# $^{14}$C-glyphosate mineralization and follow up of the dynamics of *Pseudomonas* sp. populations in three soils under different uses in Tolima (Colombia)

## Mineralización de $^{14}$C-glifosato y seguimiento de la dinámica de las poblaciones de *Pseudomonas* sp. en tres suelos del departamento del Tolima (Colombia) sometidos a diferente uso

**Liliana Figueroa del Castillo[1, 3], Myriam Rocío Melgarejo P.[1], Cilia L. Fuentes de Piedrahíta[1], and Amanda Lozano de Yunda[2]**

**ABSTRACT**

The capacity of *Pseudomonas* sp. to degrade different pesticides has been the object of numerous studies; due to this, its dynamics was evaluated and the effect of the application of glyphosate (Roundup®) in soils of Tolima, taxonomically classified as Entic haplustolls, Typic ustipsamments, and Inceptic haplustalfs with coverage of secondary forest, stubble and sorghum crop, respectively, through the *in vitro* $^{14}$C-glyphosate mineralization. These soils were subjected to presence/absence of heat, and were denominated with heat treatment (HT) and without heat treatment (WHT). In both cases a solution of glyphosate Roundup SL® and radioactive was applied. The statistical design was used at random and the response parameter was the quantification of *Pseudomonas* sp. viable cells (cfu) during the mineralization process, for the three soils up to 120 days. It was found that adding glyphosate stimulated the increase of *Pseudomonas* sp., correlated significantly with the production of $^{14}CO_2$. The rates of mineralization and the bacterial populations in WHT soils were significantly ($P \leq 0.05$) greater with respect to HT soils. Finally, the higher averages for mineralization and bacterial dynamics were in the soils under use of secondary forest (63% and $26.8 \cdot 10^7$).

**Key words:** degradation, herbicide, microbial action, biometers.

**RESUMEN**

La capacidad que poseen las *Pseudomonas* sp. de degradar diferentes plaguicidas ha sido objeto de numerosos estudios, razón por la cual se evaluó su dinámica y el efecto de la aplicación del glifosato (Roundup®), en suelos del Tolima, clasificados taxonómicamente como Entic haplustolls, Typic ustipsamments e Inceptic haplustalfs, con cobertura de bosque secundario, rastrojo y cultivo de sorgo, respectivamente, a través de la mineralización de $^{14}$C-glyphosate *in vitro*. Estos suelos fueron sometidos a presencia/ausencia de calor, y se denominaron con tratamiento térmico (CT) y sin tratamiento térmico (ST); en ambos casos se aplicó solución de glyphosate Roundup SL® y radiomarcado. Se utilizó un diseño estadístico completamente al azar, y el parámetro de respuesta fue la cuantificación de células viables (ufc) de *Pseudomonas* sp., durante el proceso de mineralización, para los tres suelos hasta 120 días. Se encontró que la adición de glifosato estimuló el incremento de las *Pseudomonas* sp., correlacionado significativamente con la producción de $^{14}CO_2$. Las tasas de mineralización y las poblaciones bacterianas en los suelos ST fueron significativamente ($P \leq 0,05$) mayores con respecto a los suelos CT. Finalmente, los promedios más altos para mineralización y dinámica bacteriana fueron en el suelo con cobertura de bosque secundario (63% y $26,8 \cdot 10^7$).

**Palabras clave:** degradación, herbicida, acción microbiana, biómetros.

## Introduction

In general terms, according to world literature, glyphosate has been considered an environmentally safe compound because of its rapid inactivation in the soil, attributed to its degradation and its strong adsorption to colloids. However, recent studies in France, in 2004, indicate that this is the fourth most frequent agricultural pesticide in surface waters, and the tenth in subterranean waters (Calvet *et al*., 2005). Once the herbicide is applied, the main routes of degradation and dissipation are water and soil. The dissipation processes that can occur are the formation of complexes with $Ca^{2+}$ and $Mg^{2+}$ ions, adsorption on clays, organic matter, sediments, and suspended particles in water or in the soil solution (Grossbard and Atkinson, 1985).

Different research has found that glyphosate is used by bacteria as the sole source of phosphorus or carbon, and that the herbicide is mineralized from the first moment of application, *i.e.*, during the first week (Araújo *et al*., 2003;

Recceived for publication: 14 July, 2009. Accepted for publication: 13 November, 2010.

