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Toxic Effects of Pentachlorophenol and Other Pollutants on the Mineralization of Acetate in Several Soils

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The mineralization of [^{14}C]acetate was studied in bottles with fresh soil and groundwater. Addition of toxicants inhibited the formation of $^{14}CO_2$ and dose–effect curves were obtained. The acetate mineralization was not inhibited by zinc, cadmium, $K_2Cr_2O_7$, chloropyrifos, and paraquat in an acid sandy soil at 1000 mg/kg dry soil. The IC_{10} is the toxicant concentration which inhibits 10% of the initial mineralization rate. The IC_{10} concentrations for 3,4-dichloroaniline, triphenyltin, and orthoxylene were 48, 96, and 730 mg/kg, respectively, in the acid sandy soil. The IC_{10} of pentachlorophenol was measured in samples from the acid sandy soil and in several other soil and subsoil samples. The geometrical mean of the 13 IC_{10} values was 16 mg pentachlorophenol/kg. A statistical method was used to calculate the PCP concentration above which 5% of the most sensitive acetate-mineralizing communities in all soils are influenced. The best estimate of this concentration is 0.3 mg PCP/kg but to be on the safe side the 95% confidence level of this concentration is 25 μg/kg. © 1993 Academic Press, Inc.

INTRODUCTION

Soil pollution is a major environmental problem in The Netherlands and in many other countries. In Dutch legislation it is determined that the soil should not be polluted in such a way that the normal functioning of the soil is suppressed. The mineralization of organic matter is a major function of the soil and a partial impairment of this activity can have major effects on plant growth because plant nutrients become stored in a growing litter layer (Hahn, 1990).

Acetate is a readily degradable substrate for most organisms (Hobbie, 1973) and it is an important intermediate in the carbon cycle (Thompson and Nedwell, 1985). Only low concentrations of readily biodegradable compounds are present in soil which limit growth of the microbial community most of the time (Scow *et al.*, 1989). The use of high concentrations of readily degradable compounds in toxicity tests can give a large underestimation of the sensitivity of the microflora under natural conditions (Barnhart and Vestal, 1983; Van Beelen *et al.*, 1991). Soil is a very heterogeneous medium and many activities of the soil microflora show seasonal variations. In order to study the spatial and temporal differences of the sensitivity of the microflora in several soils for a single pollutant, soils were tested at several times with pentachlorophenol (PCP).

In order to find out whether the 1 μg/liter acetate mineralization test is suitable to study the effects of a number of pollutants and pesticides, a number of very different pollutants or pesticides were tested in one sandy soil.

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MATERIALS AND METHODS

Preparation of Toxicant Solutions

Pentachlorophenol (95% pure, Merck) was dissolved at 100 mg/ml 0.5 *N* NaOH; chloropyrifos is *O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)phosphothioate (99% pure, Riedel de Haën) 100 mg was dissolved in 1 ml dimethylsulfoxide (DMSO); $CdCl_2$ (> 99% pure, Merck), 100 mg Cd was dissolved in 1 ml double distilled water; $ZnCl_2$ (> 98% pure, Merck), 100 mg Zn was dissolved in 1 ml 0.1 *N* HCl; triphenyltinhydroxide (97% pure, Duphar) 117 mg was dissolved in 1 ml DMSO; paraquat is 1,1′-dimethyl-4,4′-bipyridylium dichloride (Riedel de Haën) 170 mg was dissolved in 1 ml double-distilled water; $K_2Cr_2O_7$ (99.5–100% pure, Merck), 290 mg $K_2Cr_2O_7$ was dissolved in 10 ml double-distilled water; orthoxylene is 1,2-dimethylbenzene (>98% pure, Fluka) and was added as a liquid diluted with DMSO; 3,4-dichloroaniline (98% pure, Merck) 100 mg was dissolved in 1 ml DMSO.

