African Journal of Biotechnology Vol. 10(83), pp. 19419-19424, 21 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2440
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

The effect of glyphosate application on soil microbial activities in agricultural land

Mozhgan Partoazar[1], Mehran Hoodaji[1]* and Arezoo Tahmourespour[2]

[1]Department of Soil Science, Islamic Azad University, Khorasgan (Isfahan) Branch, Iran.
[2]Department of Microbiology, Islamic Azad University, Khorasgan (Isfahan) Branch, Iran.

Accepted 14 November, 2011

In recent years, intensive use of herbicides has increasingly become a matter of environmental concern partially because of the effects of these chemicals on soil microorganisms. Glyphosate [N-(phosphonomethyl) glycine] (GP) is a broad-spectrum, non-selective, post emergence herbicide that is widely used in agriculture. In this study, glyphosate effects as N, P and C nutrient sources on microbial population and the effect of different concentration of it on dehydrogenease activity and soil respiration were investigated. The results show that in a soil with a long historical use of glyphosate (soil 1), the hetrotrophic bacterial population was significantly ($p<0.05$) increased. Also, by increase in the bacterial population, the herbicide existence as the possible nutrient source is enhanced. According to results, bacterial populations in the presence of glyphosate as P source was significantly ($p<0.01$) higher than N and C sources. The application of GP to the soil led to a significant increase in dehydrogenase activity with respect to untreated control soil samples. Also, respiration rates increased with increasing glyphosate application up to 50 mM but in 500 mM the inhibitory effect of glyphosate was observed. It can be concluded that glyphosate application may alter (increase) soil microbial activity and population. Increased microbial activity may be beneficial or detrimental toward plant growth, soil microbial ecology, and soil quality.

Key words: Glyphosate, bacterial population, dehydrogenease activity, soil respiration.

INTRODUCTION

The intensive use of herbicides is an environmental problem, partially because of the potential hazardous effects of these chemicals on soil biological processes and non-target organisms. Biological and biochemically mediated processes in soils are of the utmost importance to ecosystem function. Soil microbes are the driving force behind many soil processes including transformation of organic matter, nutrient release and degradation of xenobiotics (Zabaloy et al., 2006). Several biological parameters have been used to assess soil quality and health as affected by agricultural practices (Beneditti and Pilly, 2006; Filip, 2002; Anderson, 2003). Among them, microbial activity is expected to be more efficient indicators than physical and chemical parameters as they are able to respond immediately to environmental change (Nannipierietal, 2002; Avidano et al., 2005).

Glyphosate is the systemic herbicide that is commonly used to control a broad spectrum of weed in crops and pastures worldwide (Zabaloy et al., 2006). Glyphosate is a polar compound known for its strong adsorption to Fe and Al oxides and clay minerals (Buss et al., 2001; Ratcliff et al., 2006). Among the patterns and the measurement of enzymatic activities of the whole microbial community, it appears to be a useful means to evaluate the impacts of pollutants on soils (Brohon et al., 2001). Number and mass of microorganisms are basic properties of ecological studies, and which can be related to parameters describing microbial activity and soil health (Bolter et al., 2006). Substrate induced respiration is a commonly used, sensitive parameter for the observation of pollutant impacts on soil microorganisms (Brohon et al., 2001). Dehydrogenases exist as an integral of intact cells and represent the oxidative activities of soil microbes by transferring hydrogen or electrons from substrates

*Corresponding author. E-mail: M_Hoodaji@khuisf.ac.ir.

Table 1. Characteristics of the investigated soils.

Soil number	Texture	Sand (%)	Silt (%)	Clay (%)	pH	Total N	K (ppm)	P (ppm)
1	Loam	19	35	46	7.64	0.082	285	5.43
2	Loam	22	35	43	7.26	0.082	290	8.35
3	Loam	19	39	42	6.94	0.098	305	9.7
4	Sandy loam	12.5	5	82.5	7.34	0.022	185	4.8

to acceptors and may be used as a measurement of overall microbial activity and the mineralizing capacity in soil and are suitable to assess broad – spectrum biological activity in the short – term (Nannipieri et al., 2002).