[1] Department of Agronomy, Faculty of Agronomy, Universidad Nacional de Colombia. Bogota (Colombia).
[2] Department of Chemistry, Faculty of Sciences, Universidad Nacional de Colombia. Bogota (Colombia).
[3] Corresponding author. lfigueroad@unal.edu.co

De Andréa *et al*., 2003). This rapid degradation of the herbicide, during the initial phase, is attributed to microbial action and occurs on the fraction of the glyphosate available; the subsequent decrease in the rate of degradation may be the product of microorganisms attacking the glyphosate adsorbed (Franz *et al*., 1997; De Andréa *et al*., 2003), *i.e.*, if the glyphosate is available for the microorganisms, it can be degraded, but if the adsorption in the soil is strong and rapid, and the desorption is slow, the degradation decreases (Araújo *et al*., 2003; Gimsing *et al*., 2004).

Robertson and Alexander (1994) found that glyphosate was rapidly mineralized during the first application, as well as during the second. After the second application, the bacterial populations remained high and the herbicide disappeared more rapidly than during the first application.

This may be because pesticides serving as carbon sources are affected by accelerated biodegradation, which is typical of organisms that have adapted to continuous exposure to the product and, hence, the decomposition of the glyphosate is so quick that its residual effect and its risk of contamination of soil and groundwater is drastically reduced.

It is worth highlighting that studies of glyphosate-degrading microorganisms in the laboratory commonly point to bacteria of the gender of *Pseudomonas* sp., which are characterized by their enormous metabolic potential; this permits their occupying a predominant place in the cycling of elements, primarily of carbon and nitrogen, or as promoters of plant growth. Several species of the *Pseudomonas* sp. gender are, apparently, capable of growing in the presence of some pesticides as sole sources of carbon and energy; they are also the most studied organisms as far as their capacity to degrade sugars, amino acids, alcohols, and aldoses (Paul and Clark, 1998; Madigan *et al*., 2000).

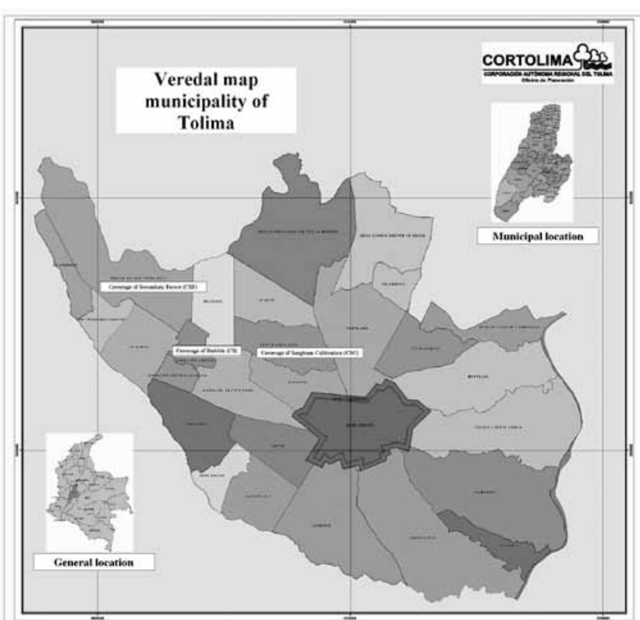


**FIGURE 1.** Location of the veredal map municipality of El Espinal. Scale: 1:100.000. Source: Cortolima (n.d.).

For those reasons, this work found it important to evaluate the behavior of the bacterial population of *Pseudomonas* sp. as possible participants of metabolism of herbicide glyphosate in soils of the department of Tolima, with diverse vegetal coverage: secondary forest, stubble, and sorghum crop, through follow up of the dynamics of said population during $^{14}C$-glyphosate mineralization for 120 d.

## Materials and methods

### Sampling of soils

The municipality of El Espinal, in the department of Tolima, constitutes an area of great agricultural interest with cultivation of rice, where glyphosate is frequently used in the so-called "chemical burnings" for weed control. To conduct this research, we selected three kinds of soils from this municipality (Fig. 1), contrasting in coverage and in history of pesticide application, among them glyphosate. The composed samples were taken at a depth of 20 cm.

The characteristics for each of the three soils evaluated were as follows:

### Coverage of secondary forest (CSF)

Located on the road to the municipality of Coello, town of Potrerillo, its geographical coordinates are: 04°13'36.6" N and 74°58'37.9" W, at a height of 400 m a.s.l. There is no record of application of any pesticide, or agronomic management.

### Coverage of stubble (CS)

Belongs to the Usocoello irrigation district, adjacent to the Corpoica – Nataima IV-6 lot; southwest boundaries with Dindalito and Guasimal, northwest boundaries with La Trinidad and San Francisco; its geographical coordinates are: 04°10'54,2" N and 74°57'46,4" W, at a height of 395 m a.s.l. For the last four years, it has not presented direct application of glyphosate.

