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Effect of the pesticide 2,4-D on microbial activity of the soil monitored by microcalorimetry

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Abstract

Microcalorimetry was applied in a series of experiments to follow the effect caused by the pesticide 2,4-dichlorophenoxiacetic acid (2,4-D) on microbial activity of red Latosol soil. The activity of the soil was stimulated by addition of 6.0 mg of glucose and 6.0 mg of ammonium sulfate under a 35% controlled humidity in a soil sample of 1.50 mg. Power–time curves recorded on calorimeter were followed by the increasing amount of the pesticide, from 0 to 10.0 mg, which affected directly the total thermal effect evolved by microorganisms. The curves showed a synergism on total thermal effect obtained by addition of 1.0 mg of pesticide, causing a consumption of 2,4-D by microorganisms, as a new source of nutrients. However, above this mass, the total thermal effect decreased exponentially. The increase of the amount of pesticide is associated to a decrease on the microbial activity of the soil, probable due to the increase of the toxic products formed from degraded 2,4-D, affecting strongly the life in this ecosystem.

Keywords: Soil; Microcalorimetry; 2,4-D; Microorganisms; Pesticide

1. Introduction

Soil can be considered as a multicomponent, forming an open biochemical system where several physical, chemical and biological reactions may occur inside and on its surface [1,2]. In this system, both matter and energy can also be exchanged with the surroundings, which are composed of a conglomerated of solids, liquids and gases [2–4].

The activities developed by microflora in soil contribute significantly to the nutritional and physical state of the soils through many biochemical reactions, that are important in the renewing and also in changing the original composition of the soils [4,5].

During the development of the microbial activity stimulated by the presence of nutrients, a flow of thermal effect is generated, which is directly related to an increase or decrease in the energy release by a variety of sources. To follow the evolution of energetic content, the microcalorimetry technique showed to be a useful tool [3]. This method has the advantage to be specific to such kind of systems, which demands only the knowledge of the initial and final energetic states and also is independent on the organisms and reaction pathways [6]. From the experimental point of view, another important advantage associated to this technique, which is of the extreme significance, is connected with the kinetic features of the reactions

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involved. Thus, slow reactions can be easily followed by a continuous recording of the signal for longer times, without any disturbance of the system. In this procedure a complete determination of a given microbial activity can be outlined, which reflects the thermal effect that is largely derived from the catabolic breakdown of substrate and also the anabolic reaction that has another contribution to the overall balance of the system [7].

An excessive diversity of man-made agrochemicals is associated, nowadays, to current agricultural practices. The use of pesticides, especially herbicides, is part of common procedure in the extensive modern agriculture [8]. Such kind of xenobiotics is spread in applications to eliminate different undesirable forms of life in agriculture and in urban areas.

The main objective is focused here on pesticide 2,4-dichlorophenoxiacetic acid (2,4-D) that belongs to this special kind of compounds. It is a systemic herbicide and is used for control of a wide range of broad leafed weeds and grasses in plantation crops such as sugar cane, oil palm, cocoa, rubber crops and weeds along highways [1,9,10]. In particular, this specific herbicide is commonly used in Brazilian agriculture, mainly in sugarcane plantations, which covers large areas to produce not only sugar, but also ethanol as a source of fuel of engines [1,9].

As a normal agricultural procedure, the direct application of these synthetic agrochemicals on soil can affect the microbial activity and also can cause an overall toxic effect on the environment [10–13]. Therefore, in this process the residual matter becomes a pollutant and may act as a potential environmental hazard, with a great tendency to accumulate in soil, to disturb the natural ecological equilibrium [13].

The microbiota activity of soil was calorimetrically monitored by recording typical power–time curves in the employment of the selected chemical 2,4-D on soil samples in the present investigation. The disturbance and thermal effects caused by the regular increment of 2,4-D in presence of nutrients are now reported.

2. Materials and methods

2.1. Reagents

All chemicals such as glucose (Hoescht), ammonium sulfate (Baker) and potassium chloride (Carlo Erba) were used as received. The herbicide 2,4-D (Sigma) has technical grade with a degree of purity of 98%.

2.2. Soil samples

Red Latosol soil, which covers approximately 15% of the State of São Paulo, was collected on the campus of the State University of Campinas. Samples were collected to a depth of 5–10 cm, after removal of the top surface layer [14–16]. These were air dried for 10 days in standard conditions of pressure and temperature and homogenized by sieving to less than 2 mm, to separate roots and large particles [1,16]. The soil was stored in polyethylene bags at 293$\pm$5 K for at least three months before being used in the calorimetric experiments.

For organic matter determination, triplicate samples of dry soil were placed in a muffle furnace to follow the decrease in mass at a temperature of 823 K for 24 h, as recommended [9,17]. Under these conditions organic matter is combusted, to leave only the inorganic component of the soil.