Determination of soil respiration is forming a large portion of primary gross product rate in land ecosystem so its estimation and measurement is very important in implementing and budging of ecosystem's carbon. The soil microbial respiration is affected by soil humidity, temperature, availability of nutrient and soil construction. The aim of this study was to investigate the GP effects as P, C and N sources on microbial population and the effect of different concentration of it on soil respiration and dehydrogenease activities.

MATERIALS AND METHODS

Soil sampling and analysis

Four soil samples were collected from a citrus garden located in the city of Darab (Iran) with the
following characteristics: soil 1, It was polluted by glyphosate (GP) at a slow rate (in 12 years time) with agricultural activities; Soil 2, the GP was added to soil 1, one month before sampling; soil 3, GP was added to soil 1 in the day of sampling; soil 4, non polluted, as a control.

All the soil samples were taken from the surface layer to a depth of 10 cm and moved to the laboratory in the sterilized containers. Air-dried sub-samples were sieved (< 2 mm) for physical and chemical analyses (Table 1).

Bacterial population

Initially, the bacteria were enumerated by the plate counts method at the end of incubation period. For this purpose, 1 g (dry weight) of each soil samples was used to provide a dilution series of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. For bacterial counts, 0.1 ml of each dilution was added to each of nutrient agar plates by spread-plate method. The plates were incubated at 30°C for 72 h. The second bacterial enumeration was done following nine days of enrichment with 500 mg/L GP: MS_1 media supplemented with GP as carbon resource; MS_1 media supplemented with GP as phosphorus resource; MS_1 media supplemented with GP as nitrogen resource. MS_1 medium contained (g/L): NH_4Cl, 2.0; $MgSO_4$.$7H_2O$, 0.2; K_2SO_4, 0.5; Na_2HPO_4, 0.03; and trace elements (mg/L): $FeSO_4$. $7H_2O$, 2.5; $CaCl_2$.$6H_2O$, 10.0; $CuSO_4$. $5H_2O$, 2; H_3BO_3, 0.06; $ZnSO_4$. $7H_2O$, 20.0; $MnSO_4$. H_2O, 1.0; $NiCl_2$. $6H_2O$, 0.05; Na_2MoO_4. $2H_2O$, 0.3. Sodium glutamate was used as the carbon source (10 g/L). The same amino acid could serve as a nitrogen source, along with NH_4Cl. Na_2HPO_4 was used as a phosphorus source (Ermakova et al., 2007). After nine days incubation in the aforementioned media, bacterial population were enumerated by the plat count method on agarized LB medium containing (g/L): Bacto peptone, 10; yeast extract, 5; NaCl, 5; pH 7.

Soil microbial activity

Soil microbial activity was evaluated by measuring dehydrogenase activity and respiration rate. Tests of microbial activities on soil 4 (control) were done before and after adding different concentrations of glyphosate. The soil samples were wetted with distilled water (10% w/w) and pre-incubated for six days at 30°C (Zabaloy et al., 2008), then treated with different GP concentrations (0, 25, 50, 500 mM) (Bass et al., 2001), humidified again to 20% (w/w) and incubated for two weeks in 30°C. Soil moisture content was maintained throughout the incubation by weighing and correcting for any water loss, using distilled water. Dehydrogenase activity and respiration rate was measured after 3, 7 and 14 days of incubation.

Dehydrogenase activity

3 g of soil sub-samples were weighed into a 50 ml polypropylene centrifuge tube and mixed with 4 ml phosphate buffer (pH 7.6) and 1 ml 3% 1,3,5-triphenyiltetrazolium chloride (TTC) solution. The centrifuge tubes were incubated for 24 h at 37°C in the dark. After incubation, triphenylformazan (TPF) formed by reduction of TTC was extracted with 10 ml acetone. Tubes were shaken in an orbital shaker at 300 rpm for 30 min, centrifuged (2000 rpm, 5 min), and the supernatant was filtered with filter paper (S&S N°859). Blanks without the addition of TTC were carried out in the same manner. The concentration of TPF was determined by spectrophotometrical measurements at 485 nm, and the results were expressed as g TPF g^{-1} soil 24 h^{-1}.

