



## Original Research Article

# Impact of glyphosate application on the microbial activity of two Algerian soils

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## ABSTRACT

### Keywords

Glyphosate;  
Saharan soil;  
Forest soil;  
Microbial respiration;  
Microbial enumeration.

The large scale use of glyphosate to control weeds emphasizes the need to understand its effects on soil microbial communities. The herbicide may change the soil environment due to toxicity to soil microorganisms. This study evaluated the impact of glyphosate treatments on microbial community structure and function in vitro of two Algerian soils with different physicochemical properties (a forest soil and Saharan soil) untreated with glyphosate. Glyphosate was applied at a rate of 2.16 mg kg<sup>-1</sup> of soil and microbial activity was assessed by soil basal respiration and microbial enumeration. Glyphosate addition to the forest soil had no effects on culturable microbial community. While an increase in basal respiration was observed from the 4<sup>th</sup> to 16<sup>th</sup> days of incubation for the glyphosate treated samples, this was followed by a decrease that reached a close level to that observed for the control. Saharan soil had a strong response in microbial activity and a marked increase in total culturable microorganisms after 30 days of incubation. These initial findings suggest that glyphosate have no negative effects on microbial activity and it can improve soil quality. Further investigation is required to evaluate possible long-term ecological risks of using glyphosate in these regions.

## Introduction

Glyphosate (N-phosphonomethylglycine) is the most commonly used herbicide worldwide (Franz *et al.*, 1997). It is the active ingredient in Roundup and other weed-killing formulations especially used in agriculture and gardens maintenance. A broad-spectrum, post-emergence, non-selective herbicide.

The side effects caused by the use of glyphosate on non-target organisms are an environmental concern. The potential disruption of soil microbial communities and processes has attracted interest because of the herbicide mode of action. Glyphosate inhibits the enzyme 5-enolpyruvate - shikimate - 3-phosphatase

esyntase (EPSPS) in the shikimic acid pathway blocking the synthesis of essential aromatic amino acids (Duke *et al.*, 2003). Most living organisms, with the exception of plants, do not have this path and are therefore not directly affected by glyphosate. However, shikimic acid is ubiquitous in microorganisms (Bentley, 1990).

Reports of adverse effects on microorganisms in laboratory studies are numerous (Quinn *et al.*, 1988; Santos and Flores, 1995). Microbial growth in artificial media containing glyphosate as the sole source of C or N is rare, and only a limited number of bacterial species and fungi are able to grow when glyphosate is provided as the sole source of P (Liu *et al.*, 1991). Unlike the results of laboratory, studies on field have shown either no effect or a slight stimulation of soil microorganisms by glyphosate.

The increase in the number of culturable bacteria and fungi (Rueppel *et al.*, 1977), soil respiration (Carlisle and Trevors, 1986; Haney *et al.*, 2000), N mineralization (Haney *et al.*, 2000) and soil enzyme activity (Gianfreda *et al.*, 1995) are reported, while negligible changes in N cycling processes (Carlisle and Trevors, 1986; Olson and Lindwall, 1991; Muller *et al.*, 1981) and microbial biomass (Wardle and Parkinson, 1990, 1992) have been observed. The differences between laboratory and field studies can be explained in part by the high concentrations of the herbicide used in numerous laboratory studies and by the herbicide chemistry (Wardle, 1995).

Most information on the non-target effects of glyphosate comes from studies on agricultural soils. Knowledge of the

response of forest soil microorganisms to glyphosate is limited, and there is no report of the response of Saharan soil microorganisms to glyphosate.

The objective of this paper is to assess the effects of glyphosate on certain microbiological variables (microbial activity as measured by soil basal respiration and the enumeration of soil microorganisms), as well as soil physicochemical properties for two Algerian soil types without a previous history application of glyphosate.

## **Materials and Methods**

### **Chemicals and media**

The isopropylamine salt of glyphosate known as Roundup® (containing 450 g active ingredient/L of glyphosate, Monsanto) was purchased from a local store supplier of agricultural products in Constantine, Algeria.

Bacteria, fungi and actinomycetes were enumerated by the plate counts method for viable cells. For the enumeration of bacteria, Plate Count Agar (PCA) medium was used. Its composition in gram per liter of distilled water, pH (7.0, 7.2) is: Peptone (5), yeast extract (2.5), glucose (1), Agar (15). For the enumeration of fungi, Sabouraud medium was used. Its composition in gram per liter of distilled water, pH (7.0, 7.2) is: Peptone (10) Glucose (40), Agar (15). And for the enumeration of actinomycetes, Olson medium was used. Its composition in gram per liter of distilled water, pH (7.0, 7.2) is: Sodium Caseinate (2), Asparagine (0.1), Sodium propionate (4), K<sub>2</sub>HPO<sub>4</sub> (0.5), MgSO<sub>4</sub> (0.1), FeSO<sub>4</sub> (0.001), Agar (15), glycerol (5mL).

