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The toxic effect on soil microbial activity caused by the free or immobilized pesticide diuron

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Abstract

The pesticide diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) was anchored onto silica gel to yield a new surface. Both free and immobilized pesticide were applied on typical Brazilian agricultural soil and the toxic effects on microbial activity of this red Latosol soil was followed by microcalorimetry. The activity of the microorganisms on 1.50 g of soil sample was stimulated by addition of 6.0 mg of glucose plus 6.0 mg of ammonium sulfate under 34.8% controlled humidity at 298.15 ± 0.02 K. The activity was recorded through power–time curves for increasing amounts of the active principle, varying from 0 to 333.33 $\mu g\, g^{-1}$. The increasing amounts of diuron, either free or immobilized, caused a decrease of the original thermal effect. The calorimetric data showed that the anchored pesticide presented a much lower toxic effect than free diuron on microbial activity.

Keywords: Diuron; Silica; Calorimetry

1. Introduction

Worldwide population growth outdistances the annual increase in agricultural production and, consequently, increases the global imperative for more food production. Food shortages lead not only to starvation and malnutrition but also to social, political and economical instability [1]. A viable alternative way to surpass the foreseeable crisis in agriculture is to increase the crop yield per acre. However, the means to this increase may lead to serious, perhaps grave, environmental consequence [1].

The present methods of growing foodstuffs cannot achieve both increased agricultural output and assure a high quality environment. Thus, the need for herbicides, insecticides, fertilizers, fungicides, and predator control is required [1]. In this direction, the use of pesticides in agriculture is a matter of environmental concern because these chemicals are recognized as a source of potential adverse impacts and, consequently, their presence in surface and ground waters has considerably grown [2–4].

Soil applied pesticides reach surface and ground waters by processes associated with run-off and leaching processes [5,6]. The natural process of sorption can attenuate the losses by soil solids, mainly those constituting the soil colloid fraction [7]. The greatest pesticide losses take place shortly after application, because the molecules need time to diffuse into soil aggregates and reach sorption sites in the soil colloid [8]. To minimize these losses pesticide immobilization on silica particles seems to be the most available method [9]. Among a series of pesticides, diuron

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(3-(3,4-dichlorophenyl)-1,1-dimethylurea) is one of the most extensively used in Brazilian agriculture [10]. From the agricultural point of view, this pesticide is normally used for total control of weeds and mosses on non-crop area [11]. On the other hand, herbicides can cause toxic effects that dramatically affect the microflora of the soil [12,13].

The main feature associated with agrochemical immobilizations on silica gel is to yield new surfaces, which could be used for controlled release as previously explored [14–16]. Taking into account these abilities, a decrease in the applied quantities of chemical in crops, without decreasing the inherent bioactivity, could be foreseen. In addition, the contamination of the environment is lower due to the decreased mobility of pesticide towards rivers and underground waters. Unless such conditions, a decrease in the total amount of the desired agrochemical to be used on plantations may be observed. Such kinds of immobilizations are an important feature to be explored, due to the fact that pesticides have become an integral component of modern agriculture [17].

The present investigation reports the effects caused on soil microbial activity, as evaluated by calorimetric determinations, whose increasing amounts of the free herbicide diuron and also its form when immobilized onto silica gel.

2. Materials and methods

2.1. Reagents

All chemicals used, such as glucose (Hoescht) and ammonium sulfate (Baker), were reagent grade. The pesticide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) (Sigma) and the silylant agent 3-trimethoxysilylpropylamine (APTS) (Fluka) were used without purification. Silica gel (Merck) was activated in a stream of dry nitrogen, to give a specific area of $387.1 \pm 21.9\,m^2\,g^{-1}$, determined by the BET method [18].

