ELSEVIER

Thermochimica Acta 371 (2001) 169–174

thermochimica acta

www.elsevier.com/locate/tca

# Microcalorimetry of the degradation of the herbicide 2,4-D via the microbial population on a typical Brazilian red Latosol soil

Alexandre G.S. Prado, Claudio Airoldi*

*Instituto de Química, Universidade Estadual de Campinas, Caixa Postal 6154, 13083-970 Campinas, São Paulo, Brazil*

Received 27 July 2000; received in revised form 22 December 2000; accepted 22 December 2000

**Abstract**

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was applied to a typical Brazilian red Latosol soil. The thermal effects evolved by 2,4-D degradation were followed in a series of microcalorimetric experiments. The power–time curves were recorded for increasing doses of herbicide, varying from 0 to 6.67 mg $g^{-1}$ under a 34.8% controlled humidity. The herbicide was used as a food source by soil microbes, the degradation causing the thermal effect. The thermal effect increases to 2.67 mg $g^{-1}$. Above this concentration, a decrease of the thermal effect was observed due to toxicity of 2,4-D to the microbes.
© 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Pesticide; Soil; Microcalorimetry; Biodegradation; 2,4-D

## 1. Introduction

Many chemicals have been extensively used during the last 50 years. Thus, great interest exists in improving the ability for predicting the consequences of chemical compounds in the environment [1]. During recent years, the use of pesticides has become ubiquitous. An understanding of the interactions of pesticides with the environment plays a key role to test environmental models to predict impacts and thus, to implement corrective actions in a short time [2]. Therefore, research concerning the impact of chemicals on the environment is a main priority for the 21st century [1].

Herbicides are normally used on the majority of Brazilian crops. They represent around 42% of the total pesticides used, while 2,4-dichlorophenoxyacetic acid (2,4-D) constitutes 40% of the total herbicides applied. This pesticide is normally used in weed control for crops such as sugar cane, palm oil, cocoa and rubber crops, as well as for weeds along highways [3–5]. Thus, the mobility, toxicity and biodegradability of 2,4-D in local soil applications merits special study. In particular, red Latosol soil is typical of the Brazilian regions dedicated to sugar cane plantations. This soil covers approximately 15% of the State of São Paulo, where most of the sugar cane plantations in Brazil are found [5].

Some pesticides are good carbon sources for microorganisms. 2,4-D belongs to this class, being metabolized by a great variety of soil microorganisms [6–9]. The biodegradation of such compounds, generates a thermal effect as a consequence of metabolic activity of the microorganisms [10–12]. During the process, any changes in metabolism will affect the thermal power [13,14]. This complex process can be followed

* Corresponding author. Tel.: +55-19-3788-3023; fax: +55-19-3788-3055.
*E-mail address*: airoldi@iqm.unicamp.br (C. Airoldi).

0040-6031/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0040-6031(01)00424-5

via microcalorimetry which is a useful technique for microbial and metabolic growth studies [15–17]. The metabolism can be evaluated to determine, whether the thermal effect depends solely on the initial and the final states of the system [18].

The procedure is independent of the type of microorganism activity. The effect can be continuously monitored to acquire the activity of a metabolic process in situ for a long period [18]. This procedure has rarely been used to investigate microbial growth in soil. Other techniques are limited from the practical point of view of applications for such heterogeneous systems [19–22].

In the present investigation, the degradation of 2,4-D in soil was followed calorimetrically. This pesticide was chosen because it is widely used on Brazilian crops, and its addition in the soil disturbs the equilibrium of this ecosystem. This disturbance is reflected in the thermal-power generated by microbial degradation of 2,4-D, for which data is now reported for a soil/herbicide system.

## 2. Materials and methods

### 2.1. Reagents

All chemicals were reagent grade. The herbicide 2,4-D (Sigma) was of technical grade, with 98% purity.

### 2.2. Soil samples

Red Latosol soil from the campus of the State University of Campinas (47.1°W longitude, 23.8°S latitude) was used. Samples were collected from a depth of 5–10 cm, after removal of the top surface layer [18]. The soil was air dried for 10 days and homogenized by sieving to less than 2 mm, to separate roots and large particles [13,18]. The soil was stored in polyethylene bags at $293 \pm 5$ K and samples were used for all operations [13,18].

