

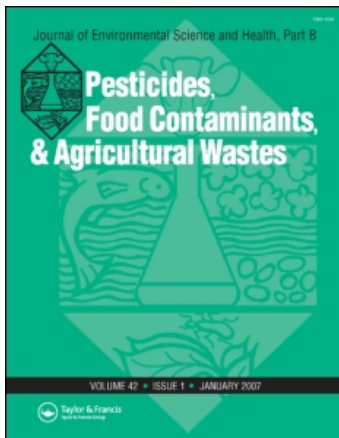
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IMPACT OF LONG-TERM PESTICIDE APPLICATIONS ON SOME SOIL BIOLOGICAL PARAMETERS

Key Words: Dehydrogenase, iron-reduction, microbial biomass

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ABSTRACT

The soil oxidative and anaerobic processes, as well as, the microbial biomass were followed during three years in a cotton farm (Tatuí) where the recommended pesticides have been used for several years, and in an experimental field (São Paulo) treated first time with the same pesticides. The oxidative process was monitored by the dehydrogenase (DHA)-activity using triphenyltetrazolium chloride (TTC) as substrate. The anaerobic process was followed by the iron-oxide reduction, and the microbial biomass was estimated by the substrate (glucose)-induced respiration. Increases in DHA-activity and in the microbial biomass occurred only in the farm soil, with concomitant decreases in iron-reduction. In the experimental field soil, the increases in DHA-activity were followed only by decreases in iron-reduction. Soil characteristics were the determining factor for different biological parameters after pesticide inputs. All the pesticides produced at least one clear but transient effect.

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INTRODUCTION

The use of pesticides has proved so far as the only mean to protect crops in large scale. However, the effects of pesticides in the environment must also be considered in the context of soil pollution and sustainability of the agroecosystem. Some crops, as cotton, needs heavy repeated applications of pesticides of different chemical groups that reach the soil (Papp et al., 1992; Schuster and Schröder, 1990a). As pesticides are designed to be biologically active (Schuster and Schröder, 1990a), they may act also on metabolic activity of soil microorganisms.

Soil microorganisms play a key role on important cycles of essential elements for soil fertility (Topp et al., 1997; Vallaeys et al., 1997). As consequence, when pesticides reach the soil environment, they may disturb the local metabolic activity (Topp et al., 1997), the soil fertility (Nannipieri et al., 1990), and also the pesticide degradation processes (Kearney and Kellogg, 1985). Although abiotic processes are very important and difficult to be separated from the biological transformations, it is known that the biological degradation of pesticides in soil is quantitatively more important than the abiotic ones (Barriuso et al., 1996).

Thus, the behaviour of the total soil microflora under pesticide inputs is an important point of the agricultural ecology. There are many reports on the determination of the best soil bioindicators in order to evaluate the effects and consequences of soil contamination (Vallaeys et al., 1997). However, data obtained by the individual tests to measure the effects of pesticides on microorganisms have limited value (Johnen and Drew, 1977), and there are only few studies that have simultaneously evaluated a variety of aspects of soil microflora's behaviour under pesticide contamination (Vallaeys et al., 1997).

This study investigated two physiological parameters, the oxidative and anaerobic processes. In addition it also evaluated the microbial biomass variation as estimated by soil respiration. The oxidative process reflects a general bioactivity of a large part of soil microorganisms that can be measured by soil-dehydrogenase activity (Trevors, 1984; Nannipieri, 1984), and the iron-reduction reflects the

physiologic reactions of anaerobic microorganisms (Zelles et al., 1986). Moreover, it has been pointed that the impact of toxic substances on the dehydrogenase activity and C-biomass has a correlation with the soil-Carbon cycle (Russell et al., 1997).

MATERIALS AND METHODS

Soil from an experimental station which is being used for cotton crops for several years (Tatuí, SP) was collected from 1995 to 1998, after different pesticide applications and in the interval between crop seasons (Fig. 1). The same application schedule used in Tatuí was followed in half of an experimental field area in São Paulo city. The other half did not receive pesticides. Soil characteristics are presented in Table 1.

