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Microbial Degradation of Terbuthylazine in Surface Soil and Subsoil at Two Different Temperatures

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Although the widespread use of pesticides in agricultural has improved crop production, it has caused soil and water pollution. This is an environmental and public health concern, particularly where groundwater is used for drinking purposes (Vanderheyden *et al.*, 1997; Funari *et al.*, 1998). Triazine-based compounds and their metabolites may be leached from soil and contaminate groundwater, with this largely depending on their behaviour in the soil system. In this respect degradability, usually expressed as degradation rate (DT_{50}), is one of their most significant characteristics, because it determines their persistency. Microorganisms have an important role in the degradation of pesticides in soil (Digrak and Ozcelik, 1998; Gebendinger and Radosevich, 1999). As the composition and abundance of microbiota (Kordel *et al.*, 1995; Veeh *et al.*, 1996), and temperature may vary considerably with soil depth (Veeh *et al.*, 1996; Holden and Firestone, 1997), degradation rates may be different between the surface and subsoil layers.

This paper reports the results of laboratory studies on degradation in surface soil and subsoil of the triazine-based herbicide Terbuthylazine (TBA) with two different temperatures.

MATERIALS AND METHODS

Soil samples were collected from a field located in Manerbio (province of Brescia) in which TBA was being applied regularly. For each of the two experiments a steel tube (12 cm in diameter and 65 cm long) was forced vertically into the ground to isolate a core. In the laboratory two parts of soil were selected: the upper part (5 to 25 cm depth) was representative of the geopedologic horizon A (surface soil) and the lower part (40 to 60 cm depth) was representative of the horizon Bc (subsoil). The surface samples had a sandy loam texture and a high organic carbon content (1.8%). The subsurface samples had a higher clay texture component and a low organic carbon content (0.1%).

The soil was handled ensuring that was no-contamination between the layers, all solutions and instruments utilized were sterilized and all the phases of the experiments were performed in a sterile cabinet.
A set of sterilized flat dishes (2 for the surface soil and 2 for the subsoil) was prepared, each containing the same quantity (about 350g) of fresh soil.

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TBA dissolved in sterile water was added to each of them to achieve a finaconcentration. A set of sterilized flat dishes (2 for the surface soil and 2 for the subsoil) was prepared, each containing the same quantity (about 350g) of fresh soil. TBA dissolved in sterile water was added to each of them to achieve a final concentration of 1 mg/Kg. Two control soils were treated only with sterile water in order to obtain the same final moisture content (35%). At each sampling the moisture content of the soil was checked and kept constant during the entire period of the experiments by weighing the soil batches periodically and replacing any losses by adding sterile water.

Two sets of experiments were carried out, at temperatures of 15 (±1)°C and 22 (±1)°C, respectively. The first temperature reproduced the conditions of the site subsoil environment and the second one was the temperature suggested by SETAC (1995) for pesticide soil degradation tests. In both experiments the set was kept in the dark.

At each sampling two replicates for each condition were collected respectively of 2 g of surface-soil/subsoil for the chemical analysis and of 1 g for microbial analysis. Sampling of soils was performed at increasing intervals starting from TBA contamination, to assess both the half disappearance time (DT_{50}) of the TBA and the microbial density (N. bacteria/g soil) over the course of the experiment.
Analysis of TBA concentration was performed by accelerated solvent extraction followed by Liquid Chromatography Mass Spectrometry with an Electrospray interface (Di Corcia *et al.*, 1999) and that of microbial density (N. bacteria/g soil) by epifluorescence direct count method (Yu *et al.*, 1995; Barra Caracciolo *et al.*, 1999; Di Corcia *et al.*, 1999).

RESULTS AND DISCUSSION

At 15 °C the half disappearance times (DT50s) were similar, in surface soil (about 180 days) and in subsoil (about 200 days), (Fig. 1). The counts of microbial analysis showed a significant difference (t test, $p< 0.01$) in bacterial numbers (N/g soil) in treated samples versus untreated samples (controls), (Fig. 2 A/B).
On the contrary, at 22 °C the herbicide degradation occurred with very different times. In fact the DT_{50} was 30 days in the surface soil and about 180 days (not significantly different from the value at 15°C) in the subsoil, (Fig. 3). The differences in bacterial numbers between treated and control samples were significant (t test, $p< 0.01$) only in the case of surface soil (Fig. 4 A/B).
These results would indicate that the TBA degradation rate was positively influenced by the temperature in the case of the surface soil, but not in the case of the subsoil.