Respiration rate

10 g of each soil subsamples, adjusted to 60% of water holding capacity in glass containers, were amended with 10 ml of 0.5 N sodium hydroxide to trapped evolved CO_2 and incubated at 30°C for 3, 7 and 14 days. Following the incubation period, the Na_2CO_3 formed in the vial containing NaOH was precipitated as $BaCO_3$ by addition of 1 M $BaCl_2$. The remaining NaOH in each vial was titrated to the phenolphthalein endpoint with a standardized 0.5 N HCl solution.

Statistical analysis

Statistical analyses were performed using SPSS 16 software and comparison of means was done with Duncan test at 5% level.

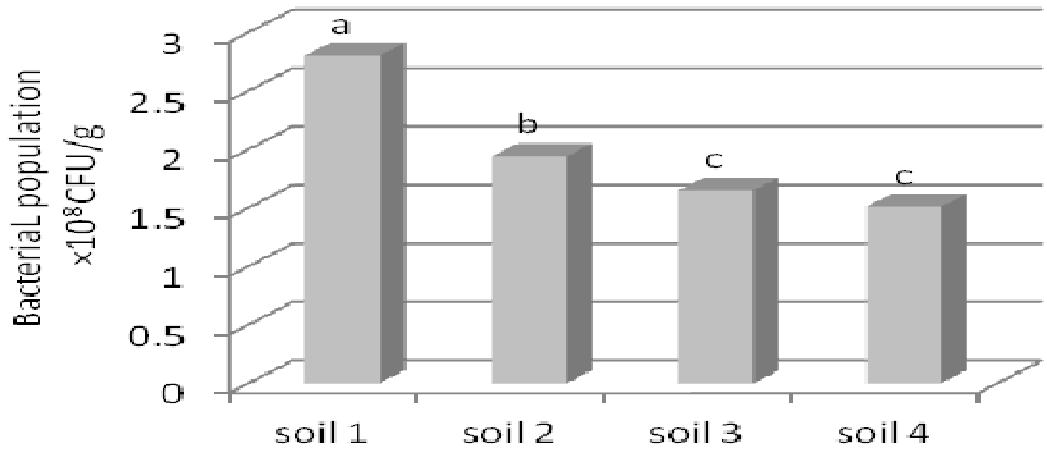

Figure 1. Heterotrophic bacterial population in the tested soils.

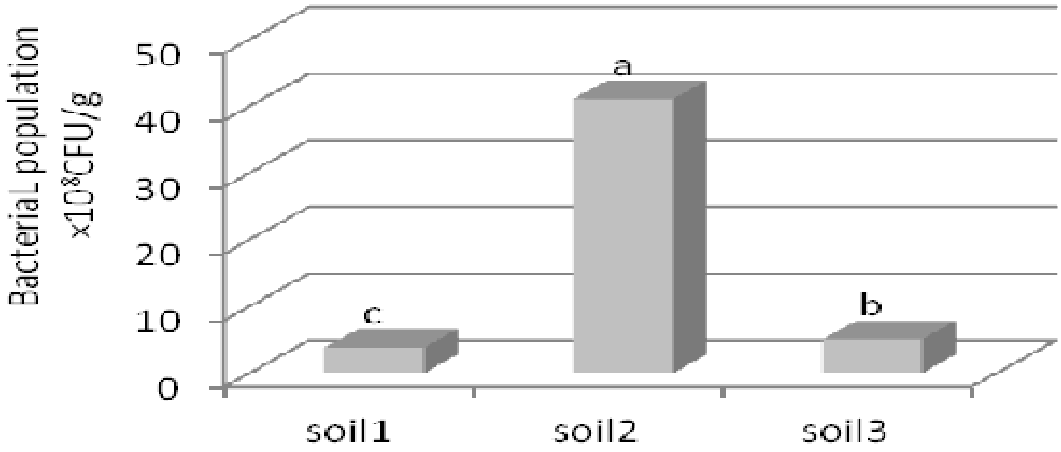

Figure 2. Effect of GP on soil bacterial population in GP containing media.

RESULTS

The principal characteristics of the soils are shown in Table 1.