2.2. Soil samples

Red Latosol soil, which covers approximately 15% of the state of São Paulo, was collected on the campus of the University (47.1°W longitude and 23.8°S latitude) [19]. Samples were collected to a depth of 5–10 cm, after removal of the top surface layer, air dried for 10 days at 25 °C and homogenized by sieving to less than 2 mm, to separate roots and large particles [20]. The soil was stored in polyethylene bags at 293 ± 5 K for at least 3 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 [20]. Under these conditions organic matter is combusted, to leave only the inorganic component of the soil.

Carbon, nitrogen, hydrogen and sulfur contents of the soil were determined in triplicate by elemental analysis by using a Fisons Instruments CHNS-O model 1110 Elemental Analyzer. Measurements of pH were obtained in triplicate by means of a Digimed DMPH-2 pH-meter. For these determinations, 2.0 g of soil sample were suspended in 5.0 cm^3 of a strong electrolyte such as 1.0 mol dm^{-3} calcium chloride in a proportion of 1:2.5 for soil:solution [19,20].

Microbial activity is related to the main characteristics of the soil. Thus, the inherent physical chemical properties such as pH, organic matter content and elemental constitution are important features to be considered. The soil assayed contained 5.50 ± 0.22% of organic matter, 5.22 ± 0.11, 0.92 ± 0.04 and 2.73 ± 0.14% of carbon, nitrogen and hydrogen, respectively, and pH was 5.84 ± 0.06 [19].

2.3. Organofunctionalization

A sample of 45.0 g of activated silica gel suspended in 100.0 cm^3 of dry xylene was refluxed and mechanically stirred for 1 h under dry nitrogen. To this suspension 15.0 cm^3 of APTS was added dropwise. The mixture was kept under reflux at boiling temperature of the solvent for another 72 h and the solid was filtered, then washed with water and ethanol [18,21]. The resulting immobilized surface, named SiAPTS, was dried in vacuum at room temperature for several hours.

2.4. Immobilization

A sample of 5.0 g of organofunctionalized silica, SiAPTS, was suspended in 100.0 cm^3 of dry xylene, refluxed as before while mechanically stirred with 6.76 g of diuron and 1.5 cm^3 of tri-*n*-butylamine for

72 h under dry nitrogen [18]. The surface containing the immobilized pesticide, named SiDi was filtered. Washing with water and ethanol eliminated the excess of diuron. This final anchored surface was dried in vacuum at room temperature.

2.5. Characterization

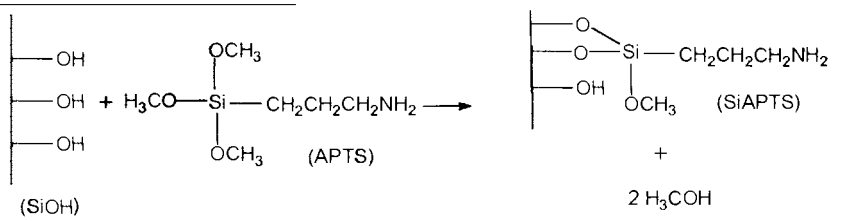
Silica surfaces were characterized by elemental analysis, surface area, infrared spectra and nuclear magnetic resonance spectra of ^{29}Si and ^{13}C nuclei, as previously described [18].

2.6. Microcalorimetry

A LKB 2277 thermal activity monitor heat-flow microcalorimeter was used for all measurements as previously described [19]. The thermal effect was obtained by using 5.0 cm^3 stainless steel ampoules with Teflon sealing discs to avoid evaporation [19] and the experiments were carried out at 298.15 ± 0.02 K [19,20]. All determinations of the thermal effect were performed in triplicate in ampoules charged with 1.50 g of soil plus 0.80 cm^3 of solution, containing 6.0 mg of glucose plus 6.0 mg of ammonium sulfate at 298.15 ± 0.02 K [19]. The toxic effect on microbiota was followed by using nutrient solutions with increasing amounts of the herbicide diuron and SiDi, varying from 0 to 333.33 μg g^{-1} [10]. Under these conditions, the moisture applied in the experimental system was controlled at 34.8%, which induces the highest microbial activity, as reported before [22]. 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 [19,20].