Preparation of the Microcosms

Fresh surface soil samples (depth 1–10 cm) were obtained from sandy soils in the vicinity of the institute in Bilthoven, The Netherlands. The Dune soil was located in a fir wood and the S soil was a sheep meadow. The soil samples were sieved and homogenized (ISO, 1991). Portions of 10 g soil and 10 ml filter-sterilized ground water were incubated in bottles with [^{14}C]acetate (1 μg/liter) and the produced $^{14}CO_2$ was measured as described previously (Van Beelen *et al.*, 1991). By incubating several bottles for different times in a shaker at 10°C, the half-life of the acetate mineralization was determined.

Determination of the Toxic Effects

Toxicants were added as a solution 2 hr prior to the addition of [^{14}C]acetate at increasing doses. The highest added concentration of the solvent DMSO was also added and compared with the control. The bottles with toxicant were incubated at 10°C for a fixed time, the $^{14}CO_2$ and [^{14}C]acetate were measured, and a dose–effect curve was obtained (Van Beelen *et al.*, 1991). The EC_{50} and EC_{10} are the toxicant doses that give a decrease of 50 or 10% of the final percentage of acetate mineralized after the incubation time. At longer incubation times the EC_{50} and EC_{10} increase because the unintoxicated part of the microflora continues to mineralize the acetate. The IC_{50} and IC_{10} are defined as the toxicant concentrations which inhibit 50 or 10% of the initial mineralization rate and are independent of the incubation time. At very short times the EC_{10} becomes equal to the IC_{10} but then only a very small percentage of carbon dioxide is formed. At longer incubation times the IC_{50} and IC_{10} can be calculated from the EC_{50}, the EC_{10}, the incubation time, and the half-life of acetate as described previously (Van Beelen *et al.*, 1991). Standard deviations were calculated from the duplicate measurements.

RESULTS AND DISCUSSION

An Example of a Dose–Effect Curve

Figure 1 reflects the effect of pentachlorophenol (PCP) on the mineralization of acetate in a sample taken from the sandy soil S on May 29, 1989. At the highest

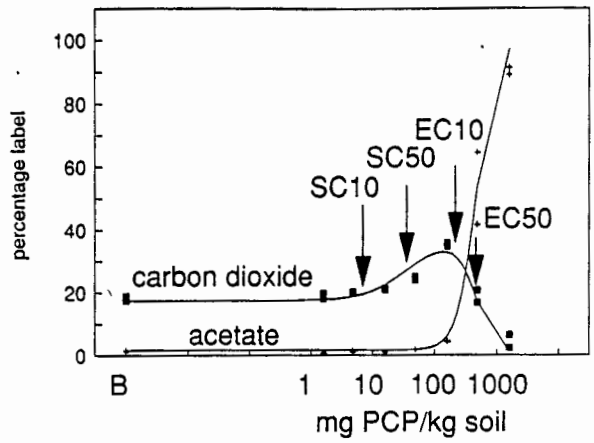

FIG. 1. The effect of pentachlorophenol on the mineralization of [^{14}C]acetate after 43 hr at 10°C.

concentration of PCP, 100% of the acetate is left and no carbon dioxide is formed. In the control measurement without added PCP, 1.5% of the label is present in the water phase and 18% is identified as $^{14}CO_2$ as is demonstrated at the concentration marked B on the *X* axis. At subinhibiting concentrations of PCP the percentage $^{14}CO_2$ increased significantly to 36% (Student's *t* test on duplicate data). The drawn lines are a model simulation of the data using the equations derived previously (Van Beelen *et al.*, 1993). The PCP concentrations which give 10 and 50% of the maximal increase of the percentage carbon dioxide are called SC_{10} and SC_{50}, respectively. SC stands for stimulatory concentration. This stimulating effect of low doses of a toxicant is called hormesis and is often observed (Stebbing, 1982). In this experiment SC_{10} = 7 mg PCP/kg and SC_{50} = 35 mg PCP/kg, which is much lower than the EC_{10} = 210 mg PCP/kg and EC_{50} = 460 mg PCP/kg (see Fig. 1).

