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Comparative Effects of Organic Waste Formulation and Inorganic Fertilizer on the Microbial Activity and Biomass of Glyphosate contaminated Soil.

S.A. Adejoro and D.S. Sonoiki.

Department of Crop, Soil and Pest Management,
The Federal University of Technology Akure. Nigeria.

Email: solomonajoro@gmail.com

Abstract

The toxicity of pesticides to the environment can be ameliorated if accompanied by other sustainable agricultural practices. The practices which will confer enhanced degradation on these chemicals are expected to be more complementary when a sustainable and healthy environment is the priority. This study compared a compost formulation and an inorganic fertilizer in their abilities to cushion the ripple effects of the glyphosate herbicide on soil microbial biomass and activity. Compost formulation and NPK mineral fertilizer were separately added to soil samples contaminated with the field rate of glyphosate to compare the effects on soil microbial activity and microbial biomass in the laboratory. The treated samples were incubated for 28 days and soil microbial activity and microbial biomass were measured. Soil microbial metabolic quotient and carbon mineralization quotient were also determined. Glyphosate applied alone gave transient inhibition of soil basal respiration, soil microbial biomass and soil mineralization quotient and was followed by their mild stimulation. The addition of fertilizer increased these parameters and the highest figures were obtained when NPK was present at the early stage of incubation. The boost in soil microbial activities, biomass and carbon mineralization quotient was however sustained to the end of incubation by the addition of compost. The addition of NPK fertilizer significantly reduced CO_2 compared to the other treatments at the beginning of incubation but NPK significantly raises it at the end. Therefore, the addition of organic fertilizers would be preferred as bio-stimulants in pesticide bioremediation programmes.

Key words: Glyphosate, soil, microbial biomass, biostimulation, mineral fertilizer, compost

Introduction

The presence of herbicides in the soil environment would pose problems relating to soil health because of the hazardous effects of these chemicals on soil biological processes and non-target organisms. The biological and biochemically-mediated processes in soils are of utmost importance to ecosystem functions because of the roles of microbes as the driving forces in the transformation of organic matter, nutrient release and degradation of xenobiotics (Zabaloy *et al.*, 2008). Thus, microbial activity is a more efficient parameter used to assess soil quality than the physical and chemical parameters because of the ability of microbes to respond immediately to changes in the environment (Nannipieri *et al.,* 2002; Avidano *et al.*, 2005).

Glyphosate (N-(phosphonomethyl)glycine), a non-selective post-emergence herbicide released for commercial use in 1974 has become one of the world's most widely-used pesticides (Cox, 2004). The use is desirable in arable crop fields because it controls a broad spectrum of annual and perennial grasses, broad leaves and sedges thereby preventing arable crops from early weed competition when applied pre-plant. Glyphosate acts by enzymatic inhibition of the shikimic acid pathway, resulting in depletion of essential aromatic amino acids needed for plant growth and survival (Haney *et al.*, 2000).

Most living organisms do not have this pathway which is, however, ubiquitous in microorganisms (Busse *et al.*, 2001).

The direct application of glyphosate to the soil is not recommended but a significant amount may reach the soil during early-season or pre-plant applications (Haney *et al.*, 2000). The soil half-life of glyphosate varies from few days to two or three months and is mostly smaller than one growing season but there are reports of soil persistence for 100 to 1000 days (Heinonen-Tanski, 1989; Feng and Thompson, 1990) and persistence of the phytotoxic activity for more than 19 weeks after the application (Weber, 1994).

Glyphosate degradation in soil has been extensively studied. Similar to other members of the phosphonate class which are compounds characterised by a C-P bond, glyphosate is relatively resistant to chemical hydrolysis, thermal decomposition and photolysis. Although degradation of glyphosate in natural environments, such as the soil ecosystem, is mainly considered a non-specific, co-metabolic process, the predominant mechanism of glyphosate transformation in soil is microbial degradation. Microbial strains capable of growing in artificial media using glyphosate as the sole C, N or P source have been isolated such as *Pseudomanas spp*. The main metabolic pathway is degradation to aminomethylphosphonic acid (AMPA) which is further mineralized to CO_2 (Grundmann *et al.*, 2008). Therefore, the practices capable of enhancing soil microbial activity would be helpful to achieve rapid degradation of glyphosate in soils. Dobbs (1992) and Hyman *et al.* (1990) have shown that microbial populations change after fertilization and soil microbial indices are influenced by both organic and mineral fertilizers. The fertilizer supplies nutrients which directly stimulate the growth of microbal populations and may affect the composition of individual microbal community in soil (Khonje *et al.*, 1989; Sarathchandra *et al.*, 1989 and Khamis *et al.*, 1990). Similarly, the accumulation of organic carbon as a result of manure addition not only results in increased microbial biomass but has also been linked to changes in microbial community structure and increased functional diversity. Jangid *et al.* (2008) found that bacterial diversity, in terms of species richness and evenness, was higher in soils amended with poultry litter than in those treated with inorganic fertilizers. Furthermore, Gomez *et al.* (2006) obtained a positive linear relationship between microbial diversity and soil organic carbon, suggesting that the increases in functional diversity can be explained by the higher amounts of available carbon resulting from manure amendments.