The rate and order of all pesticide applications (per hectare) in the 1995-1996 season were: monocrotophos (1.0 L); dimethoato (0.5 L); dimethoato again (0.5 L); endosulfan (1.2 L); deltamethrin (0.5 L); endosulfan (2.0 L); deltamethrin (300 mL); methyl parathion (1.0 L); endosulfan (2.0 L), and carbaryl (2.5 kg). Trifluralin ($2.0 \text{ L}\cdot\text{ha}^{-1}$) was applied between the two crop seasons, and in 1996-1997, the order was: monocrotophos (1.0 L); monocrotophos again (1.3 L); endosulfan (1.25 L); methyl parathion (1.2 L); endosulfan (1.2 L); endosulfan (1.0 L) plus methyl parathion (1.0 L); endosulfan (1.5 L) plus methyl parathion (1.5 L); endosulfan (2.0 L) plus methyl parathion (2.0 L); deltamethrin (1.0 L); again deltamethrin (250 mL); endosulfan (1.2 L), and after the cropping, deltamethrin (250 mL) plus methyl parathion (1.25 L). Again trifluralin was applied between crop seasons, and in 1997 - 1998 the order was: diuron (4 kg); methyl parathion (1.2 L); endosulfan (1 L); endosulfan again (1 L); methyl parathion (1 l) plus endosulfan (1 L); deltamethrin (350 mL); methyl parathion (1 L); deltamethrin (1 L); and methyl parathion (2.5 L) plus deltamethrin (1.25 L).

Soil samples were obtained from the 0 - 15 cm layer of the soil profile before the first pesticide application (Sampling 0); after the first treatments with:

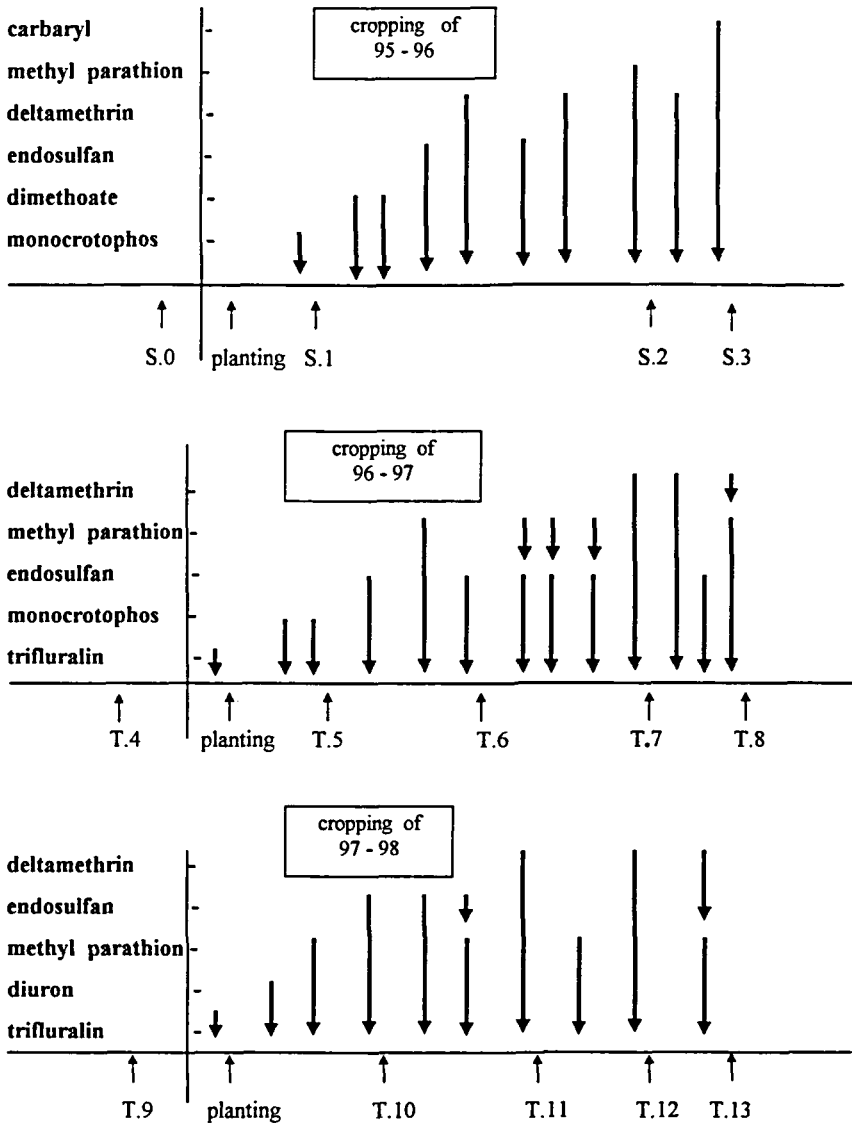


FIGURE 1
 Schedule of pesticide applications and samplings (S) during three crop seasons.

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TABLE 1
Soil Characteristics During the Experimental Time

Name	Soil type	Physico-chemical characteristics			pH	
		%				
		Silt	Clay	Sand	Organic matter (g dm ⁻³)	
Tatui	Clayey	8	51	41	20	4.9 to 5.7
São Paulo	Heavy clay	8	66	28	31 to 36	4.7 to 5.3

monocrotophos (S. 1); methyl parathion (S. 2) and carbaryl (S. 3), and after the first cropping (S. 4). In the second year of the study the samplings were done after the two applications of monocrotophos (S. 5); the first application of methyl parathion (S. 6); the first application of deltamethrin (S. 7), and after the mixture of methyl parathion and deltamethrin (S. 8). Another sampling between the crop seasons (S. 9), and then, after the first application of endosulfan (S. 10); deltamethrin (S. 11); the mixture of methyl parathion + deltamethrin (S. 12), and between another crop season (S. 13), as shown in Fig. 1.

The estimations of the oxidative process were done by measurements of dehydrogenase (DHA) activity in soil. The procedure was basically that of Schuster and Schröder (1990a) using (3 × 3 g) soil subsamples immediately after sampling and sieving in a 2 mm mesh. The 2, 3, 5-triphenyltetrazolium chloride (TTC) was used as substrate to be reduced to Formazan in presence of dehydrogenase. Formazan formation was measured in a spectrophotometer (Hitachi U-1100) at 485 nm.

The soil anaerobic bioactivity was evaluated by the iron-oxide reduction test, according to Zelles et al. (1986). The method used (3 ×) 5 g of sieved and moistened (55% of the water maximum holding capacity - WMHC) soil previously maintained at 22° C for two days, and then treated with 4 mg of glucose. Soil samples were kept under anaerobic conditions during 5 days and O-phenanthroline was used as chelating agent. Measurements of Fe²⁺ were also done by spectrophotometry at 512 nm.

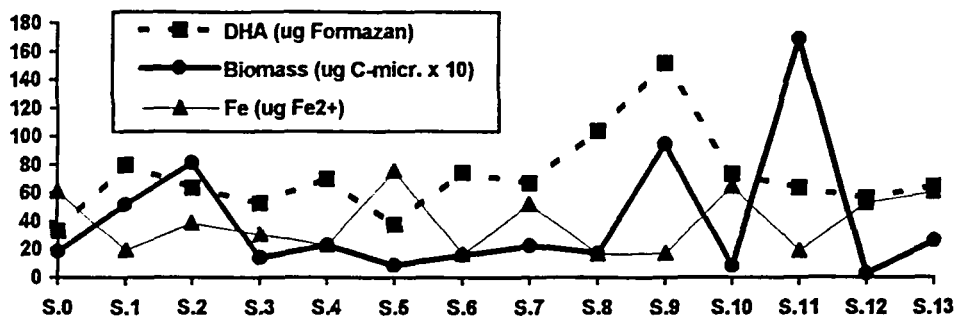


FIGURE 2

Tatuí soil-dehydrogenase activity (DHA), -biomass, and -iron reduction (Fe) after different pesticide applications and samplings (S).