### Coverage of sorghum cultivation (CSC)

Commercial lot located in the town of Agua Blanca, Guayabal Creek, El Edén farm; its geographical coordinates are: 04°10'36,7" N and 74°55'03,4" W, at a height of 355 m a.s.l. It has an intense history (over 30 years) of pesticide application, among them glyphosate, *i.e.*, there is permanent anthropic and agronomic management.

To prove that the degradation of glyphosate occurs mainly through microbial action, two treatments were used in each soil, a non-thermal and a thermal treatment. The latter procedure consisted of subjecting the soil to autoclave for one hour at 120°C and pressure at 20 psi (137 kPa), during three consecutive days.

The soils with their treatments were identified in the following manner:

- **CSFHT**: Coverage secondary forest without heat treatment.
- **CSFWHT**: Coverage secondary forest with heat treatment.
- **CSHT**: Coverage stubble without heat treatment.
- **CSWHT**: Coverage stubble with heat treatment.
- **CSCHT**: Coverage sorghum cultivation without heat treatment.
- **CSCWHT**: Coverage sorghum cultivation with heat treatment.

Ten different times were established to take the samples during the mineralization process of $^{14}$C glyphosate, and the viable cell count of the gender of *Pseudomonas* sp., thus: (day) 1, 4, 8, 14, 28, 42, 70, 84, 98 and 120.

### Glyphosate mineralization

For the mineralization experiment, we quantified the formation of $^{14}CO_2$ from $^{14}$C-glyphosate (IAEA, 1991). We took 100 g of soil, applied a mixture of commercial glyphosate (Roundup SL®, Monsanto, 480 g $L^{-1}$ of glyphosate isopropylamine salt and radio-labeled glyphosate) (Sigma Chemicals, glyphosate in its acid form, 0.1 mCi $mL^{-1}$, 2.2 mCi $mmol^{-1}$) to reach a value near the commercial dose of the product, and these were placed, in triplicate, inside sealed biometers. Then, flasks with 10 mL of NaOh 2M were incorporated to trap the $^{14}CO_2$ formed during the process, and they were incubated at 25°C during 120 d. An initial observation was made of the amount of $^{14}$C placed in each biometer. On the days established to gather samples, the flasks with NaOH were extracted and replaced again.

From each incubated flask, 1 mL of NaOH 2M was taken and mixed con 5 mL of scintillation liquid (Scintisafe Plus$^{TM}$ 50%, Fischer Chemical) in vials for liquid scintillation reading in an LS 6500 Beckman scintillation counter. The percentage of $^{14}CO_2$ was evaluated through the ratio between the disintegrations per minute (dpm) read each time and the amount initially placed as $^{14}$C-glyphosate.

### Quantification of cultivable populations of *Pseudomonas* sp. during the $^{14}$C-glyphosate mineralization process

A count was made of initial cultivable bacterial populations of the soils collected in the three sampled zones, with different history of glyphosate (Roundup®) application and corresponding to coverage of secondary forest, stubble, and sorghum through the technique proposed by Madigan *et al.* (2000) of dilution series ($10^5$ and $10^6$) and surface seeding in King B (KB) media.

The results of the total count were reported in cfu/g of soil; hereinafter called "bacterial population control" (day 0). Parallel to measuring the $^{14}$C glyphosate mineralization rate, a sample was extracted from each of the biometers on days 1, 4, 7, 14, 28, 42, 70, 84, 98, and 120 to count bacterial populations in the King B (KB) culture medium, through the technique proposed by Madigan *et al.* (2000) of dilution series ($10^5$ and $10^6$), and surface seeding, in triplicate.

Later, the samples were incubated at 25°C during 3 d; then, a fluorescent pigment reading was done of the colonies with the aid of ultraviolet light, and the macroscopic and microscopic descriptions were conducted. A control was used for each of the three study zones, which did not receive a dosage of glyphosate or thermal treatment in the laboratory.

## Results and discussion

### Physicochemical characteristics of soils

Table 1 shows the results obtained from some physicochemical properties of soils tested. The pH values for the three soils permit inferring appropriate conditions for growth of mesophilic microorganisms. Regarding the textural conditions, it is worth mentioning that soils with stubble and sorghum coverage, with high sand content and low content of organic matter, can facilitate the herbicide wash and its subsequently reaching groundwater. This situation may occur in lower proportions in forest soils, as a consequence of lower sand content, higher percentage of organic matter and cation exchange capacity, which permits suggesting higher adsorption of glyphosate into the soil.