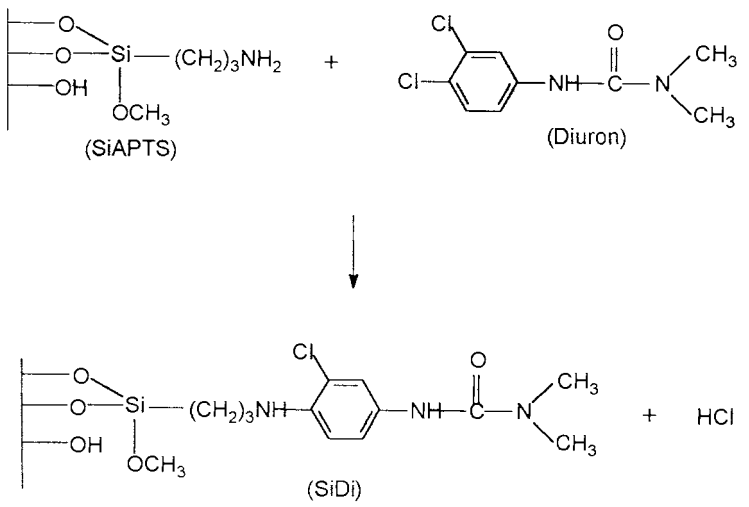
3. Results and discussion

The sequence of immobilization consisted in reacting, in a first stage, the silica gel (SiOH) with 3-trimethoxysilylpropylamine (APTS) to give the modified surface, SiAPTS, as shown in Eq. (1)



(1)

This precursor was reacted with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) to form the product SiDi in the sequence presented in Eq. (2)



(2)

Elemental analysis data for diuron immobilized onto the SiAPTS surface showed 0.79 ± 0.06 mmol of diuron per gram of silica. The full characterization of this surface was previously described [18].

The microbial population in the red Latosol soil sample manifests itself when assayed as an exothermic effect caused by degradation of nutrients [19,20,22]. Such activity involves two processes: an exothermic catabolic effect due to the degradation of nutrients and an endothermic anabolic effect resulting from microbial growth. The activities are directly related to the rate of oxygen consumption and carbon dioxide production [23]. Thus, the consistent relationship between the thermal effect and respiration rate can be followed by the use of microcalorimetry, a useful technique which allows the detection of changes in the system, such as those connected with microbiological actions in the soil [24–26].

In normal conditions, the lack of suitable substrates usually limits the microbial activity. Other important factors that can affect the soil microbiota are mineral nutrients, moisture, aeration, pH and the presence of toxic compounds, which are often not investigated in the course of a particular investigation. However, the evaluation of the complete system usually requires an additional source of energy. In this condition, the power–time curves for red Latosol soil samples were recorded while the increasing doses of diuron and SiDi were applied. Fig. 1 shows the development of the total thermal effect with time, following the application of a series of SiDi, ranging from 0 to 333.33 μg of active principle per gram of soil.

Addition of the xenobiotic SiDi in different amounts causes direct toxic effects on the original microbial activity. The thermal effect recorded from the curve without pesticide is shown in Fig. 1A, represents the normal metabolism of glucose by soil microorganisms under experimental conditions inside the ampoules. Other thermal effects recorded, as a function of time are shown in curves B–E of Fig. 1, which exhibited the effect of SiDi on the activity generated by microorganisms residing in the soil. Data about free diuron were previously reported [10]. Both free and anchored xenobiotics affect the metabolism of the microflora in the soil, producing a change in the profile of the power–time curves, which reflects in the activity of the microorganisms.

The total thermal effect, Q; the microbial growth rate constant, μ; and the peak-time value, PT, were calculated from power–time curves for all samples in order to obtain quantitative data to explore better the obtained results [19,20].