The microbial biomass was studied by the method of substrate-induced respiration according to Anderson (1982). Soil (6×50 g) subsamples were placed in biometer flasks (Bartha and Pramer, 1965), remoistened to 55% WMHC, and pre-incubated at 28°C for two days. Three subsamples were then amended with $4\text{ mg glucose} \cdot \text{g}^{-1}$ soil, and CO_2 formation in all soil subsamples was measured in the KOH ($0.01\text{ mol} \cdot \text{L}^{-1}$) of the side arm of the flasks. KOH was changed after 6 hours, 1, 2, and 3 days, and the CO_2 produced by basal respiration (soil subsamples without glucose amendment) and induced (by addition of glucose) respiration was determined by titration with HCl ($0.05\text{ mol} \cdot \text{L}^{-1}$) after addition of phenolphthalein and back-titration after addition of methyl orange. The metabolic quotient (basal / substrate-induced respiration per hour) was multiplied by 20.6 to account for the biomass of soil subsamples.

RESULTS AND DISCUSSION

Results on DHA-activity, iron-oxide reduction, and biomass of soils from Tatuí, and São Paulo untreated and treated with the different pesticides in three crop seasons are shown in Figs. 2, 3 and 4. The data are expressed on per gram of soil basis (dry weight equivalent).

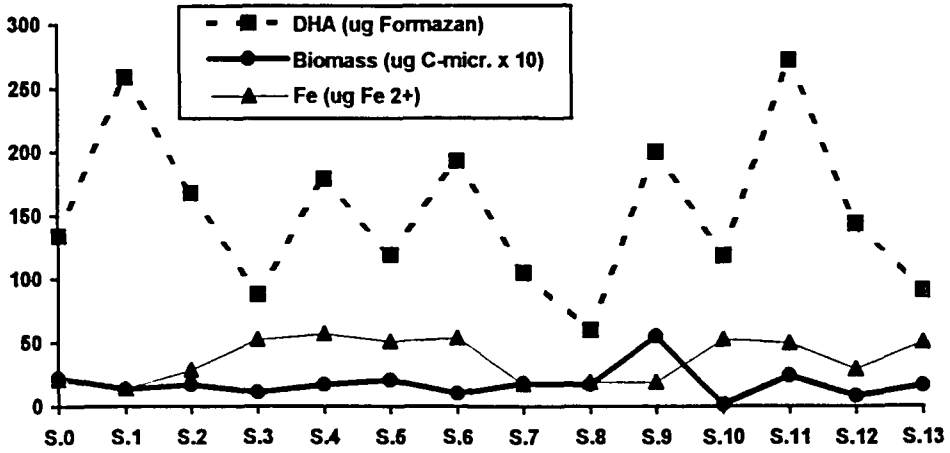


FIGURE 3

São Paulo treated first time soil-dehydrogenase activity (DHA), -biomass, and -iron reduction (Fe) after different pesticide applications and samplings (S).

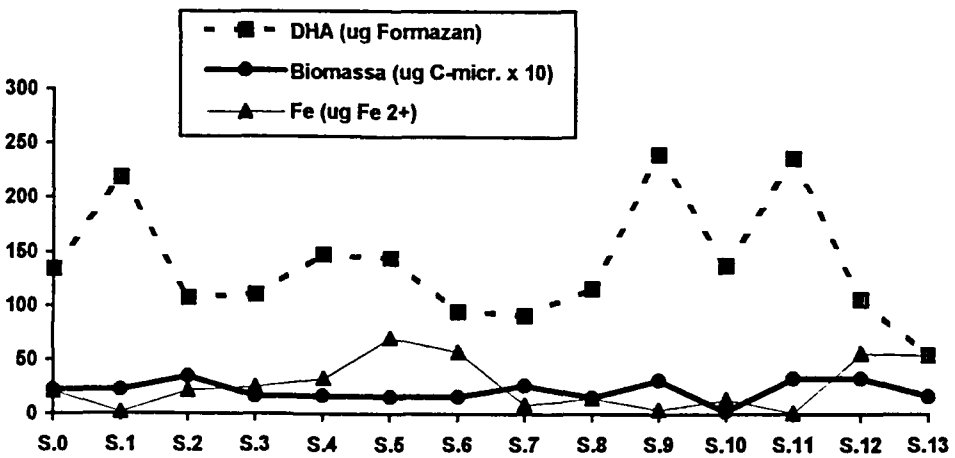


FIGURE 4

São Paulo pesticide untreated soil-dehydrogenase activity (DHA), -biomass, and -iron reduction (Fe).