**TABLE 1.** Physicochemical characteristics of soils evaluated.

| Property | Coverage secondary forest (CSF) | Coverage stubble (CS) | Coverage sorghum (CSC) |
|---|---|---|---|
| pH (potentiometric 1:1) | 6.7 | 7.4 | 5.7 |
| Sands (%) (Bouyoucos) | 65.6 | 73.9 | 77.9 |
| Organic matter (%) (Walkley - Black) | 2.93 | 0.93 | 1.06 |
| CIC (cmol $kg^{-1}$) (ammonium acetate 1N pH 7) | 10.70 | 2.90 | 3.00 |
| Electrical conductivity (dS $m^{-1}$) | 0.55 | 0.32 | 0.20 |
| P (mg $kg^{-1}$) (Bray II) | 61 | 15 | 30 |
| Structural stability (DPM: mm) | 4.5 | 4.2 | 0.4 |
| State of aggregation (mm) | 80 | 76 | 25 |

It should be mentioned that the phosphorous content of forest soil, whose value is notably greater than that of the two other tested soils and which, according to Gimsing *et al.* (2004), can promote glyphosate desorption by competing with the common sites where it could be adsorbed while facilitating herbicide degradation by soil microorganisms.

Regarding structural stability values and the state of aggregation for forest and stubble, it may be inferred that they fulfill the appropriate conditions to host microorganisms, while for sorghum conditions are much more limited due to soil management, which is subjected to high mechanization.

### $^{14}$C-glyphosate mineralization

Figure 2 shows the evolution of glyphosate mineralization over time. Note that in the three soils, the greatest mineralization rate is present in those without heat treatment with relationship to those with heat treatment; a situation showing the degradation of this herbicide, primarily via microbial processes.

In soils with heat treatment, the highest mineralization rate was present in the stubble soil (CSWHT), reaching average values of 18%, in contrast to soil cultivated with sorghum (CSCWHT) where the values obtained do not exceed 1%. Regarding soils without heat treatment, the mineralization rate in the soil cultivated with sorghum (CSCHT) with a history of glyphosate application was the lowest, with percentages below 10%, while in the forest soil (CSFHT) without history of pesticides, it was the highest, with values close to 63%.

In the three soils without heat treatment, there was a high rate of glyphosate mineralization during the first 14 d and diminished over time, according to that found by Gimsing *et al.* (2004). In soils without heat treatment the forest soil (CSFHT) stands out; the amount of herbicide initially applied is reduced by approximately 50% at 32 d (Fig. 2), which is related to its physicochemical properties and to the high populations of *Pseudomonas* sp. The least favorable conditions for glyphosate degradation were manifested in soil cultivated with sorghum, probably because of soil management along the years, which has led to its deterioration; that is, tillage and continuous seeding of yearly crops (rice-sorghum-cotton), high use