Moreover the greater bacterial numbers in the treated soils would suggest that not only TBA, when it is at a level of concentration as in the experiments, does not have a toxic effect on soil microorganisms, but also that the microbial activity might have a significant role in its degradation. Microorganisms might have degraded the TBA both by using it as a source of carbon and nitrogen for growth (Dousset et al., 1997; Yanze Kontchou and Gschwind, 1999; Gebendinger and Radosevich, 1999) and simply through transforming it with co-metabolic reactions (in which micro-

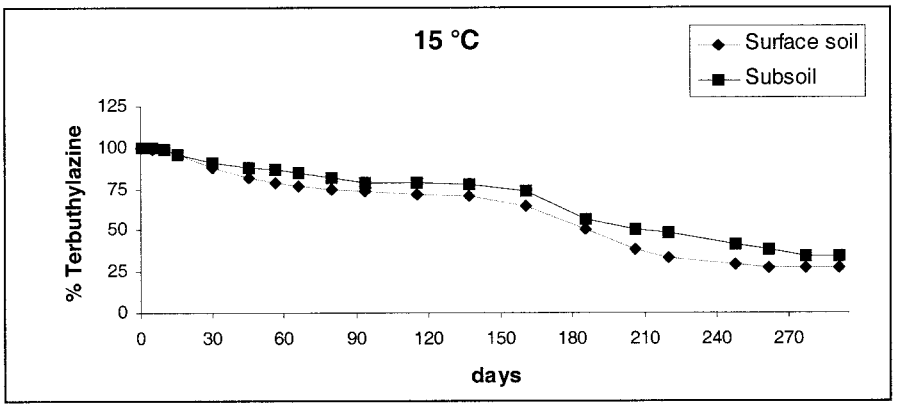

Figure 1. Degradation of terbuthylazine at 15°C in surface soil (5-25 cm) and in subsoil (40-60 cm).

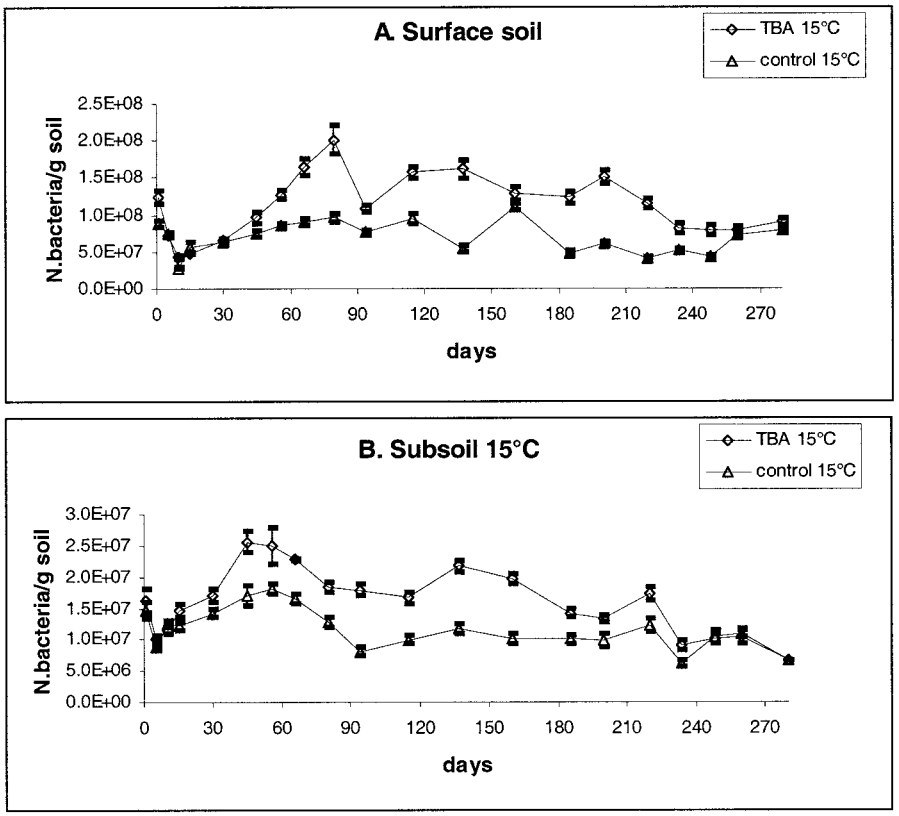

Figure 2 A/B. Trend in bacterial numbers (N/g soil) samples treated with TBA (continuous line) and untreated (dashed lines) at 15°C. The vertical bars are the standard errors. (A= surface soil: 5-25 cm; B= subsoil: 40-60 cm).