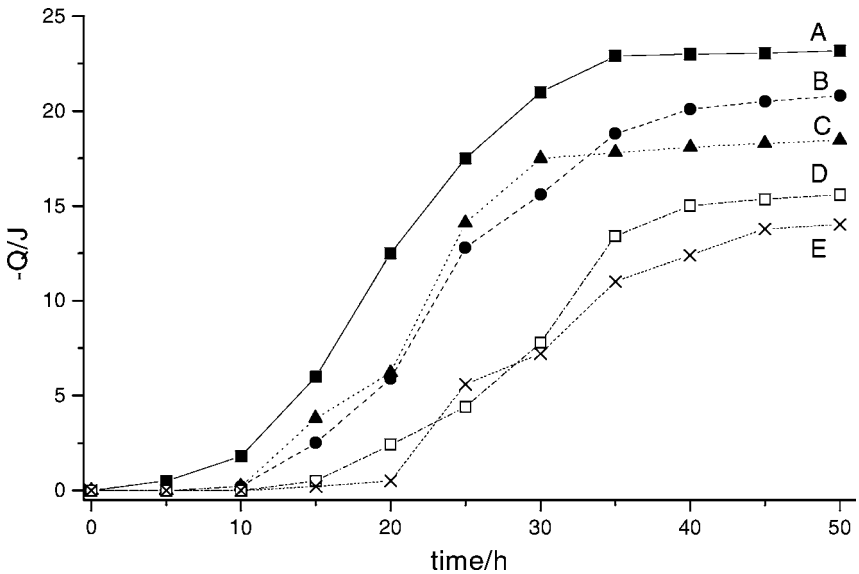


Fig. 1. The total thermal effect, Q, as a function of time for different doses of SiDi applied to soil: (A) 0, (B) 1.67, (C) 3.33, (D) 66.67, and (E) 333.33 μg g^{-1}.

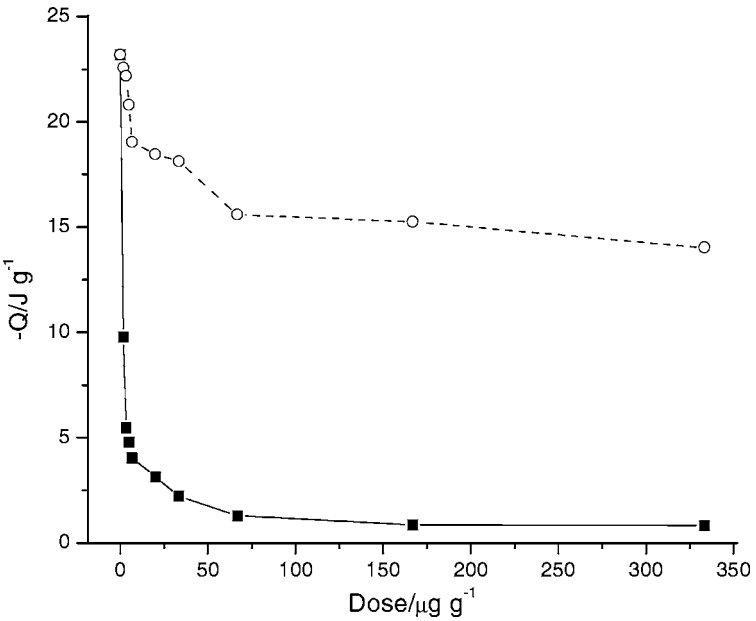


Fig. 2. The total thermal effect, Q, calculated from the metabolism of soil as a function of diuron (■) and SiDi (○) doses.

The effect of diuron and SiDi on soil microbial activities is shown in Figs. 2–4. The effect of free and anchored pesticide applied on soil resulted in thermal effects, which are plotted as function of dose in Fig. 2. The activity decreases quickly with increasing doses of diuron up to a level of 66.67 $\mu g\,g^{-1}$. Above this value the activity decreases smoothly and becomes constant above 66.67 μg of diuron per gram of soil. On the other hand, the toxic effect of SiDi is much lower than that caused by free pesticide, with a slow decrease in thermal effect, becoming stable above 66.67 $\mu g\,g^{-1}$. The diuron application of 333.33 $\mu g\,g^{-1}$ causes a decrease of 96.4% of total microbial activity, while for SiDi this dose causes a decrease of only 39.5%. This large difference in action suggests that the attached pesticide has much lower toxicity than free diuron, due to inhibition of metabolism caused by the toxic effects of xenobiotics on the microorganisms of soil. This behavior can be connected to the death of soil microorganisms, as was reported before for others chemicals [24].