The DHA-activity varied from about 23 (S. 0) to 151 μg (S. 9) of Formazan formed in Tatuí. Values decreased after S. 9, but at S. 13 they were nearby the same as in the beginning of the study. In São Paulo the detected values were a little higher in the soil treated first time than in the untreated area. The variation was from about 60 (S. 8) to more than 250 μg (S. 1) in São Paulo treated area, and in the untreated area it was from about 55 (S. 13) to 240 μg (S. 9), and again decreased to the initial values. Some variations (S. 1, S. 2, S. 4, and S. 9) were also observed in the untreated area, likely due to the seasonal factors as no pesticide was applied in this soil.

A clear inhibition of DHA-activity was detected in both treated soils after monocrotophos treatment (S. 5), and a stimulus after endosulfan (S. 6), as occurred in others soils (Tu, 1995). But, the inhibition caused by the application of deltamethrin + methyl parathion (S. 8) was detected only in the firstly treated soil of São Paulo (Fig. 2), which may indicate an inhibition of the local microbiota.

Anaerobic metabolism was always small but followed the opposite way of DHA-activity, i. e, the highest values of Fe^{2+} were mostly detected in soil from Tatuí. Between crop seasons (S. 9) small inhibition was observed in Fe^{3+} -reduction in all the soil samples, but the activity was recovered in the treated soil samples after planting and application of endosulfan (S. 10). In São Paulo treated area the anaerobic metabolism was a little stimulated by carbaryl (S. 3), endosulfan (S.10 and S. 11), but inhibited by the mixture deltamethrin + methyl parathion (S. 12), as compared with the untreated area.

The highest values of soil microbial biomass were detected in the repeatedly treated soil of Tatuí, which also presented the highest variations (Fig 2). A small stimulus was detected after application of methyl parathion (S. 2) and deltamethrin (S. 11). However, after the application of the mixture deltamethrin + methyl parathion (S. 12) a clear inhibition was detected not only in Tatuí, but also in São Paulo treated area, but not detected in the untreated area. As methyl parathion applied alone stimulated, this inhibition may indicate the action of the mixture of

the two insecticides also on this parameter. The soil biomass-detected values were always very small in São Paulo.

In the farm usually treated soil (Tatuí) the increased DHA-activity was also followed by increased values of microbial biomass, similarly to Schuster and Schröder (1990a). Nevertheless, these increases were clearly inversely related with the reduced-Fe contents. On the other hand, although the soil of São Paulo (mainly the untreated one) had presented higher DHA-activity than the Tatuí soil, the microbial biomass increases were small and the biomass itself was always very small. However, the increases of detected-Formazan were also followed by observable decreases in the amount of Fe-reduced (Fig. 2).

In the area of São Paulo treated first time, the amounts of detected-Formazan were even higher than in the untreated soil. This may indicate that pesticide applications contributed to the enhancement of the indigenous activity of the soil. However, the increases of detected-Formazan were also followed by observable decreases in the amount of Fe-reduction (Figs. 3 and 4). These observations indicate that microorganisms in the soil of São Paulo, although very small in number, were very efficient on the C-substrates of the soil. Moreover, as previously found by others (Schuster and Schröder, 1990b), that DHA-activity was the more meaningful biological measured parameter, mainly because it may be used as an index of the physiologically active biomass (Rossell et al., 1997).

Results also indicate that soil characteristics may induce different activities of the microbiological parameter studies in this investigation. As reported by Tu (1981), Schuster and Schröder (1990b) and Felsot and Dzantor (1995), the usual pesticide treatments produced some clear but transient effects. Then, it is essential to maintain favorable conditions for soil microorganisms to have their continuous activity and short-lived effects of pesticide applications.

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