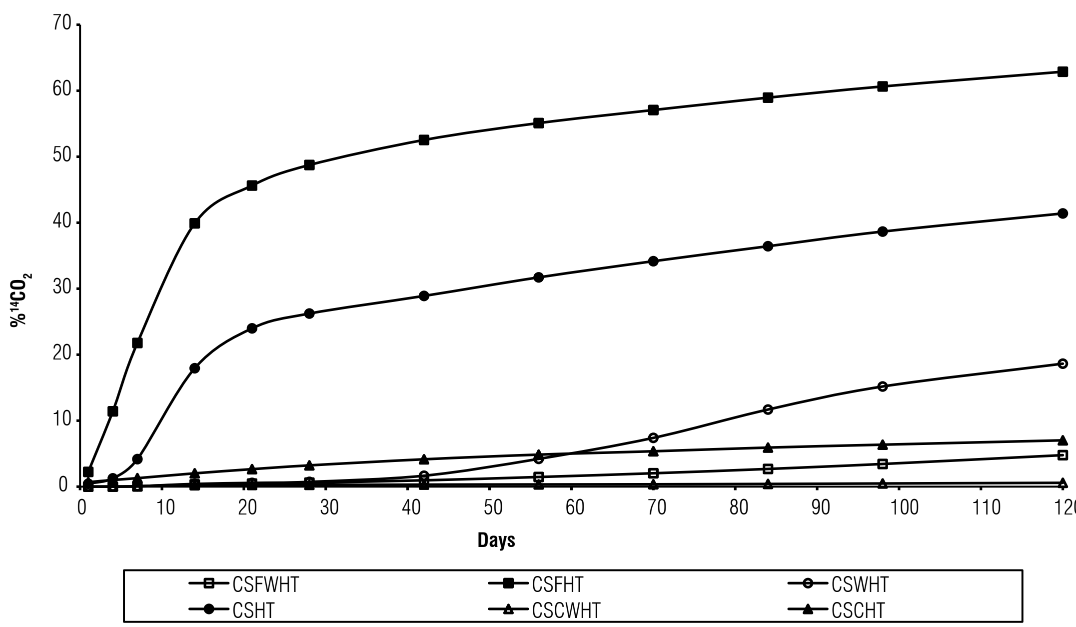


**FIGURE 2.** Accumulated $^{14}$C-glyphosate mineralization rate. CSFHT, coverage secondary forest without heat treatment; CSFWHT, coverage secondary forest with heat treatment; CSHT, coverage Stubble without heat treatment; CSWHT, coverage Stubble with heat treatment; CSCHT, coverage sorghum cultivation without heat treatment; CSCWHT, coverage sorghum cultivation with heat treatment.

of agricultural inputs and lack of practices to restitute organic matter, among others. De Andréa *et al.* (2003), for example, report the significant decrease of glyphosate mineralization in soil treated with repeated applications of the herbicide, against its mineral-ization with only one application.

### Relationship between cultivable *Pseudomonas* sp. populations and the $^{14}C$ glyphosate mineralization process

Figure 3 illustrates changes in the size of the *Pseudomonas* sp. populations, without heat treatment (Fig. 3a) and with heat treatment (Fig. 3b), during the process of $^{14}C$ glyphosate mineralization, on the ten sampling dates. Note that day 14 is shown as the date of maximum growth of bacterial populations (Fig. 3a), from which the values of viable cells, expressed in colony formation units (cfu), decrease until day 120. Considering this result, we conducted an analysis of variance (SAS® System version 9) with a completely randomized design, seeking to identify significant effects of soil coverage or thermal treatment on the *Pseudomonas* sp. populations at this date of the process of $^{14}C$ glyphosate mineralization (Tabs. 2 and 3). Additionally, we conducted a Duncan multiple compar-ison test ($P \leq 0.05$) to find significant differences among viable cell bacterial populations in King B (KB) medium, according to the distinct coverage and the treatments evaluated (Tab. 4).

**TABLE 2.** Effect of soil coverage on *Pseudomonas* sp. populations on day 14 of the $^{14}C$ glyphosate mineralization process.

| Description of treatment | N | Mean |
|---|---|---|
| Coverage of secondary forest | 6 | 4.17689 a |
| Coverage of stubble | 6 | 3.81128 b |
| Coverage of sorghum cultivation | 6 | 2.89083 c |

Means with different letters indicate significant differences according to the Duncan test ($P \leq 0.05$).

**TABLE 3.** Effect of thermal treatment on *Pseudomonas* sp. populations on day 14 of the $^{14}C$ glyphosate mineralization process.

| Description of treatment | n | Mean |
|---|---|---|
| Without heat treatment | 9 | 3.8159 a |
| With heat treatment | 9 | 3.4367 b |

Means with different letters indicate significant differences according to the Duncan test ($P \leq 0.05$).

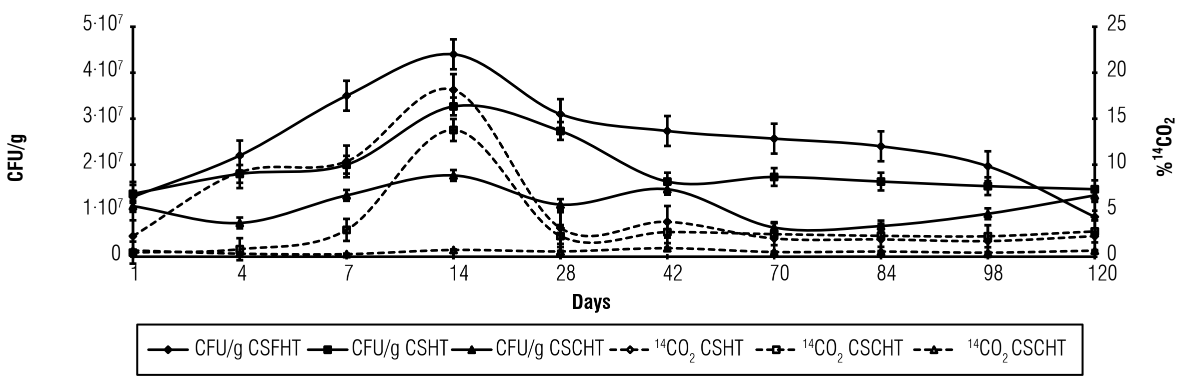


**FIGURE 3A.** *Pseudomonas* sp. populations without heat treatment during $^{14}C$-glyphosate mineralization at 120 d. CSFHT, coverage secondary forest without heat treatment; CSHT, coverage Stubble without heat treatment; CSCHT, coverage sorghum cultivation without heat treatment.