The variation of the thermal effect as a function of the logarithm of the applied dose is plotted in Fig. 3. From this linearized form the maximum dose (MD) can be predicted, which is the dose capable of eliminating all soil microbial activity [25,26].

In both cases, when the total thermal effect reaches a null value, MD, is obtained. Based on the data presented linear and angular coefficients of 6.28 and −2.42 for diuron and 23.34 and −3.67 for SiDi applications were obtained, respectively. Thus, the dose able to eliminate the soil microbial activity (MD) was 398.5 $\mu g\,g^{-1}$ for diuron and 2.29 $g\,g^{-1}$ for SiDi. Another feature to be explored is the effect caused by application of these xenobiotics on the delay in metabolism, which can be better visualized when the peak-time is plotted as a function of the dose of chemicals applied is shown in Fig. 4. These results demonstrate that an increase in the amount of xenobiotics reflects in changing the PT values, as a consequence of metabolism modification. In both cases the activity is delayed, although, the application of SiDi presents a lower change in the original PT values, contrasting with diuron, that dramatically alters the PT values.

The increase in diuron and SiDi levels in the soil can also change the microbial growth rate constant, μ. An increase in μ values up to a dose of 333.33 $\mu g\,g^{-1}$ is observed in Fig. 5 for different doses of diuron application. This fact is a good indication of the pesticide stress to the microorganisms in soil [27]. Above

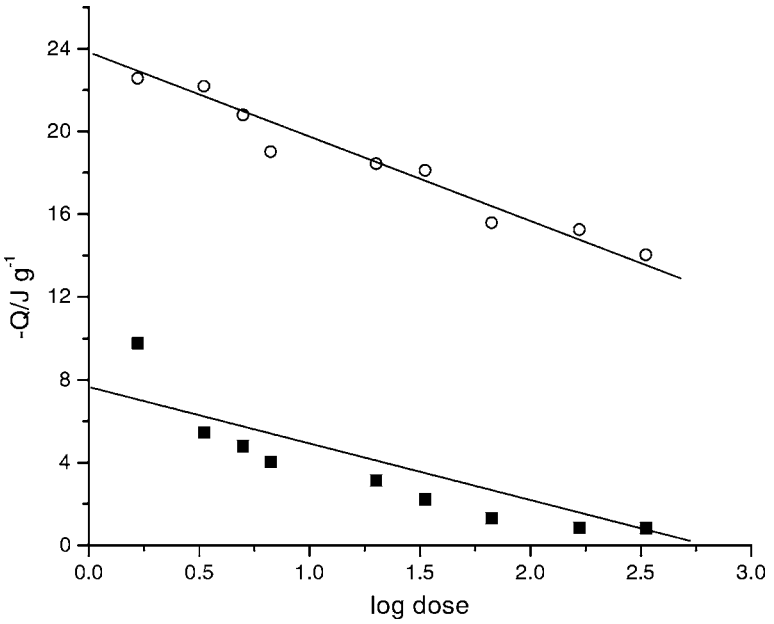


Fig. 3. The total thermal effect, Q, as a function of the logarithm of the diuron (■) and SiDi (○) doses applied.