The Influence of Incubation Time

The incubation time of 43 hr (1.8 days) of the experiment reflected in Fig. 1 is much longer than the half-life of 24 min (0.02 days) for the mineralization of acetate which was measured for this soil sample. Therefore, the uninhibited part of the microflora had a relatively long time to mineralize the acetate and the EC_{50} and EC_{10} concentrations are much higher compared with the incubation time independent IC_{50} and IC_{10} toxicant concentrations. Table 1 demonstrates the EC_{50} and EC_{10} values of 460 and 210 mg PCP/kg soil and the calculated IC_{50} and IC_{10} of 24 and 6 mg PCP/kg soil. These concentrations are of the same order of magnitude as the SC_{50} and SC_{10} of 35 and 7 mg/kg. This also exemplifies that toxic effects can occur below the EC_{10} concentration of 210 mg PCP/kg.

The Effect of Other Pollutants

Table 1 indicates that triphenyltinhydroxide, 3,4-dichloroaniline, and orthoxylene are less toxic for the acetate mineralizing community in soil Dune compared to PCP.

The herbicides diuron and propanil can be degraded to 3,4-dichloroaniline which is able to inhibit the nitrification at concentrations above 5 mg/kg (Thompson and

TABLE 1

THE TOXICITY OF PENTACHLOROPHENOL, TRIPHENYLTIN, 3,4-DICHLOROANILINE, AND ORTHOXYLENE ON THE MINERALIZATION OF ACETATE

Toxicant	Soil	Date	EC_{50}	EC_{10}	Incubation time	Half-life	IC_{50}	SD	IC_{10}	SD
PCP*	S	29-5-89	460	210	1.8	0.02	24	5	6	2
PCP	sub-S	29-5-89	60	41	50	2.5	27	38	15	31
PCP	sub-S	25-9-90	69	27	8	0.5	39	32	14	17
PCP*	Dune	19-6-89	1500	800	3	0.03	150	245	50	208
PCP	Dune	28-6-89	47	8	2	0.14	2	1	0.1	0.1
PCP	Dune	1-8-89	30	5	2	0.5	10	3	1.4	0.7
PCP	Dune	2-8-89	15	3	2	0.3	2.7	1	0.3	0.1
TPT	Dune	30-10-89	1700	640	2	0.2	400	40	96	17
DCA*	Dune	28-11-89	480	260	2	0.1	130	39	48	46
OXL	Dune	28-11-89	5500	3300	2	0.1	1700	1400	730	1200

Note. EC_{50}, EC_{10}, IC_{50}, and IC_{10} are in mg toxicant/kg dry soil. Incubation time and half-life are in days. The sub-S samples demonstrated a lag time of 6 days and the Dune sample from 19/6 demonstrated a lag time of 0.04 days. The experiments marked with an asterisk demonstrated a significant hormesis effect. PCP, pentachlorophenol; TPT, triphenyltinhydroxide; DCA, 3,4-dichloroaniline; OXL, orthoxylene.

Corke, 1968). The IC_{10} of 48 mg/kg (see Table 1) is higher than this reported NOEC on nitrification.

The following compounds did not indicate an effect on the acetate mineralization at the highest concentrations tested; 1110 mg Zn/kg, 1110 mg Cd/kg, 2900 mg $K_2Cr_2O_7$/kg, 1110 mg chloropyrifos/kg, and 1700 mg paraquat dichloride/kg. The insecticide chloropyrifos seems to have few side effects on different communities of bacteria since also the reductive dechlorination of chloroform in anaerobic sediments was not inhibited by chloropyrifos (van Beelen *et al.*, 1994).

The relatively low toxicity of zinc for the microflora in acid soils has been observed more often (van Beelen *et al.*, 1993; Bewley and Stotsky, 1983; Wilke, 1990). The Zn^{2+} ion forms $Zn(OH)^+$ and $ZnCO_3$ plus $Zn(OH)_2$ which precipitate above pH 7 (McComish and Ong, 1988). At neutral pH values zinc is more toxic to various microbial activities in soil but a high clay content and a high amount of organic material can reduce the toxicity of zinc (Kawabata *et al.*, 1983; Brookes *et al.*, 1986; Doelman and Haanstra, 1986; Doelman and Haanstra, 1989; Haanstra and Doelman, 1991).

