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

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

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**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* 14C-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 14CO₂. The rates of mineralization and the bacterial populations in WHT soils were significantly (P≤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 26.8·10⁷).

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

**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 Ca²⁺ and Mg²⁺ 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;
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).

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 14C-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, Garraybal 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 perma-
nent 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 fol-
lowing 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™ 50%, Fischer Chemical) in vials for liquid scintilla-
tion 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 dif-
ferent 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 physicoche-
nical properties of soils tested. The pH values for the three
soils permit inferring appropriate conditions for growth of
mesophilic microorganisms. Regarding the textural con-
ditions, 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.

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

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.

14C-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
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 comparison 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.

<table>
<thead>
<tr>
<th>Description of treatment</th>
<th>N</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coverage of secondary forest</td>
<td>6</td>
<td>4.17689 a</td>
</tr>
<tr>
<td>Coverage of stubble</td>
<td>6</td>
<td>3.81128 b</td>
</tr>
<tr>
<td>Coverage of sorghum cultivation</td>
<td>6</td>
<td>2.89083 c</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Description of treatment</th>
<th>n</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without heat treatment</td>
<td>9</td>
<td>3.8159 a</td>
</tr>
<tr>
<td>With heat treatment</td>
<td>9</td>
<td>3.4367 b</td>
</tr>
</tbody>
</table>

Means with different letters indicate significant differences according to the Duncan test ($P \leq 0.05$).
TABLE 4. Accumulated values of the percentage of 14C-glyphosate mineralization at 120 d and of cfu/g of soil in the treatments tested.

<table>
<thead>
<tr>
<th>Identification</th>
<th>14C- glyphosate mineralization (%)*</th>
<th>CFU/g (10^7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSFHT</td>
<td>63.00</td>
<td>26.8</td>
</tr>
<tr>
<td>CSFWHT</td>
<td>5.00</td>
<td>16.2</td>
</tr>
<tr>
<td>CSHT</td>
<td>41.4</td>
<td>25.3</td>
</tr>
<tr>
<td>CSWHT</td>
<td>19.00</td>
<td>19.1</td>
</tr>
<tr>
<td>CSCHT</td>
<td>4.15</td>
<td>11.1</td>
</tr>
<tr>
<td>CSCWHT</td>
<td>0.58</td>
<td>6.80</td>
</tr>
</tbody>
</table>

* Total accumulated from the relationship between DPM (disintegrations per minute) and the amount with which it was initially treated like 14C-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 14C 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 14C-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 14C glyphosate mineralization (expressed in percentage) over time, in the three tested soils and with thermal and without heat treatments.

The percentage of 14CO$_2$ glyphosate and the number of colonies were transformed to validate the normality assumptions, through Equation (1).

$$r^2 = \frac{\gamma^3 - 1}{\lambda}$$

Figure 4. Correlation between the *Pseudomonas* sp. populations during 14C-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.

<table>
<thead>
<tr>
<th>Description of treatment</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSFHT</td>
<td>0.74</td>
</tr>
<tr>
<td>CSFWHT</td>
<td>0.73</td>
</tr>
<tr>
<td>CSHT</td>
<td>0.78</td>
</tr>
<tr>
<td>CSWHT</td>
<td>-0.27</td>
</tr>
<tr>
<td>CSCHT</td>
<td>0.51</td>
</tr>
<tr>
<td>CSCWHT</td>
<td>0.55</td>
</tr>
</tbody>
</table>

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 thermosensitive 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**