The present study aims at comparing the ability of compost (Sunshine Organic Fertilizer ® produced by the Ondo State Waste Management Board, Akure Nigeria) and inorganic fertilizer (NPK 15-15-15) to restore soil microbiological quality in a glyphosate-contaminated soil.

Materials And Methods

The study was conducted in the Soil Laboratory of the Department of Crop, Soil and Pest Management of the Federal University of Technology Akure, Ondo State (7º16´N, 5º12´E) located in the rainforest vegetation zone of Nigeria. The soil used was collected from a field in the Experimental Station of the Department of Crop, Soil and Pest Management in the Teaching and Research Farm that had no herbicide treatment history during the last two decades.

Surface (0-15 cm) soil samples were randomly collected in the field, bulked and brought to the laboratory in sealed polyethylene bags. The soil samples were sieved (< 2 mm) and subsampled for the determination of chemical properties using standard methods (AOAC, 1990): pH (1:2 soil-water) was measured with pH meter; organic carbon by dichromate oxidation method; total N by macro-Kjeldahl digestion and distillation method; available phosphorus by the Bray 1 extraction and measured by colorimetry, exchangeable K, Ca and Mg were extracted with neutral 1N ammonium acetate and K in the extract was determined by flame photometer while Ca and Mg were determined on atomic absorption spectrophotometer. Soil is acidic (pH = 5.7), contains 33.4, 47.6 and 190.0 g kg^{-1} of clay, silt and sand respectively, 7.89 g kg^{-1} organic carbon, 1.1 g kg^{-1} of soil total nitrogen, 8.5 mg kg kg^{-1} available phosphorus and 0.05 Cmol kg kg^{-1} available potassium. The sieved soil was homogenized, wetted to field capacity and oven-dried to adjust the moisture content to 60% water holding capacity and 500 g soil weighed into plastic jars and stabilized at 30 ºC in the dark for one week. The treatments applied to the stabilized soil samples were: glyphosate at the recommended field rate of 2 litres ha^{-1} alone

(GLY-NF); glyphosate combined with 300 kg ha^{-1} Sunshine Organic Fertilizer ® (GLY-ORG), glyphosate combined with 200 kg ha^{-1} NPK 15-15-15 (GLY-NPK) and a control (CONTROL) in three replicates. The glyphosate was dissolved in water and applied directly to the soil inside the jars, while the fertilizers were added directly to the soil and homogenized. The plastic jars were covered tightly using paper tapes and the soils incubated at a constant temperature of 30ºC for a period of 28 days. The organic fertilizer contained 7 % N according to the manufacturer's label.

Measurement of soil microbial respiration:
The gas entrapment method developed by Hutchinson and Mosier (1981) was used to measure the soil microbial respiration. A 10 ml solution of 0.5M NaOH was dispensed into a 50 ml beaker and placed inside the plastic jars containing the treated soil to trap CO_2 evolved from the soil. The trapping solution were replaced after titration at seven-day intervals and covered back with lids (air tight seal). At the end of each week, 5 ml of 1.0M $BaCl_2$ were added to the NaOH solutions to precipitate the carbonates to facilitate determination of CO_2 evolved from the treated soil. The evolved CO_2 was determined by titration the NaOH in solution against 0.5M HCl using phenolphthalein indicator.