Characterization was carried out by routine methods. For organic matter determination, samples of dry soil were placed in a muffle furnace to follow the decrease in mass for 24 h at a temperature of 823 K, as recommended [23]. Under these conditions organic matter is combusted, to leave only the inorganic component of the soil [23]. Carbon, nitrogen, hydrogen and sulfur contents in soil were determined from elemental analysis by using a Fisons Instruments CHNS-O model 1110 Elemental Analyzer. Measurements of pH were performed by means of a Digimed DMPH-2 pHmeter in a suspension of 2.0 g of soil sample with 5.0 $cm^3$ of a strong electrolyte of 1.0 mol $dm^{-3}$ calcium chloride, having a 1:2.5 soil:-solution ratio [18,24]. The results expressed are for triplicate assays.

### 2.3. Microcalorimetry

An LKB 2277 Thermal Activity Monitor heat-flow microcalorimeter, Thermometric AB, Sweden, was used for all measurements and has been described previously [19,24]. The thermal effect was obtained using 5.0 $cm^3$ stainless steel ampoules. Teflon sealing discs which avoid evaporation inside the apparatus [19], hermetically close these ampoules. Experiments were carried out at $298.15 \pm 0.02$ K [12,18]. All determinations of the thermal effect were performed in triplicate in ampoules containing 1.50 g of soil and 0.80 $cm^3$ of solution with different doses of 2,4-D. Under these conditions, moisture applied to the experimental system was controlled at 34.8%, which induces the highest microbial activity [18]. The thermal effect associated with nutrient degradation was recorded as a function of time. The final value was calculated by comparing the integrated area of the power–time curve for each experimental determination [12,18].

## 3. Results and discussion

The development of the microbial activity in a chosen system can be affected by the properties associated with the soil. Thus, the inherent physical chemical properties such as pH, organic matter content and elemental constitution are important features to be considered in such kinds of investigations. The composition of the soil system selected for the present study $5.50 \pm 0.22\%$ of organic matter with the following elemental results: $5.22 \pm 0.11$; $0.92 \pm 0.04$ and $2.73 \pm 0.14\%$ for carbon, nitrogen and hydrogen, respectively. This composition leads to a natural pH of $5.84 \pm 0.06$.

Effective illustrations about the stimulus caused on microorganism populations of the selected red Latosol soil, through the addition of nutrients, were clearly demonstrated before [12,18,19,24]. Stimulation of the metabolism is directly promoted by the addition of an amount of the desired nutrients to the ecosystem [12]. Unequivocal evidence of the degradation of 2,4-D by microbial metabolism is shown by a series of power–time curves obtained from a sequence of assays in which only the herbicide 2,4-D was added (Fig. 1).