Carbon, nitrogen, hydrogen and sulfur contents in soil were determined in triplicate by elemental analysis by using an Elemental Analyzer Fisons Instruments CHNS-O model 1110.

Measurements of pH were obtained in triplicate by means of a pHmeter Digimed DMPH-2. In such determination 2.0 g of soil sample was suspended in 5.0 cm^3 of a strong electrolyte such as 1.0 mol dm^3 calcium chloride in a 1:2.5 for soil:solution proportion [17,18].

2.3. Microcalorimetry

A heat-flow microcalorimeter LKB 2277 Thermal Activity Monitor, Thermometric AB, Sweden, was used for all measurements and was described previously [19,20].

A typical representation of a power–time curve can be observed in Fig. 1, which shows in part A different phases of the development of the microorganisms. Each regime of the curve is associated to a characteristic phase and is labeled to better describe the evolution of the thermal effect, such as: (a) adaptation period where the microorganisms are recognizing the source of nutrients, (b) acceleration period that

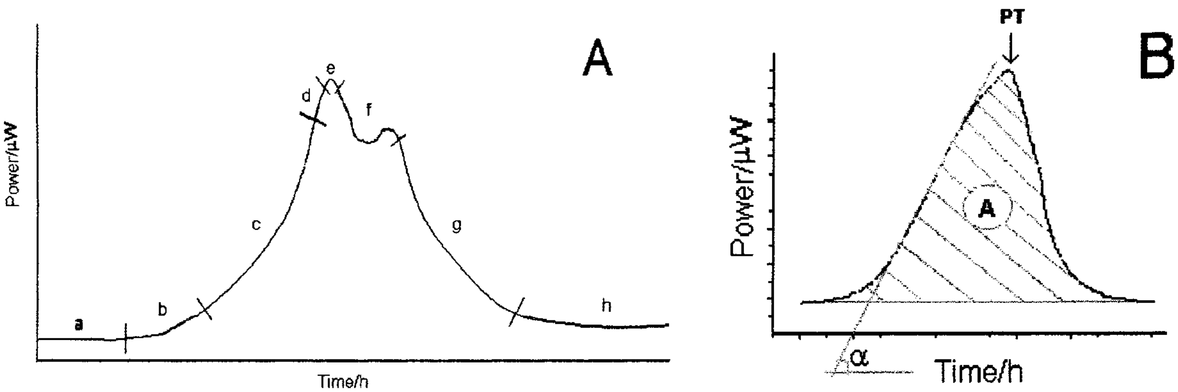

Fig. 1. Typical power–time curve of red Latosol soil, different phases of soil metabolism (A) and the proposed thermal effect calculation (B).

occurred in the beginning of the growth of microorganisms, (c) an exponential growth with a maximum rate, (d) delay period due to the scarce of nutrients, (e) exhaustion period when the applied nutrients are going to end, (f) a decrease with endogenous growth, where the microorganisms use other sources of food like the available organic matter, (g) a typical decrease period and (h) latent stage when the microorganisms returned to the natural activity [6,21].

The general method of calorimetric data determination consisted in considering all thermal effect, which is related to the area indicated under the curve as represented in part B of Fig. 1. Thus, the total thermal effect obtained for a given experiment can be calculated by the integration of the area of power–time curve. The constant growth rate is obtained from the slope corresponding to the experimental growth, whose value is determined when the maximum activity is displayed [22].

In the present series of experiments, the thermal effect was obtained using 5.0 cm^3 stainless steel ampoules. These ampoules were hermetically closed by Teflon sealing discs to avoid evaporation inside the apparatus. Experiments were carried out at 298.15±0.02 K. 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 containing 6.0 mg of glucose plus 6.0 mg of ammonium sulfate [3,23]. Under these conditions, moisture applied on experimental system was controlled at 35%, which induces the highest microbial activity [20]. The thermal effect associated with nutrient degradation was recorded as function of time. The final value was calculated from the integrated area of the power time curve for each experimental determination [3,4,14,18].

3. Results and discussion

The development of the microbial activity in a chosen system can be affected by the properties associated to the soil. Thus, the inherent physical chemical properties such as pH, organic matter content and elemental composition are important features to be considered. In the present system, the selected soil is constituted by 5.50±0.22% of organic matter, 5.22±0.11% of carbon; 0.92±0.04% of nitrogen and 2.73±0.14% of hydrogen, resulting in a natural pH of 5.84±0.06.