Determination of soil microbial biomass:
Soil microbial biomass C (MBC) was determined by the fumigation and extraction method described by Vance *at al.* (1987). 10 g portion of non-fumigated soil was extracted with 50 mL of 0.5 M K_2SO_4 by shaking for 45 min with a rotary shaker at 180 rpm and the suspension filtered using a Whatman No. 2 filter paper. A separate portion of soil was fumigated by placing it in a 50 mL beaker inside a desiccator alongside with another beaker containing ethanol-free chloroform. The desiccator was covered and evacuated with a vacuum pump until the chloroform boiled vigorously for 5 min. The evacuation was repeated three times at intervals of 15 min, letting air pass back into the desiccator to facilitate the

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distribution of the chloroform throughout the soil. The desiccator was evacuated a fourth time until the chloroform boiled vigorously for 2 min and 24 hours later, the chloroform was removed by vacuum extraction and the fumigated sample extracted. Organic carbon in the extract was determined by the wet dichromate oxidation method of Walkley and Black. MBC was calculated as the difference between the fumigated and non-fumigated samples divided by the K_2SO_4 extract efficiency factor (Kc = 0.35) (Sparling *et al.*, 1990).

Eco-physiological indices
qCO_2 (the community respiration per biomass unit or the metabolic quotient) and qM mineralization quotient) were measured at the end of incubation. The qCO_2 was determined as the ratio of CO_2-C (µg CO_2-C g^{-1} soil) to biomass carbon while qM was determined as the ratio of CO_2-C (µg CO_2-C g^{-1} soil) to organic carbon (mg g^{-1} soil).

Data Analysis
The data collected were subjected to analysis of variance while treatment means were compared using the Tukey tests.

Results
The microbial respiration in the treatments is shown in Table 1. There were significant variations in the responses of soil microbial respiration to the fertilizer-glyphosate treatments during the 28 days of incubation. Glyphosate applied alone caused significant reduction in soil respiration within the first 7 days of incubation and the repressive effect compared to the non-amended soil samples appeared to disappear at the 14^{th} day of incubation and beyond. The GLY-NPK treatment gave the highest concentration of CO_2-C g^{-1} soil which differed significantly from GLY-ORG at 7 and 14 days after application but both were different from GLY-NF and CONTROL. The significant boost in CO_2-C production caused by the compost to highest values at 21 days was sustained up to 28 days of incubation whereas microbial activity dwindled at 14 days and reduced further to similar concentrations at 21 and 28 days of incubation in the GLY-NPK treatment

Table 1: The effects soil amendments and glyphosate on soil respiration (CO_2-C mg kg^{-1} soil)

Treatments	Days after treatment application			
	7	14	21	28
GLY-NF	2.16^{d}	1.36^{c}	1.84^{b}	1.80^{b}
GLY-ORG	3.96^{b}	2.06^{b}	3.36^{a}	2.34^{a}
GLY-NPK	4.86^{a}	2.38^{a}	1.88^{b}	1.88^{b}
Control	3.06^{c}	1.32^{c}	1.74^{b}	1.70^{b}

Means in each column with the same letter (s) are not significantly different by Tukey's test (p 0.05)

Table 2 shows the cumulative production of CO_2-C as affected by the glyphosate-fertilizer treatments. The cumulative production of CO_2-C was significantly lowered by glyphosate treatment compared to the control until 21 days of incubation while the reduction was not significant at 28 days. The addition of organic or inorganic fertilizer significantly raised the cumulative CO_2-C at each sampling time compared to GLY-NF and CONTROL but GLY-NPK only differed from GLY-ORG at 7 days of incubation.

Table 2: The effects of glyphosate-soil amendments on cumulative soil respiration (CO_2-C mg kg^{-1} soil)

	Days after treatment application			
Treatments	7	14	21	28
GLY-NF	2.16^{d}	3.48^{c}	5.32^{c}	7.12^{b}
GLY-ORG	3.96^{b}	6.02^{a}	9.38^{a}	11.72^{a}
GLY-NPK	4.86^{a}	7.24^{a}	9.12^{a}	11.00^{a}
Control	3.06^{c}	4.42^{b}	6.16^{b}	7.86^{b}

Means in a column with the same letter (s) are not significantly different by Tukey's test (p 0.05)

The soil MBC was significantly reduced at 7 days after treatment with glyphosate alone and subsequently recovered to be significantly higher than the control 21 days after treatment application (Table 3). The control showed a reduction at 14 days and recovery subsequently. The addition of fertilizer, irrespective of the type, increased MBC significantly from the beginning up to 21 days of incubation but with the concentration highest from GLY-NPK while the highest value was observed in GLY-ORG at 28 days after treatment application. At the end of the experiment, GLY-ORG increased MCB by 55.8 percent while GLY-NF and GLY-NPK caused 9.06 and increases 13.16 percent respectively compared to the unamended soil samples

