P-value <0.001, 0.002 and 0.001, respectively.

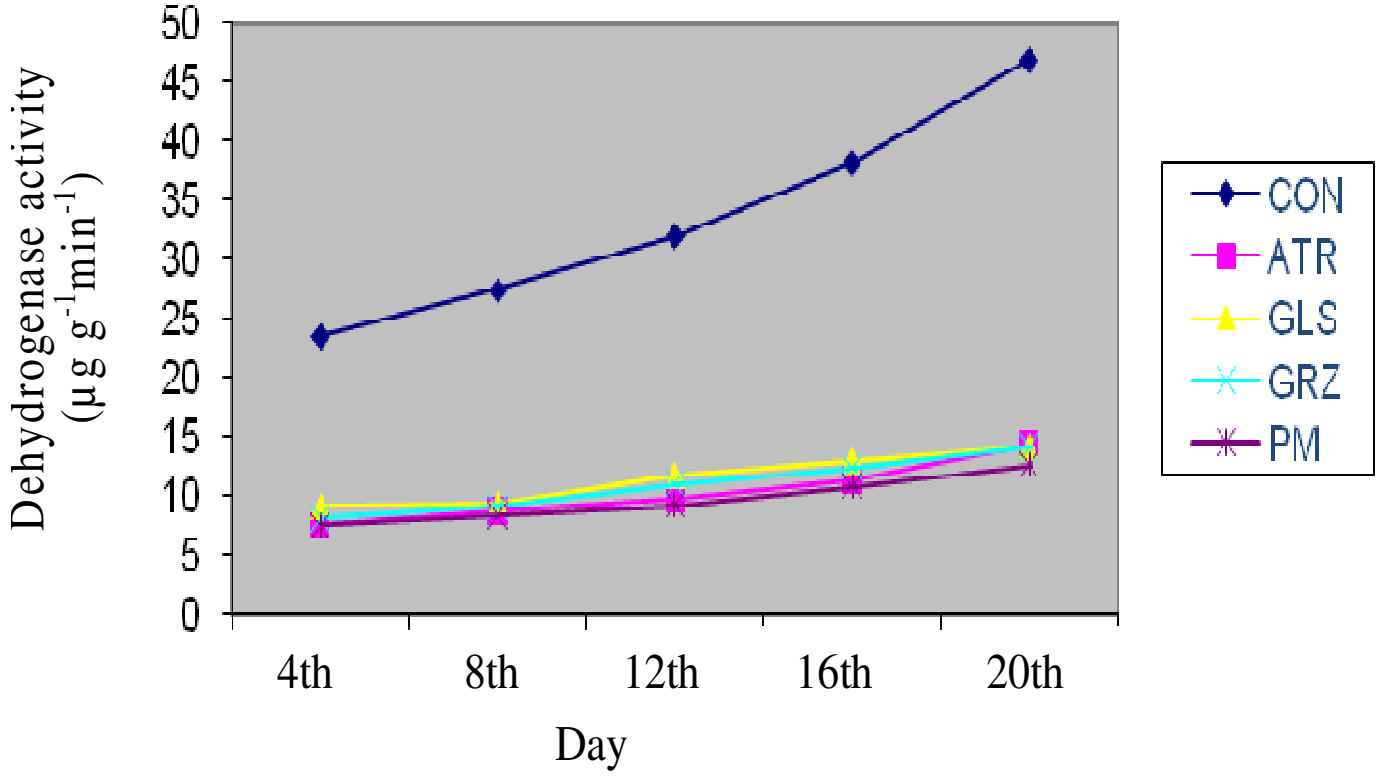

Figure 6. Effect of herbicide treatment on soil dehydrogenase activity after four weeks of treatment. For herbicide treatment, weeks of herbicide treatment and days of incubation, P-value <0.001, 0.002 and 0.001, respectively.

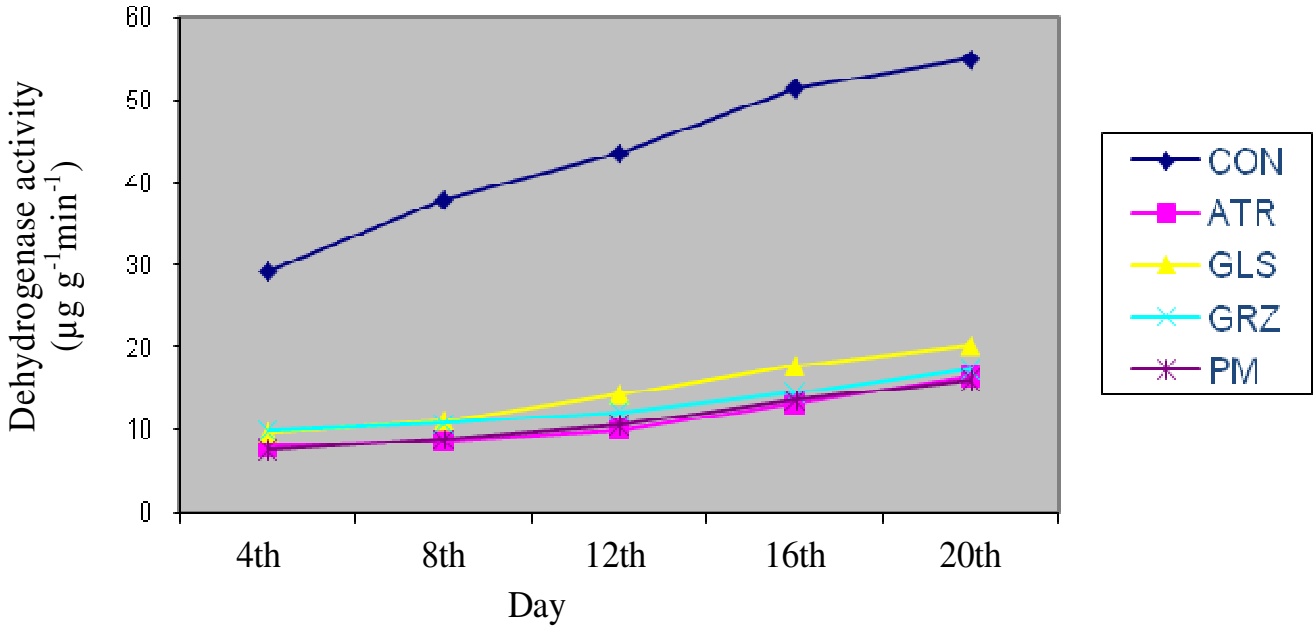

Figure 7. Effect of herbicide treatment on soil dehydrogenase activity after six weeks of treatment. For herbicide treatment, weeks of herbicide treatment and days of incubation, P-value <0.001, 0.002 and 0.001, respectively.

is a sensitive bioindicator of the microbial activity response to herbicide inputs.

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