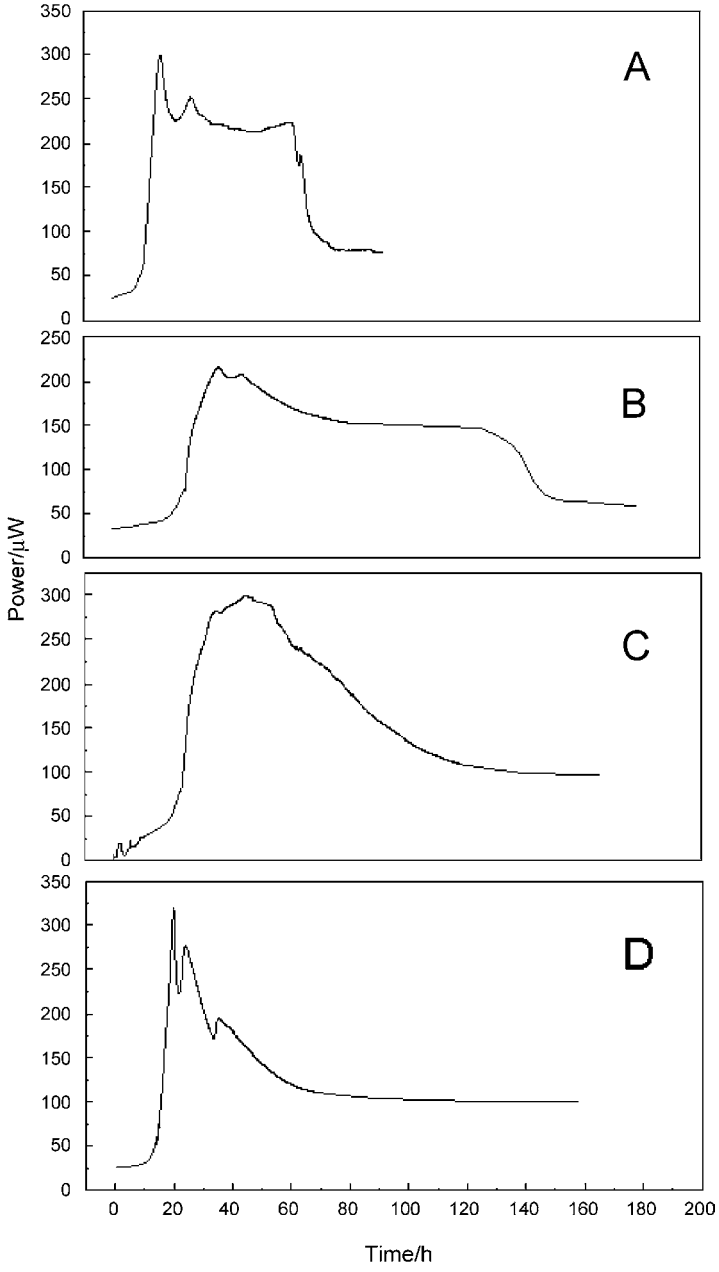


Fig. 1. Power–time curves of soil microbial activity during the degradation of the herbicide 2,4-D in 1.50 g of red Latosol soil with controlled moisture (34.8%), and with different doses of 2,4-D applied to this soil: 0.67 (A), 1.33 (B), 2.67 (C) and 6.67 (D) mg of 2,4-D per 1.0 g of soil.

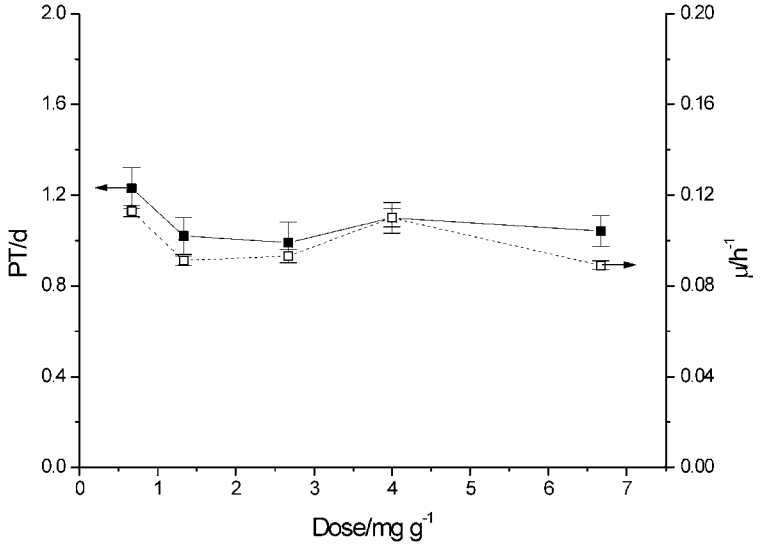


Fig. 2. The change in peak-time, PT, indicated by (■) and in microbial growth rate constant, $\mu$, indicated by (□), as a function of the dose of 2,4-D applied (mg $g^{-1}$).

The curves obtained represent only the microbial activity due to degradation of the herbicide [19].

Previous investigations on this herbicide showed that, upon its addition to a given soil sample containing glucose, a synergistic effect occurred at low concentration, reflected in an increase in the power–time curve signal. However, as the concentration of herbicide was increased a pronounced decrease in the signal takes place, due to toxic effects [24]. To confirm that 2,4-D can be used as a food source by microorganisms and this effect changes due to toxicity to microbial populations in soil. In this study, herbicide was also added to the soil sample in the absence of glucose.

The thermal effects evolved in all the power–time curves in Fig. 1 are thus only the result of metabolic activity of the soil population on 2,4-D. Any other thermal effect of physicochemical origin was compensated in the reference ampule [18].