. **Table 3: Treatment effects on microbial biomass carbon (MBC) (mg g^{-1} soil)**

Treatments	Days after treatments			
	7	14	21	28
GLY-NF	19.6^{d}	23.2^{c}	44.3^{b}	31.0^{b}
GLY-ORG	39.6^{b}	33.4^{b}	44.6^{b}	53.3^{a}
GLY-NPK	81.0^{a}	90.0^{a}	60.8^{a}	29.7^{b}
Control	30.6^{c}	23.3^{c}	26.8^{c}	34.2^{b}

Means in a column with the same letter (s) are not significantly different by Tukey's test (p 0.05)

The effects of the treatments on calculated soil eco-physiological quotients are shown in Table 4. The application of glyphosate slightly increased qCO_2 at 7 days of incubation but lowered it at 14 and 21 days. The GLY-NPK treatment produced the least soil qCO_2 within the first 21 days of incubation which differed significantly ($P < 0.05$) from other treatments but increased it to the highest value at 28 days of incubation. The addition of compost to the herbicide contaminated jar (GLY-ORG) did not significantly reduce qCO_2 within the first week and subsequently till 28 days after application compared to the control and GLY-NF treatments. The estimate of the carbon mineralization quotient (qM) based on the cumulative CO_2-C production for the 28 days increased significantly only in the soil samples to which fertilizers were added irrespective of type, but the highest value was recorded with compost (GLY-ORG) amended.

Table 4: Treatment effects on eco-physiological quotients (qCO_2 and qM)

	Days after treatment application				
Treatments	7	14	21	28	qM
GLY-NF	0.11^{a}	0.15^{a}	0.12^{b}	0.23^{b}	0.045^{bc}
GLY-ORG	0.10^{a}	0.18^{a}	0.20^{a}	0.21^{b}	0.057^{a}
GLY-NPK	0.06^{b}	0.08^{b}	0.15^{b}	0.37^{a}	0.051^{ab}
Control	0.10^{a}	0.19^{a}	0.23^{a}	0.23^{b}	0.041^{c}

Means in a column with the same letter (s) are not significantly different by Tukey's test (p 0.05)

Discussion

This study has shown that the soil used contained microorganisms that are susceptible to the toxic effects of glyphosate. This agrees with Quinn *et al.* (1988); Santos and Flores (1995) who have reported the adverse effects of glyphosate on microorganisms in laboratory studies. The recovery of metabolic activities at 14 days of incubation, as demonstrated by the increase in the production of CO_2-C and MBC in the jars in which the soil was contaminated with glyphosate however suggests that the soil also contained organisms that can metabolize glyphosate for increased activity. This can be explained by the fact that the glyphosate added to soil constitutes a source of nutrient and energy, enriching the soil, where it is immediately metabolized resulting in stimulation of activity and functional diversity of the heterotrophic microbial community (Benslama and Boulahrouf, 2013). The boost in soil microbial activity engendered by the addition of NPK suggests that mineral fertilizer was quick to release nutrients to the soil which were used by the microorganisms to cushion the repressive effects of the herbicide treatment on their metabolic activities. The flux in microbial biomass C in response to the addition of NPK (having readily available high energy containing nutrients) also suggests that the soil used for the experiment was dominated either by zymogenous microflora, copiotrophic bacteria or the r-strategists (Kennedy, 2005; Brady and Weil, 2008) which thrive better in the presence of freshly added readily-available nutrients. The depression of microbial indices noticed with NPK application as incubation progressed agrees with the well documented side effects of mineral fertilizers on soil microbial community. Doran *et al.* (1996) have shown that mineral fertilization strongly affects a number of microorganisms and the qualitative selection of whole communities of soil microorganisms. The application of mineral fertilizer alone also increased the rate of organic matter mineralization, leading to a decrease in the content of easily decomposable organic matter in the soil that is related with a reduction in microbial biomass content (Collins *et al.*, 1992; Lovell *et al.*, 1995). The consistent increase in soil respiration and MBC caused by the addition of organic fertilizer indicates sustained supply of nutrients and substrates for energy production. Research results are consistent on the fact that the addition of organic manure from animal or plant sources to soils increases soil respiration, microbial biomass and diversity (Goyal *et al.*, 1999; Fontaine *et al.*, 2003 and Jangid *et al.*, 2008). The addition of NPK fertilizer greatly reduced perturbation in the soil microbial community as indicated by the significant reduction in qCO_2 compared to the other treatments at the beginning of incubation. The significantly higher qCO_2 recorded in this treatment at the termination of the experiment indicates the imposition of stress on the soil microbial community as time went on. This agrees with earlier observations that fertilization practice can lead to seriously disturbed functions of entire agroecosystems and contribute to the formation of different compounds in the soil (e.g. nitrosamines, mycotoxins) which are harmful to soil microorganisms and cultivated plants (Smyk *et al.*, 1989). The survival of microbes may also be inhibited due to osmotic stress created by fertilizers (Bharathi *et al.*, 2011). The increase in soil carbon mineralization quotient (qM) caused by all the amendments shows that both the herbicide and fertilizers possess bio-stimulatory potentials. The stimulatory effect of glyphosate on carbon mineralization has been reported (Adejoro, 2016) while fertilizer application to soil greatly influences the soil microbiological status and may affect the composition of individual microbial communities (Khonje *et al.*, 1989; Sarathchandra *et al.*, 1989 and Khamis *et al.*, 1990)