The glyphosate effect on bacterial population

The mean comparison of heterotrophic bacteria population in four different soil samples is shown in Figure 1. Soil bacterial populations in the control sample (soil 4) was found to be significantly lower than those of herbicide treated soils. Soil 1 and 3 had the highest and lowest bacterial populations of 2.8×10^8 and 1.6×10^8 CFU/g-soil, respectively.

The effect of GP on bacterial population in the mineral MS_1 medium containing glyphosate is shown in Figure 2. According to the results, all the three polluted soil samples showed significant differences in comparison with each other and the highest bacteria population (41×10^8) was related to soil 2.

The effect of GP as C/N/P sources on bacterial population (Figures 3 and 4) showed that the bacterial population in the presence of GP as P source was significantly ($p<0.01$) higher than N and C sources.

Soil microbial activity

Dehydrogenase activity as measure as g TPF g^{-1} soil 24 h^{-1} hydrolyzed per gram of soil, increased significantly with time and GP addition in compare with control samples and it is highest amount (0.4 g TPF g^{-1} soil 24 h^{-1}) was related to Soil 1 with 25 mM GP after one week incubation (Figure 5).

Respiration rate measured as µg CO_2 g^{-1} soil, increased significantly ($p<0.01$) except 500 mM concentrations of GP in comparison with the control. The highest amount of respiration (93.3 µg CO_2 g^{-1} soil) belonged to the 50 mM GP concentration after 14 days incubation.

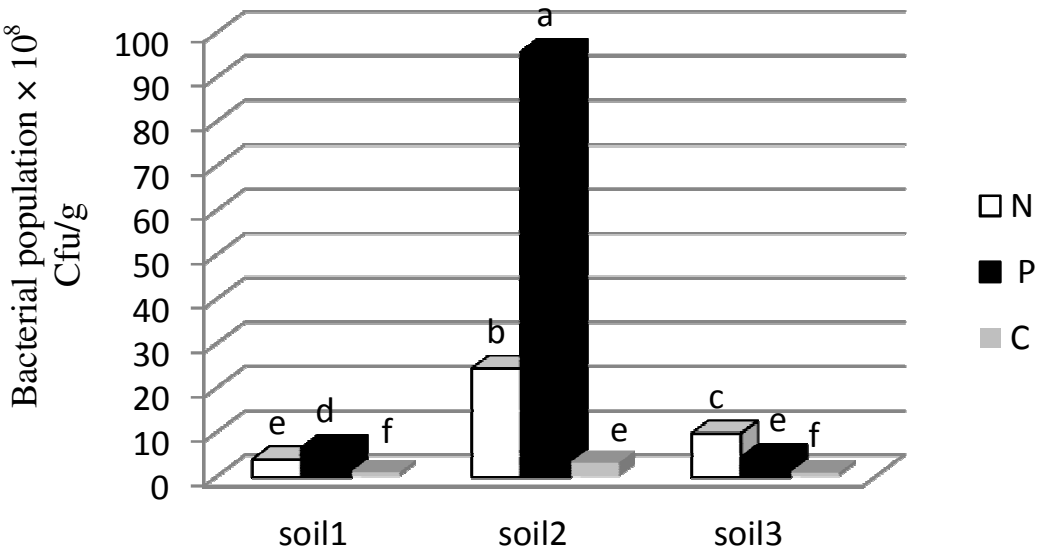

Figure 3. Effect of GP as C/N/P sources on bacteria population in each soil.

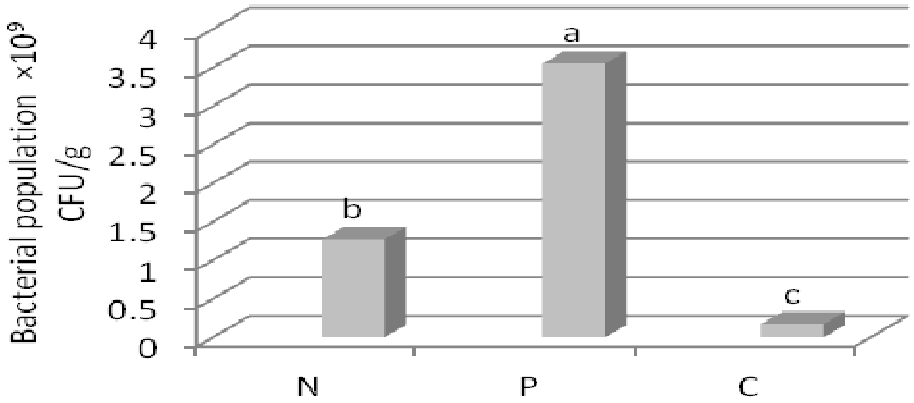

Figure 4. Effect of GP as C/N/P sources on bacterial population (mean of three polluted soils).