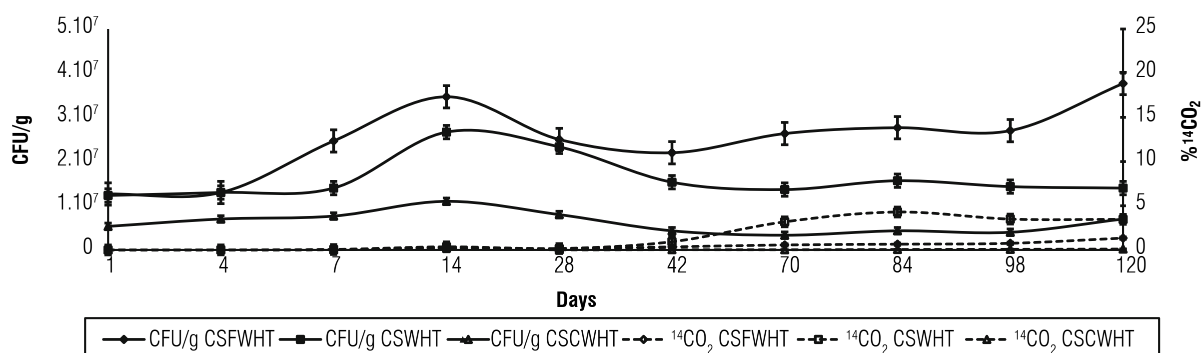


**FIGURE 3B.** *Pseudomonas* sp. populations with heat treatment during $^{14}C$-glyphosate mineralization at 120 d. CSFWHT, coverage secondary forest with heat treatment; CSWHT, coverage Stubble with heat treatment; CSCWHT, coverage sorghum cultivation with heat treatment.

**TABLE 4.** Accumulated values of the percentage of $^{14}$C-glyphosate mineralization at 120 d and of cfu/g of soil in the treatments tested.

| Identification | $^{14}$C- glyphosate mineralization (%)* | CFU/g ($10^7$) |
|---|---|---|
| CSFHT | 63.00 | 26.8 |
| CSFWHT | 5.00 | 16.2 |
| CSHT | 41.4 | 25.3 |
| CSWHT | 19.00 | 19.1 |
| CSCHT | 4.15 | 11.1 |
| CSCWHT | 0.58 | 6.80 |

* Total accumulated from the relationship between DPM (disintegrations per minute) and the amount with which it was initially treated like $^{14}$C-glyphosate, at 120 d.

The analysis of variance indicated highly significant differences between the soils and the heat treatment. Thus, the number of *Pseudomonas* viable cells was markedly different among the three soils, with the highest number in the secondary forest soil compared to the two other soils; for its part, the thermal treatment significantly reduced these bacterial populations, in contrast to not applying heat to the soil.

Figure 3a shows that the degree of mineralization agrees with the growth of the *Pseudomonas* sp. populations; that is, with a greater value of viable cells, in general, there is greater mineralization. In Fig. 3b, the $^{14}$C glyphosate mineralization presents a different behavior of bacterial growth, especially in soils with secondary forest and stubble coverage. The bacteria with the capacity to degrade the herbicide, apparently, were inactivated through the effect of heat, inactivity that ceased on day 28 when mineralization began.

Table 4 presents the values accumulated at 120 d, the percentage of $^{14}$C- glyphosate mineralization, and the bacterial cfus. The highest values of herbicide mineralization were for the CSFHT system, followed in order by the CSHT, CSWHT, CSFWHT, CSCHT, and CSCWHT systems. The same situation was obtained for the *Pseudomonas* sp. populations, evidencing microbial intervention in the glyphosate degradation process, as reported by Cheah *et al*. (1998) and Gimsing *et al*. (2004).

Correlation coefficients were calculated with a 5% significance level ($r^2$) between the bacterial populations cultivable in King B (KB) medium and $^{14}$C glyphosate mineralization (expressed in percentage) over time, in the three tested soils and with thermal and without heat treatments.