From the power–time curves in Fig. 1 the total thermal effect of metabolism, $-Q$ (J), was obtained through integration of each curve. The values of peak-time, PT (h), were obtained when the maximum activity is displayed. The microbial growth rate constant, $\mu$ ($h^{-1}$), was calculated from the slope of a semi-logarithmic plot of the exponential phase. These data are shown in Figs. 2 and 3, respectively.

Increased doses of 2,4-D has little influence on PT and $\mu$ values. Despite the small changes in PT and $\mu$ values with dose, as shown in Fig. 2, the general profile of the power–time curves is much changed by different doses.

The soil microbial activity gave a null value in the absence of the herbicide, but the presence of different amounts changed the activity of microorganisms as indicated in Fig. 3. The total thermal effect increased up to 1.67 mg $g^{-1}$. Above this dose, the total thermal effect is constant up to 4.00 mg $g^{-1}$ and then decreases. The total thermal effect is expected to increase as the amount of carbon source increases. However, the $-Q$ (J) values decrease above a dose of 4.00 mg $g^{-1}$. Thus, the degradation of 2,4-D must be followed by a toxic effect of the herbicide or its metabolites [24].

To better understand the toxic effect of the 2,4-D, in that occurs together with biodegradation of this pesticide, a representation of the total thermal effect per dose as a function of applied dose is shown in Fig. 4.

The −Qd values decrease with the increase of an applied dose of 2,4-D. This confirms toxic effect of the herbicide. The calorimetric technique detected the total thermal effect generated by the microbial population. So, the final thermal effect observed consists of

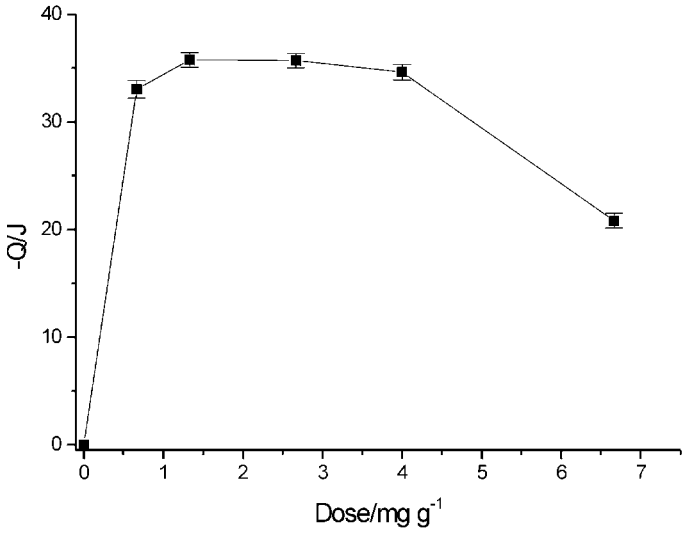


Fig. 3. The total thermal effect, $-Q$, calculated from metabolism of degradation of the herbicide 2,4-D by the microbial population.

two effects, the degradation of the herbicide together with the toxic effect.

The linearized data were obtained by plotting −Qd values against the inverse of the dose applied, as shown in Fig. 4.

The linear coefficients obtained from Eq. (1), from which the dose giving a null value of −Qd present was calculated to be 51.49 mg $g^{-1}$:

$$-\mathrm{Qd} = -0.654 + 33.674 \times \mathrm{dose}^{-1} \quad (1)$$

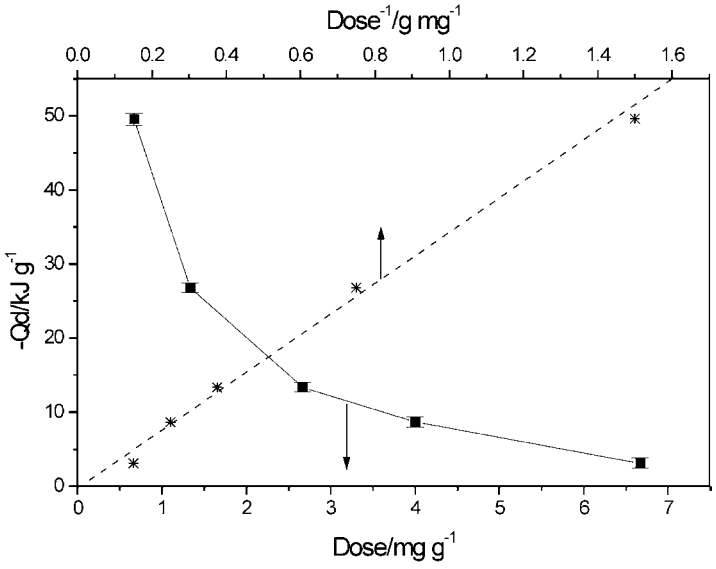


Fig. 4. The thermal effect obtained per dose applied from microbial metabolism of 2,4-D, −Qd as function of dose and so as a function of the inverse of dose.

The result shows that 2,4-D can be harmful to typical Brazilian red Latosol soil, if it is indiscriminately applied in repeated doses.

## 4. Conclusion

The herbicide 2,4-D is degraded by microbial populations of red Latosol soil, but at higher concentrations of this, biodegradation is followed by a toxicological effect which drastically affects the microflora of soil. The amount that was able to fully eliminate the microbial activity of this soil is quite large, but the indiscriminate application of herbicides can affect agriculture. Thus, calorimetric data can make a contribution to understand the effect of the applications of 2,4-D on soils and, in particular, to Brazilian soil.

## Acknowledgements

The authors are indebted to FAPESP for financial support and a fellowship to AGSP and also to CNPq for a fellowship to CA.

## References

[1] Anonymous, Environ. Sci. Technol. 31(1997) 20A.
[2] W.F. Jardim, Pure Appl. Chem. 70 (1998) 2259.
[3] E.M. Vieira, A.G.S. Prado, M.D. Langraf, M.O.O. Rezende, Quim. Nova 22 (1999) 305.
[4] A.G.S. Prado, C. Airoldi, Pest Manage. Sci. 56 (2000) 419.
[5] A.G.S. Prado, E.M. Vieira, M.O.O. Rezende, An. Assoc. Bras. Quim. 47 (1998) 239.
[6] L.T. Ou, Soil Sci. 137 (1984) 100.
[7] J.O. Ka, W.E. Holben, J.M. Tiedje, Appl. Environ. Microbiol. 60 (1994) 1121.
[8] L.E. Greer, J.A. Robinson, D.R. Shelton, Appl. Environ. Microbiol. 58 (1992) 1027.
[9] V.G. Matheson, L.J. Forney, Y. Suwa, C.H. Nakatsu, A.J. Sexstone, W. Holben, Appl. Environ. Microbiol. 62 (1996) 2457.
[10] U. Von Stockar, L. Auberson, I.W. Marison, Pure Appl. Chem. 65 (1993) 999.
[11] C. Airoldi, Quim. Nova 21 (1998) 635.
[12] S.A.M. Critter, J.A. Simoni, C. Airoldi, Thermochim. Acta 232 (1994) 145.
[13] N. Barros, S. Feijoo, J.A. Simoni, A.G.S. Prado, F.D. Barboza, C. Airoldi, Thermochim. Acta 328 (1999) 99.
[14] S. Fradette, D. Rho, R. Samso, A. LeDuy, Appl. Microbiol. Biotechnol. 42 (1994) 432.
[15] L. Gustafsson, Thermochim. Acta 193 (1991) 145.
[16] I. Wadso, Thermochim. Acta 269 (1995) 337.
[17] M.L. Ferrey, R.E. Lovrien, Talanta 40 (1993) 127.
[18] A.G.S. Prado, C. Airoldi, Thermochim. Acta 332 (1999) 71.
[19] C. Airoldi, S.A.M. Critter, Thermochim. Acta 288 (1996) 73.
[20] G.P. Sparling, Soil Biol. Biochem. 13 (1981) 93.
[21] K. Ljunghlom, B. Noren, I. Wadso, Oikos 33 (1979) 15.
[22] K. Ljunghlom, B. Noren, I. Wadso, Oikos 33 (1979) 24.
[23] A.G.S. Prado, M.O.O. Rezende, An. Assoc. Bras. Quim. 48 (1999) 186.
[24] A.G.S. Prado, C. Airoldi, Thermochim. Acta 349 (2000) 17.