DISCUSSION

Although herbicides are not intentionally designed to inhibit microbes, non-target effects on microbial activities are likely to occur in some extent if there are sensitive members in the microbial population. We used several indices of microbial population to assess the effects of GP. These included plate counts of aerobic heterotrophic bacteria, soil respiration rate and dehydrogenase activity.

In this study, the observed trends in bacteria population showed that in a soil with a long historical use of GP (Soil 1), the hetrotrophic bacteria population was significantly ($p<0.05$) increased. Also, by increasing bacteria population, the possibility of GP existence as nutrient source is enhanced. This may consequently explain the effect of GP application time on microbial population. It is likely that the GP provided nutrients for bacterial growth, as evidenced by the significant increase in bacterial population. Araujo et al. (2003) reported an increase in heterotrophic bacteria of a soil with history of GP application. In contrast, Ratcliff et al. (2006) reported transient increase in fungal propaggules and effect on culturable bacteria after GP addition (50 mg kg^{-1}). Sumalan et al. (2007) showed that the soil bacteria population after 10 days of GP application was significantly higher than 3 days of it's application (about 2.3 times).

Glyphosate treatment (500 mg/L) led to increasing bacterial population in soil 2 in comparison with other soils ($p<0.05$) because bacteria were adapted to the toxic effects of GP and were able to utilize it as nutrient source. The bacterial growth in the presence of GP as sole C source indicates that the GP was a source of energy for microbial activity. In fact, the soil with the highest long-term exposure exhibited the strongest relationship (Haney et al., 2000). This conclusion agrees with Lancaster et al. (2010), who found that after repeated application of GP, microorganisms were better

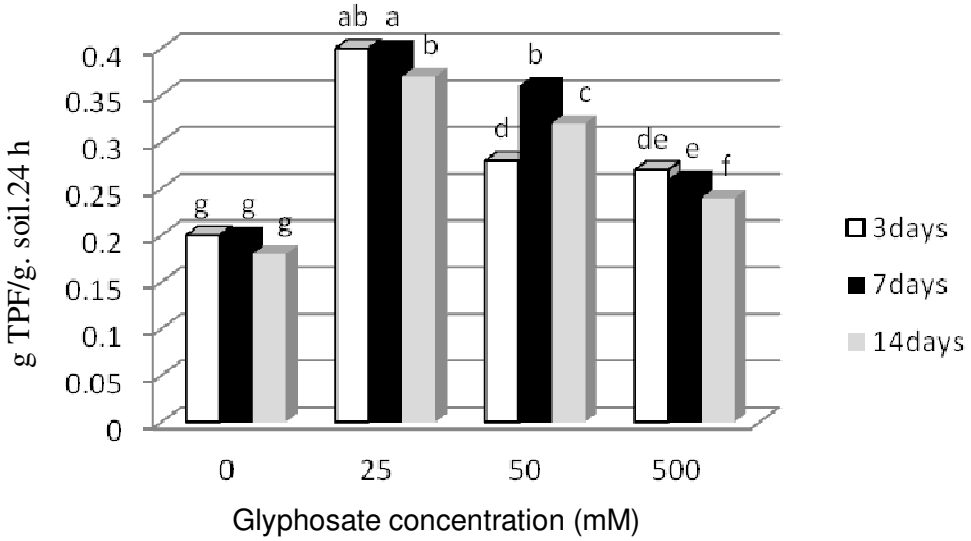

Figure 5. Dehydrogenase activity of soils after time interval incubation with different concentrations of GP.

able to utilize it.