### **Experimental soils and treatment**

Soil specimens were collected from two different untreated soils. The first sample is a forest soil taken from the forest of Chaâberssas located in the University of Constantine, between 7°35' longitude and 36°23' latitude in the center of eastern Algeria. The second sample was taken from a sandy field located in the region of Biskra between 34°51'01" north latitude and 5°43'40" east longitude in the north-eastern of Algeria on the northern edge of the Sahara, Desert. Samples of about 1 kg were taken from the first 15 cm of depth, pooled and sieved. Samples were air dried and stored in sterile plastic bags at 4 °C until use.

The treatment of soils was carried out according to Araujo *et al.*, (2003) method. A sufficient amount of Roundup to give a final glyphosate concentration of 2.16 mg kg<sup>-1</sup> was added to 75 g of each soil sample.

### **Determination of soil physicochemical parameters**

The soil pH (H<sub>2</sub>O) was determined using an electronic pH-meter in 1g soil to 2.5 mL water. Total organic matter was determined following established method (Nelson and Sommers, 1982). Particle size was determined using sieving method. The standard sieves of different mesh numbers as per specification of National bureau of standards were used.

### **Enumeration of microorganisms**

Bacteria, fungi and actinomycetes were counted after 0 and 30 days of incubation for controls and 30 days for samples treated with glyphosate. An amount of 5 g of each sample weighed into into a flask

containing 25 mL of sterile saline solution and stirred for 10 min on an orbital shaker. After settling (10 min), 3 mL of the supernatant was transferred into 25 mL of fresh sterile saline solution to make the 10<sup>-2</sup> dilution. Similarly, the 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>, 10<sup>-6</sup> and 10<sup>-7</sup> dilutions are prepared.

For total bacteria count, 0.1 mL for the last three dilutions was seeded in duplicate on PCA medium. The Petri dishes were incubated at 30°C for 48 hours. The same amount was seeded for actinomycetes on Olson medium and incubated at 30°C for 21 days and for fungi on Sabouraud medium and incubated at 30°C for 7 days.

### **Basal soil respiration (SBR)**

Basal respiration ( $\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil}$ ) was controlled for treated samples and controls by removing sub-samples at 2, 4, 8, 16, 24 and 30 days of incubation according to the alkali sorption-titration method described by Anderson and Domsch, 1990. Briefly, 20 g of each sample was weighed into a glass bottle (500 mL). The vials were closed with an airtight cap. The CO<sub>2</sub> produced by respiration is captured in 2 mL of 0.1M NaOH pipetted into a small beaker and placed in the bottom of the bottle. The experiment was performed in triplicate. Two blanks without soil were prepared to assess the amount of CO<sub>2</sub> trapped without respiratory activity. The flasks were incubated 24 hours at 22°C. The NaOH solution was transferred to a beaker which was used as the titration vessel. A 4 mL of BaCl<sub>2</sub> solution (0.05M) was added to precipitate the carbonate as BaCO<sub>3</sub>. Few drops of phenolphthalein were added as an indicator. Then, the remaining NaOH was brought to pH 8.3 by slow addition of 0.05M HCl, with magnetic stirring until disappearance of the color.

## Results and Discussion

Microbial communities are the basis of important ecosystem processes (such as nutrient recycling, transformation of pollutants and gas exchange with the atmosphere). The factors influencing these processes can affect soil health. Soil microbial properties, particularly those related to the biomass, activity and diversity of soil microbial communities can be used as indicators of the impact of disturbances on soil quality (Hernández-Allica *et al.*, 2006; Mijangos *et al.*, 2009).

### Determination of soil physicochemical parameters

The physicochemical properties of the tested soils are listed in Table 1. The forest soil is a clay-loam soil rich in organic matter. While the Saharan soil has a sandy texture with low organic matter.

### Enumeration of microorganisms

The number of microorganisms at the beginning and the end of the incubation in both soils is shown in Table 2.

For the forest soil, Plate counts and colony morphology were comparable between control and glyphosate treated sample after 30 days of incubation for all enumerated microorganisms. In studying the effect of glyphosate on the number of microorganisms in the soil, Stratton and Stewart (1992) observed no negative or positive effects in respect to the number of microorganisms. These findings are consistent with our results. The lack of negative effect on the microbiota can be explained by the fact that glyphosate is probably not directly toxic, leading to the acute death of sensitive types. Rather, it might exert a gradual effect caused by relative changes in growth efficiency

(slowly decreasing the abundance of sensitive types which waste energy due to the stress response and increasing the abundance of those adapted for rapid use of the free resources resulting from cometabolism) (Zabalay *et al.*, 2008).