The microorganism population of this red Latosol soil was shown to be very sensitive to the addition of nutrients, as observed before [3]. In general, the stimulation of the metabolism is directly promoted by addition of an amount of to the desired nutrients to the ecosystem. This stimulating source is normally composed of a mixture of glucose, ammonium sulfate and water in variable ratios. In that sense, an unequivocal evidence of the activity of the microbial metabolism in degrading glucose can be visualized in a series of power–time curves obtained for a sequence of assays. Such kind of curve records the rate of thermal effect, dQ/dt (µW), during any change in microbial activity on soil, without disturbing the system [19]. The selected condition was experimentally established to give the best recorded signal [20]. In all measure-

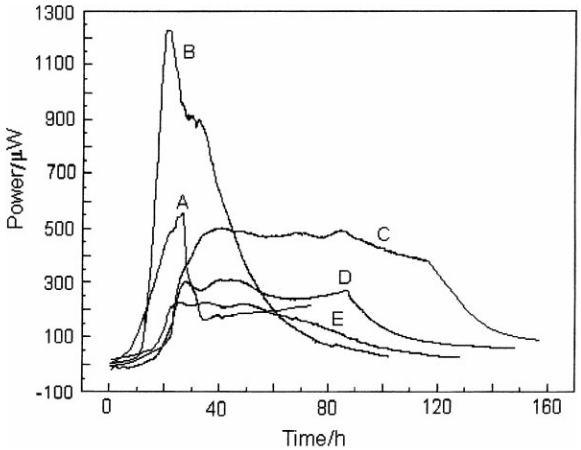

Fig. 2. Power–time curves of the soil microbial activity at 35% of total moisture in a sample of 1.50 g of red Latosol, containing 0.0 (A), 1.0 (B), 2.0 (C), 4.0 (D), 10.0 (E) mg of 2,4-D added on soil.

ments the resulting thermal effect was compared to a given reference, containing only the equivalent amount of soil and 0.80 cm^3 of water [20].

The metabolism of microbial population developed in red Latosol soil in absence and presence of distinct amounts of the pesticide is shown in a series of curves in Fig. 2. As illustrated, the power–time curves are recorded for different amount of 2,4-D, in which a great variability of the activity can be visualized. The resulting total thermal effect should be connected to the metabolic activity of soil in the presence of nutrients and pesticide as is represented in Table 1. In this established conditions, the variation of thermal effect originating from any physicochemical event is immediately compensated by the reference ampoule in the microcalorimeter, due to its operational condition [7].

The set of distinguishable profiles obtained from the power–time curves with distinct amount of 2,4-D added on soil samples gave results that clearly evidenced the variation in activity, as changing the amount of pesticide applied to the microorganisms. From these power–time curves recorded, in Fig. 2, the total thermal effects of the system, Q, were calculated through the integration of each curve. Thus, these total thermal effects evolved by the microorganisms per mass of soil, Qm, were also calculated for distinct assays, as listed in Table 1. The values of the peak-time, PT, were obtained when the maximum of activity is displayed. The microbial growth rate constant, μ, was calculated from the slope of semi-logarithm of exponential phase.

The thermal effect for the sample assayed in absence of 2,4-D showed in Fig. 2 gave a value of 23.18±0.24 J. This individual value is connected only to the degradation of glucose under experimental conditions inside the ampoule. In this aerobic condition, a total degradation of glucose is expected to produce as final products water and carbon dioxide. However, when 2,4-D is added on samples the final products could be constituted by some metabolites derived from pesticide, which are dispersed inside water and also carbon dioxide is formed due to the reaction of decomposition of the pesticide.

The application of 1.0 mg of 2,4-D in the soil sample resulted in a remarkable increase of the total thermal effect, by reaching the value of 107.97±0.56 J. This result was not initially expected because in previous reports where other pesticides were employed only a clear inhibitory effect took place [19,23]. However, this occurrence of an enhancement in the microbial activity is probably connected to fact that the microorganisms used 2,4-D as a new source of

Table 1
Influence of the mass of 2,4-D applied on soil, m (mg) on total thermal effect, Q (J), on total thermal effect per 1 g of soil, Qm (J g^{-1}); on peak-time (h) and on microbial growth rate (h^{-1})

M (mg)	Q (J)	Qm (J g^{-1})	PT (h)	μ (h^{-1})
0.0	23.18±0.24	15.45±0.24	25.32±0.52	0.093±0.003
1.0	107.97±0.56	71.98±0.56	20.20±1.02	0.151±0.003
2.0	86.77±0.62	57.85±0.62	39.10±1.02	0.108±0.004
3.0	62.94±1.00	41.96±1.00	32.69±0.96	0.090±0.002
4.0	62.25±0.97	41.50±0.97	28.85±0.38	0.092±0.002
6.0	44.94±0.57	29.96±0.57	28.59±0.33	0.091±0.003
10.0	23.58±1.02	15.72±1.02	29.28±0.72	0.089±0.003

food [24–27]. The degradation of 2,4-D in absence of glucose was detected by us in an appropriated experiment. On the other hand, other investigations are in agreement that this agrochemical started its degradation in soil around 10 h of assayed [25,26,28].