Conclusion

The study has shown that contamination of soil with glyphosate caused repressive effects that are transient on the soil microbiological indices. The addition of either organic or mineral fertilizer was able to cushion the envisaged adverse effects of the herbicide on the soil microbial community. It is, therefore, recommended that herbicide application programmes involving the use of glyphosate be accompanied with fertilizer application in order to address concerns about environmental contamination from the herbicide. However, the use of organic manures which guarantees sustained release of nutrients to maintain soil microbial activity for a longer period would be preferred.

References

Adejoro, S.A. (2016). Interaction effects of glyphosate and cypermethrin on soil basal respiration and carbon mineralization quotient. *Applied Tropical Agriculture* 21(1): 7-14

AOAC. (1990). *Official Methods of Analysis.* 15th Edition. Association of Official Analytical Chemists, Washington, DC, USA.

Avidano, L., Gamalero, E., Cossa, G.P. and Carraro, E. (2005). Characterization of soil health in an Italian polluted site by using microorganisms as bioindicators. *Applied Soil Ecology* 30: 21-33.

Benslama, O. and Boulahrouf, A. (2013). Impact of glyphosate application on the microbial activity of two Algerian soils. *International Journal of Current Microbiology and Applied Sciences* 2(12): 628-635.

Bharathi, J.M., Balachandar, D., Narayanan, R. and Kumar, K. (2011). Impact of fertigation on soil microbial community and enzyme activities cropped with maize under precision farming system. *The Madras Agricultural Journal* 98(13):84-88.

Brady, N.C. and Weil, R.R. (2008). *The Nature and Properties of Soils*. 12th Edition, Prentice Hall, Upper Saddle River, New Jersey 07458. Pp 1,530

Busse, M., Ratcliff, A., Shestak, C. and Powers, R. (2001). Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. *Soil Biology* 33:1777-1789.

Collins, H.P., Rasmussen, P.E. and Douglas, C.L. Jr. (1992). Crop rotation and residue management effect on soil carbon and microbial dynamics. *Soil Science Society of America Journal* 56: 783-788.

Cox, C. (2004). Glyphosate Herbicide Fact Sheet. *Journal of Pesticide Reform*, Winter 2004, 24: 10-15.

Dobbs, I. (1992). The changing face of soil fertility. *Dairying Today* 16, Farmhouse Publications, Auckland, New Zealand.

Doran, J.W., Sarrantonio, M. and Liebig, M.A. (1996). Soil health and sustainability. *Advances in Agronomy* 56: 1-54.

Feng, J.C. and Thompson, D.G. (1990). Fate of glyphosate in a Canadian forest watershed: Persistence in foliage and soils. *Journal of Agricultural Food Chemistry* 38: 1118-1125.

Fontaine, S., Mariotti, A. and Abbadie, L. (2003). The priming effect of organic matter: A question of microbial competition. *Oil Biology and Biochemistry* 35: 837-843.

Gomez, E., Ferreras, L. and Toresani, S. (2006). Soil bacterial functional diversity as influenced by organic amendment application. *Bioresource Technology* 97: 1484-1489.

Goyal, S., Chander, K., Mundra, M.C. and Kapoor, K.K. (1999). Influence of inorganic fertilizers and organic amendments on soil organic matter and soil microbial properties under tropical conditions. *Biology and Fertility of Soils* 29: 196-200.