According to results, bacterial population in the presence of GP as P source was significantly ($p<0.01$) higher than those of other sources. Glyphosate as an organophosphonate can be used as a source of P, C or N by either Gram-positive or Gram negative bacteria (Zabaloy et al., 2008). Some researches highlighted the positive effect on the microflora in the addition of GP case, which is an alternative source of C, N or P (Sumalan et al., 2008). The results show that GP serves as a better phosphorous source than as a carbon source (Figure 3). It is evident that the effects of GP on bacterial populations are dose-dependant and highly temporal and could be explained by a rapid enrichment of opportunistic bacteria that use the compound as a nutrient and/or C source (Ratcliff et al., 2006).

Microbial activity is an important factor in the behavior of GP in the soil. The application of GP to the soil led to a significant increase in dehydrogenase activity with respect to untreated control soil samples. This might be due to increase in microbial population with the potential of utilizing GP as carbon or other nutrient sources. Obtained results also indicate that soil treated with 25 mM GP had the highest dehydrogenase activity when compared to other GP concentrations used in this study. So, higher concentrations of GP inhibited dehydrogenase activities in respect to lower concentrations of it. In literature, such effects on several soil enzymes are reported (Gianfreda et al., 2005; Quilchano and Maranon, 2002). The effect of time on dehydrogenase activity also showed that the maximum activity was obtained in 7 days and it was reduced at 14 days of incubation. Also, Andreá et al. (2003) showed that dehydrogenase activity was higher than the control after one month after GP application. In this case, it can be suggested that glyphosate can increase dehydrogenase activity or soil oxidative processes. Conversely, some researches reported the reduction of dehydrogenase activity in comparison to the control sample (Zabaloy et al., 2008; Sebiomo et al., 2011). Accinielli et al. (2002) also found that GP at doses ranging from 20 to 200 mg kg^{-1} exerted effect on microbial activity as measured by respiration and dehydrogenase activity.

Soil respiration is usually used as an essential parameter for examining the effects of pollutants on soil micro-organism (Brohon et al., 2001). In this study, respiration rates increased with increasing GP application rate up to 50 mM at all days. The inhibitory effect of GP was observed at 500 mM. In this concentration, no significant difference was observed in respect with control. The increases in microbial respiration after GP exposure may be correlated with high levels of extractable soil phosphate. The inhibitory effect of 500 mM GP on respiration might be associated with the total microbial population that has been lowered compared to the other tested GP treatments.

The difference in the respiration response suggests that GP exposure shifts the microbial community to a population that can more readily use GP as a substrate, an observation supported by Araujo et al. (2003). Zabaloy et al. (2008) also showed that the respiration increased after a few days of starting incubation (3 days).This increase was about 42% in regard with control soil. Zabaloy et al also mentioned that, the respiration reaction continued at least two weeks after incubation in each soil and after that the respiration decreased. Asinley (2002) reported that GP the effect on respiration was positive in he GP concentrations between 20 to 200 mg/kg. Busse

et al. (2001) also showed that microbial respiration was unchanged at expected field concentrations (5 to 50 µg/g) and was stimulated by concentrations of 100-fold greater. This research confirms that glyphosate application may alter (increase) soil microbial activity and population. Increased microbial activity may be beneficial or detrimental toward plant growth, soil microbial ecology, and soil quality. Beneficial effects include optimum plant growth and production due to greater availability of nutrients, resulting from mineralization of GP mediated by soil microorganisms. Increased microbial activity and high microbial populations may also sequester plant nutrients in microbial biomass, decrease crop growth and yields, and increase susceptibility to diseases and pests (Yamada and Xe, 2000; Wolf and Wagner, 2005). Future studies should explain further if changes in microbial activity and population due to GP are beneficial or harmful for crop productivity and soil ecology by monitoring specific plant-microbe-soil interactions (that is, legume nodulation by rhizobia) rather than individual general activities (that is, microbial respiration).

REFERENCES

Accinelli C, Screpanti C, Dinelli G, Vicari A (2002). Short-time effects of pure and formulated herbicides on soil microbial activity and biomass, intern, J. Environ. Anal. Chem. 82: 519-527.