The percentage of $^{14}CO_2$ glyphosate and the number of colonies were transformed to validate the normality assumptions, through Equation (1).

From the Box Cox algorithm ($y^{\lambda}$-1/$\lambda$) (1)

Low correlation values ($r^2$ $P$<0.55 and negative) were found between el process of mineralization of herbicide and la population of *Pseudomonas* sp. subjected to thermal treatments, while with those without heat treatments, the values were over 0.7. In the first case, glyphosate mineralization occurred late, toward day 28, given that prior to this day the remnant populations of the thermal process were not evidently intervening in the degradation (Table 4).

The best correlation coefficient between the *Pseudomonas* sp. populations and mineralization was present in the CSHT soil ($r^2$ = 0.78) (Fig. 4), which indicates that said bacteria genus present in this soil is actively participating

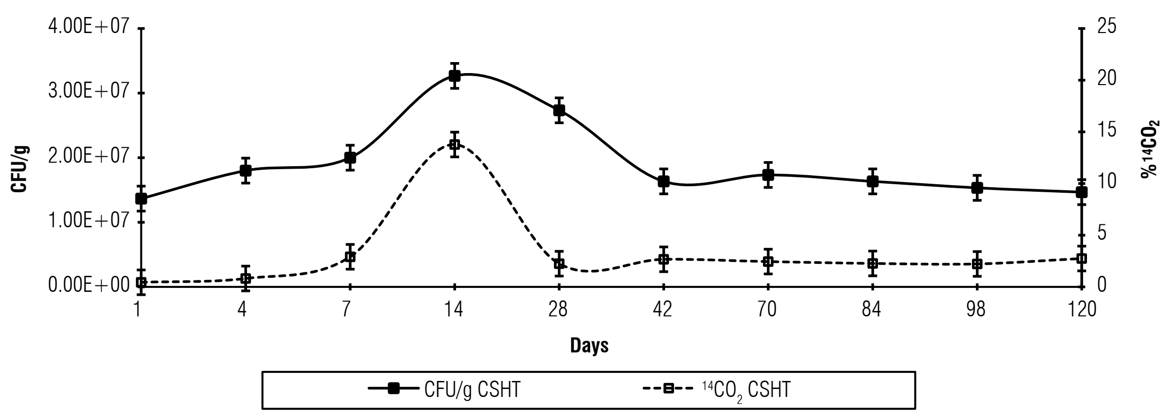


**FIGURE 4.** Correlation between the *Pseudomonas* sp. populations during $^{14}$C-glyphosate mineralization process in coverage of stubble without heat treatment (CSHT).

in $^{14}C$-glyphosate mineralization, while upon applying thermal treatment, the resistant populations of the CSWHT system ($r^2$ = -0.27) do not participate in the process of herbicide mineralization, as apparently being done to some extent by the thermal resistant populations present in the two other soils.

**TABLE 5.** Correlation coefficients ($R^2$) between the *Pseudomonas* sp. populations and $^{14}C$ glyphosate mineralization.

| Description of treatment | $R^2$ |
|---|---|
| CSFHT | 0.74 |
| CSFWHT | 0.73 |
| CSHT | 0.78 |
| CSWHT | -0.27 |
| CSCHT | 0.51 |
| CSCWHT | 0.55 |

In general, it may be said that the $^{14}C$ glyphosate was mineralized by the bacteria, and that this process is subject to the physicochemical conditions and management of the soils tested and herbicide availability. With the results obtained, it was determined that the soil with forest coverage without heat treatment registered the highest mineralization rate, probably due to the availability of the glyphosate for the microorganisms (pH neutral) and due to the greater number of viable cells compared to the two other soils, perhaps because it is a suitable environment for growth and development of bacteria, a product of the high content of organic matter (OM: 2.93%) and better physicochemical properties.

The soil with sorghum coverage presented the lowest number of viable cells and the lowest mineralization rate, likely caused by deterioration of the soil, and particularly due to the low content of organic matter and to the loss of soil structure (Tab. 1), which limited the growth of bacterial populations.

Although the works by Robertson and Alexander (1994) and Racke *et al.* (1997) revealed increased microbial activity in exposed soils for a long time to repeated applications of glyphosate (6 -11 years); a product of microorganism adaptability (accelerated biodegradation), compared to soils where bacterial populations were always lower and the pH was slightly acid, did not favor the availability of glyphosate. In this study, the microbial populations were low in soils seeded with sorghum, and the behavior found is more similar to that proposed by Karpouzas and Singh (2006), who stated that frequent applications of glyphosate will lead to the bacterial population's decreased capacity to degrade the herbicide over time.