In the case of Saharan soil, a significant increase in bacteria, fungi and actinomycetes populations was observed for treated sample. This finding is in agreement with the results of Gimsing *et al.*, (2004) and Ratcliff *et al.* (2006) who report an increase in viable soil microorganisms counts after glyphosate addition. This can be explained by the fact that the glyphosate added to soil is an available source of nutrient and energy enriching the Saharan soil, where it is immediately metabolized resulting in stimulation of activity and functional diversity of the heterotrophic microbial community.

The largest increase for microbial population of Saharan soil was observed for fungi. Several studies document effects of glyphosate on soil fungal community response and function. According to Wardle and Parkinson (1990) antagonistic interactions between the fungal species were eliminated by adding glyphosate to soil suggesting that the herbicide might influence overall soil fungal community structure. Krzysko-Lupicka and Orlik, 1997 revealed that glyphosate added to sandy clay with a history of repeated glyphosate treatment appeared to select for specific fungal species that were able to use the herbicide as a nutrient source. Means, 2004 detected a significant increase in soil *Fusarium* within 2 weeks after glyphosate was applied at recommended rates in the field. Another study verified that long-term exposure of soil microorganisms to

**Table.1** Physicochemical characteristics of tested soils

Parameter	Value	
	Forest soil	Saharan soil
pH	7.3	7.6
Clay (%)	39	0.38
Loam (%)	25	1.21
Coarse silt (%)	17	1.04
Sand (%)	10	52.15
Coarse sand (%)	09	45.22
Total CaCO <sub>3</sub> (%)	36.10	34.27
Organic matter (%)	10.12	1.07

**Table.2** Type and number of microorganisms detected in forest and Saharan soil before and after incubation for 30 days with and without added glyphosate

Microorganism and soil type	Colony forming units per gram of soil		
	Day 0	Day 30	
	Soil with no added glyphosate	Soil with no added glyphosate (control)	Soil supplemented with 2.16 mg kg <sup>-1</sup> glyphosate
<i>Bacteria</i>			
Forest soil	1.90 × 10 <sup>5</sup>	3.05 × 10 <sup>5</sup>	3.10 × 10 <sup>5</sup>
Saharan soil	0.7 × 10 <sup>3</sup>	3.24 × 10 <sup>3</sup>	6.17 × 10 <sup>3</sup>
<i>Fungi</i>			
Forest soil	2.60 × 10 <sup>3</sup>	4.41 × 10 <sup>3</sup>	5.01 × 10 <sup>3</sup>
Saharan soil	1.03 × 10 <sup>3</sup>	2.51 × 10 <sup>3</sup>	0.07 × 10 <sup>4</sup>
<i>Actinomycetes</i>			
Forest soil	0.27 × 10 <sup>4</sup>	2.12 × 10 <sup>4</sup>	2.07 × 10 <sup>4</sup>
Saharan soil	0.41 × 10 <sup>3</sup>	2.06 × 10 <sup>3</sup>	4.31 × 10 <sup>3</sup>

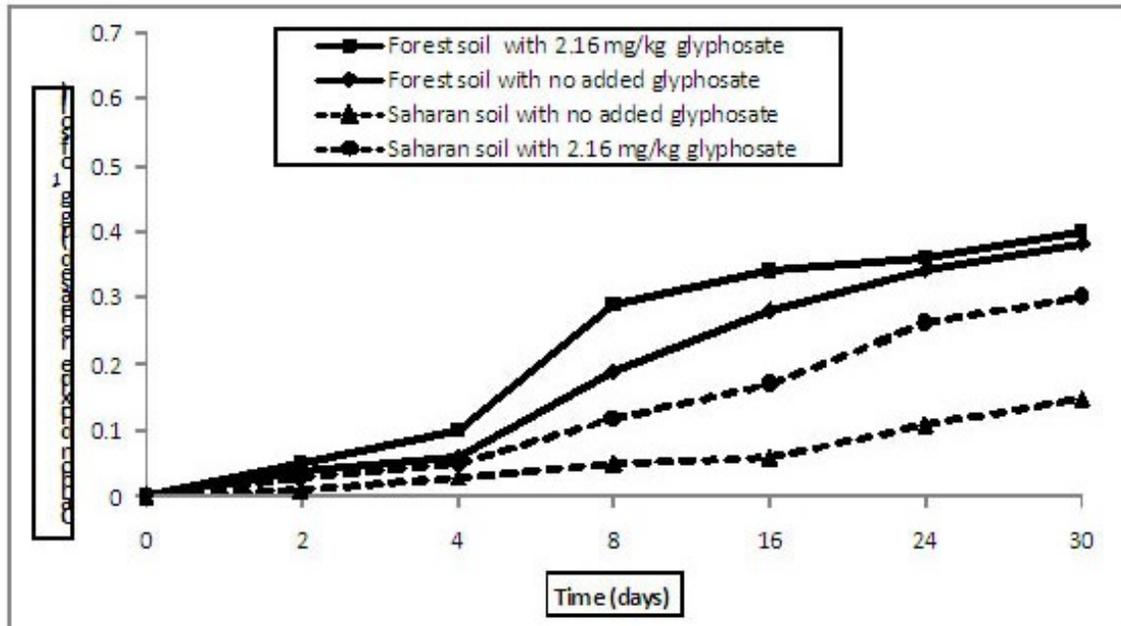
glyphosate led to a fungal community dominated by *Fusarium* spp. (Krzysko-Lupicka and Sudol, 2008).