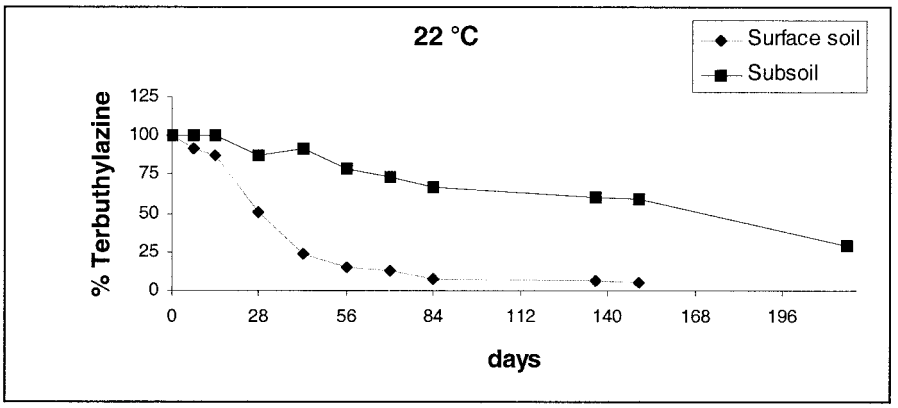

Figure 3. Degradation of terbuthylazine at 22°C in surface soil (5-25 cm) and in subsoil (40-60 cm).

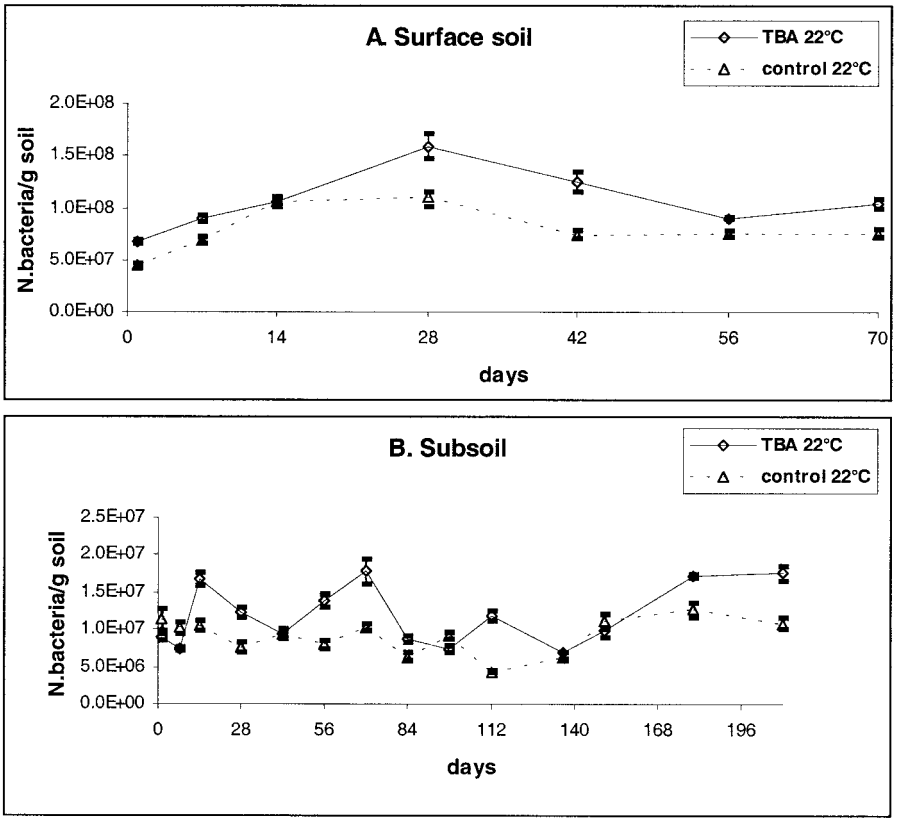

Figure 4 A/B. Trend in bacterial numbers (N/g soil) in samples treated with TBA (continuous line) and untreated (dashed lines) at 22°C. The vertical bars are the standard errors. (A= surface soil: 5-25 cm; B= subsoil: 40-60 cm).

organisms degrade the chemical with no growth). Although it was not possible to quantify the specific contribution of the different abiotic and biotic processes to TBA degradation, the different concentration of organic carbon (1.8% vs 0.1%) and the different clay content (18% vs 22%) between surface and subsoil might also have influenced the degradation rates (Fig. 3). For example a higher carbon content in soil might have supported a greater percentage of microbial populations degrading the herbicide by co-metabolic pathways. The lack of a significant increase in the degradation rate at 22°C in the subsoil might be due also to the fact that conditions were not optimal for the autochtonous bacteria since such a high temperature is unrealistic for this subsoil horizon. In any case further studies are necessary to quantify the different roles of biotic and abiotic factors in the degradation of TBA. Experiments in which sterilized soil is added are already in progress.

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