Anderson TH (2003). Microbial eco-physiological indicators to assess soil quality, Agric. Environ. 98: 285-293.

Andreá MM, Peres TB, Luchini LC, Bazarin S, Papini S, Matallo MB, Savoy VLT(2003). Influence of repeated applications of glyphosate on its persistence and soil bioactivity, Pesq. Agropec. Bras. 38:1329-1335.

Araujo ASF, Monteiro RTR, Abarkeli RB (2003). Effect of glyphosate on the microbial activity of two Brazilian soils.J.Chemosphere. 52: 799-804.

Avidano L, Gamalero E, Cossa GP, Carraro E (2005). Characterization of soil health in an Italian polluted site by using microorganisms as bioindicators, Appl. Soil Ecol. 30: 21-33.

Beneditti A, Dilly O (2006). Introduction In: Microbiological Methods for Assessing Soil Quality, CABI Publishing, Wallingford, UK. pp. 3-14.

Bolter M, Bloem J, Meiners K, Moller R (2006). Enumeration and biovolume determination of microbial cells. In: Bloem J, Hopkins DH, Benedetti A (Eds.), Microbiological Methods for Assessing Soil Quality, CABI Publishing, Wallingford, UK. pp. 93-107.

Brohon B, Delolme C, Gourdon R (2001). Complementarily of bioassays and microbial activity measurements for the evaluation of hydrocarbon-contaminated soils quality, J. Soil Biol. Biochem. pp. 889-891.

Busse MD, Ratcliff AW, shestak CJ, Powers RF (2001). Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities, J. Soil Biol. Biochem. 33: 1777-1789.

Ermakova IT, Shushkova TV, Leontevskii AA (2007). Microbial Degradation of Organophosphonates by Soil Bacteria, J. Microbiol. 5: 615-620.

Filip Z (2002). International approach to assessing soil quality by ecologically- related biological parameters, Agric. Ecosyst. Environ. 88: 169-174.

Gianfreda L, Rao MA, Piotrowska A, Palumbo G, Colombo C (2005). Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution, Sci. Total Environ. 341: 265-279.

Haney RL, Senseman SA, Hons FM, Zuberer DA (2002). Effect of glyphosate on soil microbial activity and biomass. Weed Sci. 48: 89-93.

Lancaster SH, Hollister EB, Senseman SA, Gentry TJ (2010). Effects of repeated glyphosate applications on soil microbial community composition and the mineralization of glyphosate, Pest Manage. Sci. 66: 59-64.

Nannipieri P, Kandeler E, Ruggiero P (2002). Enzyme activities and microbiological and biochemical processes in soil, in: Burns RG, Dick RP (Eds.), Enzymes in the Environment: Activity, Ecology and Applications, Marcel Dekker Inc. pp. 1-33.

Quilchano C, Maranon T (2002). Dehydrogenase activity in Mediterranean forest Soils, Biol. Fertil. Soils, 35: 102-107.

Ratcliff AW, Busseand MD, Shestak CJ (2006). Changes in microbial community structure following herbicide [1] (glyphosate) additions to forest soils, Appl. Soil Ecol. 34: 114-124.

Sebiomo A, Ogundero VW, Bankole SA (2011). Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity, Afr. J. Biotechnol., 10: 770-778.

Sumalan R, Alexa E, Negrea M, Dehelean C (2008). Researches Concerning structure and activity of edaphic microflora community when glyphosate is added.

Wolf DC, Wagner GH (2005). Carbon transformations and soil organic matter formation. In Principles *and* Applications of Soil Microbiology, 2nd Ed. Syliva DM, Fuhrmann JJ, Hartel PG, Zuberer DA (Eds.), Pearson Prentice Hall: Upper Saddle River, NJ: pp. 285-332.

Yamada K, Xe H (2000). Properties and applications of an organic fertilizer inoculated with effective microorganisms. J. Crop Prod. 3*:* 255-268.

Zabaloy MC, Garland JL, Gomez MA (2008). A integrated approach to evaluate impacts of the herbicides glyphosate, 2- 4-D and metsulfurom- methyt on soil microbial communities in the pampas region, Argentina, J. Appl. Soil Ecol. 40: 1-12.