## Conclusions

It was possible to conclude that the populations of *Pseudomonas* sp. actively participate in $^{14}C$ glyphosate mineralization, particularly in low-intervention systems: Secondary forest and stubble. The latter presented a better correlation with mineralization; said population was thermo-sensitive under experimental conditions. Glyphosate was highly mineralized in the soil with forest coverage: at 120 d of incubation, the herbicide mineralization was 1.5 and 6.0 times greater with relation to soils of stubbles and sorghum, respectively. The results obtained in the work with the soil cultivated with sorghum confirm that frequent applications of glyphosate will lead to decreased herbicide degrading capacity by the bacterial populations. Nevertheless, apparently because of the behavior of stubble soil, recovery is possible by allowing the soil to rest for several years.

It was evidenced that, under laboratory conditions, the bacterial populations were significantly stimulated with the addition of glyphosate to the study soils, with respect to the soil that did not get application of the herbicide, possibly because it was used as a substrate.

The bacterial populations that most actively participated in $^{14}C$ glyphosate mineralization were present in the secondary forest and stubble systems, possibly because of the greater amount of microorganisms and the availability of the herbicide compared to sorghum. In *in vitro* degradation tests, the use of isotopic techniques permits reliable monitoring of the degrading activity of the pesticides in the soil.

### Acknowledgements

This work was conducted at the Malherbology Laboratory of the Faculty of Agronomy, within the framework of the research group "Residual and environmental fate of pesticides in agricultural systems", from the Department of Chemistry and the Faculty of Agronomy at Universidad Nacional de Colombia, Bogotá branch, with the auspices of the branch Research Division (DIB). Likewise, we thank the International Atomic Energy Agency (IAEA) for the equipment donated, which permitted us to carry out this work.

## Literature cited

Araújo, A.S.F., R.T.R. Monteiro, and R.B. Abarkeli. 2003. Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 52, 799-804.

Calvet, R., E. Barriuso, C. Bedos, P. Benoit, M.-P. Charnay, and Y. Coquet. 2005. Les pesticides dans le sol: conséquences agronomiques et environnementales. Editions France Agricole. Paris.

Cheah, U., R. Kirkwood, and K. Lum. 1998. Degradation of four commonly used pesticides in Malaysian agricultural soils. J. Agr. F. Chem. 46, 1217-1223.

Cortolima, Corporación Autónoma Regional del Tolima. N.d. Mapa veredal municipio del Espinal. In: http://www.unibague.edu.co/sitios/biblioteca/tolima/espinal/mapa.html; consulted: December of 2011.

De Andréa, M.M., T.B. Peres, L.C. Luchini, S. Bazarin, S. Papini, M.B. Matallo, and V.L. Savoy. 2003. Influence of repeated applications of glyphosate on its persistence and soil bioactivity. Pesq. Agropec. Bras. 38(11), 1329-1335.

Franz, J.E., M.K. Mao, and J.A. Sikorski. 1997. Glyphosate: a unique global herbicide. Monograph 189. American Chemical Society, Washington DC.

Gimsing, A.L., O.K. Borggaard, O.S. Jacobsen, J. Aamand, and J. Sørensen. 2004. Chemical and microbiological soil characteristics controlling glyphosate mineralisation in Danish surface soils. Appl. Soil Ecol. 27, 233-242.

Grossbard, E. and D. Atkinson. 1985. The herbicide glyphosate. Butterworth and Co., London.

IAEA, International Atomic Energy Agency. 1991. Laboratory training manual on the use of nuclear and associated techniques in pesticide research. Technical Reports Series No. 329. Vienna.

Karpouzas, D. and S. Singh. 2006. Microbial degradation of organophosphorus xenobiotics: metabolic pathways and molecular basis. Adv. Microb. Physiol. 51, 20-225.

Madigan, M., J. Martinko, and J. Parker. 2000. Brock biology of microorganism. 8th ed. Prentice Hall, Englewood Cliffs, NJ.

Paul, E.A. and F.E. Clark. 1998. Soil microbiology and biochemistry. 2nd ed. Academic Press, New York, NY.

Racke, K.D., M.W. Skidmore, D.J. Hamilton, J.B. Unsworth, J. Miyamoto, and S.Z. Cohen. 1997. Pesticide fate in tropical soils. Pure Appl. Chem. 69(6), 1349-1371.

Robertson, B. and M. Alexander. 1994. Growth-linked and cometabólic biodegradation: possible reason for occurrence or absence of accelerated pesticide biodegradation. Pestic. Sci. 41, 311-318.