### Effect of glyphosate on microbial respiration

Total carbon mineralization of treated and untreated soils during the incubation was used as an indicator of the total microbial activity in the soils. During the incubation period of Saharan soil samples (treated triplicate and 2 controls), soil respiration

was strongly stimulated by the addition of glyphosate. More carbon dioxide was released from samples which glyphosate was added in comparison with controls. The cumulative amount of carbon dioxide released from soil samples without glyphosate (controls) at the end of the incubation period (30 days) was 0.15, while the amount of carbon dioxide released from the samples that had received an additional of 2.16 mg kg<sup>-1</sup> of glyphosate was 0.30 (Figure 1). This result indicates that glyphosate

**Figure.1** Evolution of carbon dioxide of forest and Saharan soil with and without treatment with glyphosate



has no toxic effect on Saharan soil microorganisms. Similar results were also observed by Haney *et al.*, (2000) and Weaver *et al.* (2007). This may be related to a stress response in glyphosate sensitive species, due to the energy drain resulting from the ATP used in the accumulation of shikimate and hydroxibenzoic acids (Zablotowicz and Reddy, 2004)

The large amount of carbon dioxide released in the presence of glyphosate suggest that the microbiota of the Saharan soil is able to use glyphosate as a nutrient source, these results are consistent with the results of Wardle and Parkinson (1990) who suggest that the production of carbon dioxide is related to the decomposition of glyphosate in soil.

For the forest soil, a rapid increase of carbon dioxide released in the samples treated with glyphosate was observed from the 4<sup>th</sup> day of incubation (Figure 1). After the 16<sup>th</sup> day, the amount of carbon dioxide

released decrease and reached a close level to that observed for the control. This may be due to the adsorption of glyphosate to soil particles making it unavailable for soil microorganisms. Indeed, glyphosate is a polar molecule known for its high adsorption to Fe and Al oxides as well as molecules of clay in the soil (McBride and Kung, 1989; Piccolo *et al.*, 1994; Morillo *et al.*, 2000). And as the forest soil is a clay-loam soil (Table 1), the high clay content can cause the unavailability of the herbicide.

The apparent contradiction between the lack of effect of glyphosate on the composition of the forest soil microbial community and the increased respiration from the 4<sup>th</sup> to 16<sup>th</sup> day of incubation, can be explained by the dominance of a microbial community prone to use the substrates produced from the cometabolic decay of glyphosate for respiration, rather than for assimilation.

Despite the strong increase in Saharan soil respiration, mineralization intensity for forest soil was more important for both treated samples and controls, this indicates that the forest soil has a higher microbial activity and this is probably due to the difference in organic matter between the two soils, where it is higher in the forest soil.

This investigation is the first report of the side effects of glyphosate on Saharan soil. We were able to confirm some short-term (30 days) side effects of glyphosate tested on soil microbial respiration and microbial community composition of two different soils. Contrary to forest soil, activity of Saharan soil microbial community was markedly activated in the presence of the glyphosate, not only in the increase of the microbial number, but also in the microbial activity as presented by soil basal respiration.

These effects were not large but the results indicate that the overall impact of glyphosate on soil ecosystems depended on the soil physicochemical properties. It is clear both from our results and that of other researchers that glyphosate can stimulate certain microbiological variables in the soil. Further research will be needed to determine if results from such small-scale microcosm soil incubations can be extrapolated to those in larger systems such as greenhouse and field systems.

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