Grundmann, S., Dorfler, U., Ruth, B. and Loos, C. (2008). Mineralization and transfer processes of 14C-labeled pesticides in outdoor lysimeters. *Water, Air and Soil Pollution* 8: 177-185.

Haney, R., Senseman, S., Hons, F. and Zuberer, D. (2000). Effect of glyphosate on soil microbial activity and biomass. *Weed Science* 48: 89-93.

Heinonen-Tanski, H. (1989). The effect of temperature and liming on the degradation of glyphosate in two arctic forest soils. *Soil Biology and Biochemistry, Oxford* 21: 313-317.

Hutchinson, G.L. and Mosier, A.R. (1981). Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal* 45:311–316.

Hyman, M.R., Kim, C.Y. and Arp, D.J. (1990). Inhibition of ammonia monooxygenase in *Nitrosomonas europaea* by carbon disulfide. *Journal of Bacteriology* 172: 4775-4782.

Jangid, K., Williams, M.A., Franzluebbers, A.J., Sanderlin, J.S., Reeves, J.H., Jenkins, M.B., Endale, D.M., Coleman, D.C. and Whitman, W.B. (2008). Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. *Soil Biology & Biochemistry* 40: 2843-2853.

Kennedy, A.C. (2005). Rhizosphere. In: Sylvia D.M. (Ed). *Principles and Applications of Soil Microbiology*. Pearson Educational, Inc. Upper Saddle River, New Jersey: 242-262.

Khamis, A.A., El-Sherbieny, A.E. Awad, E. and Osman, F.A. (1990). Effect of nitrogen fertilizers combined with nitrification inhibitor on cotton plants. *Zagasin Journal of Agricultural Research* 13: 195-213.

Khonje, D.J., Varsa, E.C. and Klubek, B. (1989). The acidulation effects of nitrogenous fertilizers on selected chemical and microbiological properties of soil. *Communications in Soil Science and Plant Analysis* 20: 1377-1395.

Lovell, R.D., Jarvis, S.C.and Bardgett, R.S. (1995). Soil microbial biomass and activity in long-term grassland: effects of management changes. *Soil Biology and Biochemistry* 27: 969-975.

Nannipieri, P., Kandeler, E. and Ruggiero, P. (2002). Enzyme activities and microbial and biochemical processes in soil. In: Burns, R.G. and Dick, R.P. (Eds). *Enzymes in the Environment: Activity, Ecology and Applications*. Marcel Dekker, New York, NY, USA. 1-33.

Quinn, J.P., Peden, J.M.M. and Dick, R.E. (1988). Glyphosate tolerance and utilization by the microflora of soils treated with the herbicide. *Applied Microbiology and Biotechnology* 29: 511-516.

Santos, A. and Flores, M. (1995). Effects of glyphosate on nitrogen fixation of free living heterotrophic bacteria. *Letters in Applied Microbiology* 20: 349- 352.

Sarathchandra, S.U., Perrot, K.W. and Littler, R.A. (1989). Soil microbial biomass: Influence of simulated temperature changes on the size, activity and nutrient content. *Soil Biology and Biochemistry* 21: 987-993.

Smyk, B., Rozycki, E. and Barabasz, W. (1989) Impact of application of mineral nitrogen fertilizers (N and NPK) on the occurrence of nitrosoamines and mycotoxins in the soils of mountain grassland ecosystems. *Problematic Journals of Agricultural Sciences*. 380,151

Sparling, G.P., Feltham, C.W., Reynolds, J., West, A.W. and Singleton, P. (1990). Estimation of soil microbial carbon by fumigation - extraction method. Use on soils of high organic matter content, and a reassessment of the KEc- factors. *Soil Biology and Biochemistry* 22: 301-307.

Vance, E.D., Brookes, P.C. and Jenkinson, D.S. (1987). An extraction method for measuring soil microbial biomass-C. *Soil Biology and Biochemistry* 19: 703-707.

Weber, J.B. (1994). Properties and behavior of pesticides in soil. In: Honeycutt, R.C. and Schabacker, D.J. (Ed.). *Mechanisms of Pesticide Movement into Groundwater*. Lewis, London: 15-41.

Zabaloy, M.C. and Gómez, M.A. (2008) Microbial respiration in soils of the Argentine pampas after metsulfuron-methyl, 2,4-D, and glyphosate treatments. *Communications in Soil Science and Plant Analysis* 39(3/4): 370-385