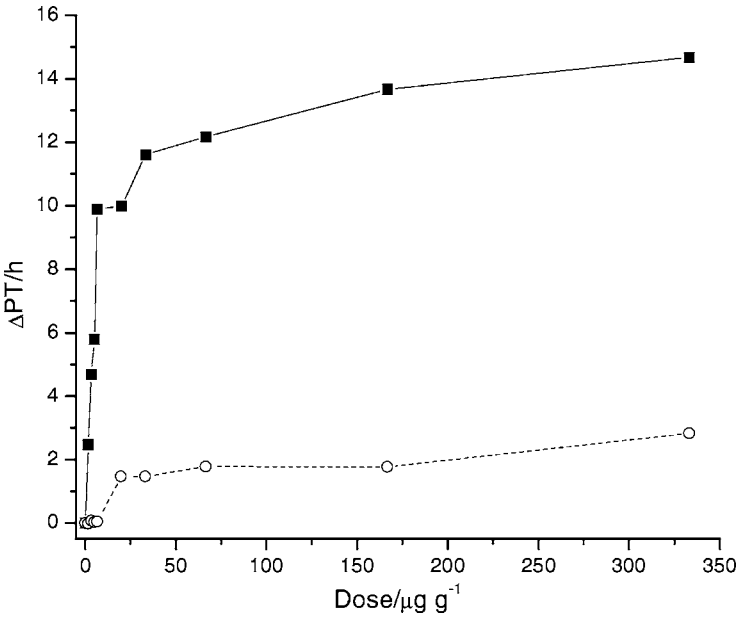


Fig. 4. The change in peak-time, ΔPT, as a function of the influence of the doses of diuron (■) and SiDi (○) applied.

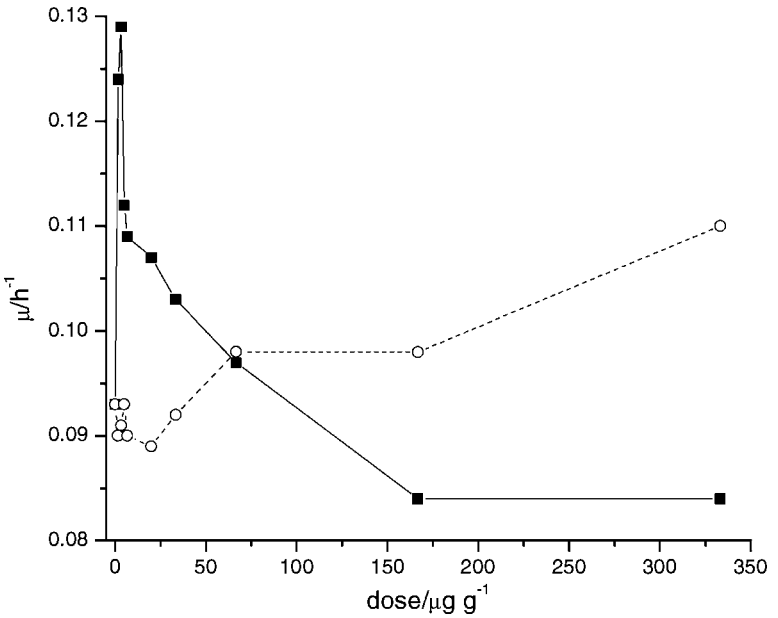


Fig. 5. The microbial growth rate, μ, as a function of the diuron (■) and SiDi (○) doses applied.

this dose of diuron, the μ values decreased as expected. The same behavior was observed for μ value in a SiDi application of 166.67 $\mu g\, g^{-1}$. Above this dose an increase is observed, an effect, which could be due to stress caused by SiDi on soil microorganisms [27].

The impact caused by immobilized pesticide SiDi on soil microbial activity is much lower than that of free diuron. The data reported here show that the immobilized pesticide form presented a low toxicity. This substance must be explored in terms of the controlled release properties and its phytotoxicity to weeds that diuron is used to eliminate.

4. Conclusion

Immobilization of diuron was obtained with success and its activity presented a lower toxicity than the free pesticide. The microcalorimetry experiments showed that SiDi displayed a toxic effect much lower than typical diuron pesticide, with the advantage that the immobilization minimizes losses by leaching and run-off.

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