From this unitary amount of pesticide applied to the system, the exponential curves decrease in value as observed for other xenobiotics. Then, the increase of the amount of pesticide is followed by an inhibition of the microbial activity as shown in Fig. 2. These results suggested that the increase of applied amount from 2.0 to 10.0 mg can cause the appearance of metabolites derived from degradation of the pesticide by the population of the microorganisms of the soil. These new components must be toxic to microorganisms, as is noticed by a drastic inhibition effect, as is demonstrated by the decrease, which reflect directly in the microbial activity. As a consequence, the lower thermal effect, resulting as due to the application of 10.0 mg of this pesticide on soil, is 23.58±1.02 J as observed in Table 1.

The microbial growth rate was calculated as $0.093\pm0.003\ h^{-1}$ in absence of 2,4-D. Due to this anomalous behaviour the first addition can induce an increase in the microbial growth rate constant to $0.151\pm0.03\ h^{-1}$. However, above this amount of pesticide the value remained stable at around $0.090\ h^{-1}$. From these data are demonstrated that the application of 1.0 mg of this pesticide increased the soil microbial growth rate up to a maximum to decrease near a constant value.

In examining the sequence of 2,4-D added on soil, the total thermal effect values suggested an occurrence of a synergism in this system, due to the presence of herbicide. However, by adding increasing amounts of it, an exponential decrease in the microbial activity was observed. From these values it is possible to infer that indiscriminated applications of this agrochemical directly on cultivated soils can affect the normal life of microorganisms. This disturbance reflects in the microbial growth and consequently causes a contraction of it, that are of fundamental importance to the fertility of soil.

By comparing the present results with those obtained for addition of other xenobiotics [10,14,17,23], a distinct behaviour was observed. In general, the first addition of a given chemical to the soil is followed by a clear inhibitory effect, which contrasts with the results obtained here, where the inhibition started to occur only above 1.0 mg of 2,4-D added. The exponential profile of this curve shows that it can be linearized through a semi-logarithm form, by considering the thermal effect as function of the added mass and Fig. 3 presented a linear behaviour. The straight line confirmed that the exponential behaviour of the decrease is related to the total thermal effect, as the amount of pesticide is applied. From this plot a general equation can be established:

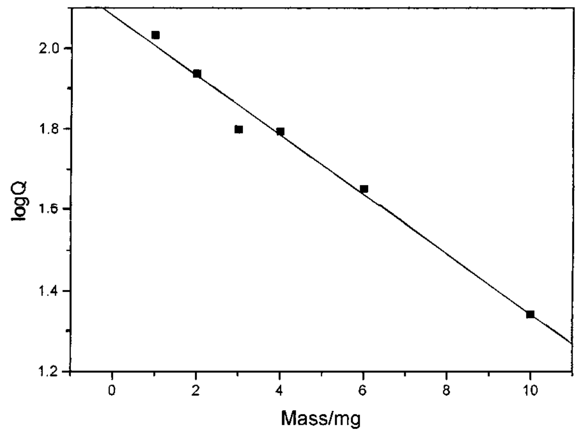

Fig. 3. Logarithm of total thermal effect calculated from microbial soil activity plot against the mass (mg) of 2,4-D.

$$\log Q = A + \alpha C \qquad (1)$$

In this expression, Q is total thermal effect, A is the linear coefficient, α is the slope, and C is the mass of herbicide used. By introducing the exponential data resulted in the expression 2:

$$Q = 10^{(2.080-0.074C)} \qquad (2)$$

Thus, the total thermal effect in the range of applied amount of 2,4-D on soil varying from 1.0 to 10.0 mg, which can be clearly inferred through the application of the above equation.

4. Conclusions

The indiscriminate application of agrochemicals on crops is one among other problems, which can affect the modern agriculture. The calorimetric results showed that the pesticide 2,4-D acts directly on the microbiology of soil. Firstly, the microbial population of soil uses this pesticide as nutrient. Subsequently, the increase of the applied amount causes a decrease on microorganisms activity, probably due to the formation of toxic metabolites yielded from the metabolism of this pesticide for microbial population of soil. These results showed that soils might have problems on renewal of organic matter when greater amounts of 2,4-D are constantly applied, by causing serious disturbance in the ecosystem. In this process, the microorganisms quickly assimilate the pesticide as a source of food better than its natural nutrient. However, the increase of toxic products are formed on degradation decreases perceptibly the microbial population of soil. All these calorimetric information on soil can give considerable contributions to important understanding features related to effect of the applications of 2,4-D on soil. The total thermal effect evolved by microbial activity on soil can be correlated to any range of application of this pesticide.

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