Cadmium can precipitate as $CdCO_3$ above pH 8 (McComish and Ong, 1988). In contrast with zinc there are also reports showing a high toxicity of cadmium at low pH (Shirazi *et al.*, 1984).

Chromium (VI) can be more toxic than Cr^{3+} which is prevalent in soil (Slooff *et al.*, 1990). The highest tested $K_2Cr_2O_7$ concentration of 2900 mg/kg or 1000 mg Cr/kg is higher than the EC_{50} of the most sensitive soils tested in other reports (Doelman and Haanstra, 1989; Haanstra and Doelman, 1991; Shirazi *et al.*, 1984).

The herbicide paraquat indicated no effects on several soil animals at doses below 1000 mg/kg since it is sorbed strongly to soil material (Dirven *et al.*, 1990). Many bacteria were also able to withstand high concentrations (Carr *et al.*, 1986). Different processes in the same soil can have very different sensitivities for paraquat. The carbon dioxide evolution of glucose amended soil was sensitive for a field application rate, while the nitrification and C_2H_2 reduction were not (Smith and Mayfield, 1977).

Stimulatory and Toxic Effects of Pentachlorophenol

The toxic effects of PCP in several soils were tested and the calculated IC_{50} and IC_{10} concentrations are reflected in Table 1. The surface soils S and the Dune sample taken on June 19, 1989 were the least sensitive for the addition of PCP. In both instances a relatively long incubation time was used compared to the short half-life of acetate in these samples. All the surface soils demonstrated an enhanced $^{14}CO_2$ production upon the addition of low concentrations of PCP but only in surface soil S and the Dune sample of June 19, 1989 this stimulation was large enough to be significant (Student's *t* test). In the latter soil the SC_{50} = 0.5 and SC_{10} = 0.1 mg/kg. These are only order of magnitude estimations but clearly lower than the EC_{50} and EC_{10} values of this sample shown in Table 1. Since the hormesis effects occur at toxicant concentrations which are in the same order of magnitude as the IC_{10} values, the latter are used for the estimation of PCP concentrations without effect on the acetate mineralization.

Sorption of Pentachlorophenol

Table 2 reflects the properties of the used soil and subsoils. The Dune soil was sampled twice to assure that the high EC_{10} value of PCP on June 19, 1989 was not due to differences in soil properties compared to the other Dune samples.

The sorption of PCP to the organic material present in soil is dependent on the pH since PCP is a weak acid (pK_a = 4.75) and above pH 7 sorption of the phenolate anion is dominant (Lee *et al.*, 1990). When the percentage organic carbon and the pH of a soil are known, the sorption coefficient K_p can be calculated, using the formula described by Lee *et al.*, (1990). Table 3 reflects the calculated K_p values for the soils where the effect of PCP on the mineralization of [^{14}C]acetate was determined. The K_p value is concentration in soil divided by the concentration in water and is dependent on the total concentration used, since sorption can decrease at higher concentrations. In

TABLE 2

PROPERTIES OF THE SOIL SAMPLES

Soil code	S	sub-S	Dune	Dune
Date	29-05-89	29-05-89	19-06-89	30-10-89
Depth in cm	0–10	250–350	0–10	0–10
pH water	4.8	5.7–6.1	4.4	4.4
pH KCl	3.7	4.5–4.8	3.9	3.8
Moisture (%)	6.6	15.2	2.5	6.5
Carbon (%)	5.2	<0.1	0.6	0.5
Nitrogen (%)	0.37	0.002	<0.002	<0.002
Clay (%)	2.5	0.5	0.4	0.5
M-50 μm	180	150–380	125	125
CEC mmol/kg		5	11	16
Density kg/liter	2.1	2.2	2.4	2.3
AODC log/g	8.5	6.3		7.5

Note. M-50, the median particle diameter; CEC, cation exchange capacity; AODC, acridine orange direct counts (SD < 0.1). The methods for soil analysis were described previously (Van Beelen *et al.*, 1991). The percentages of moisture and the density are based on fresh weight and the other values are calculated on a dry weight basis.

TABLE 3

THE EFFECT OF PENTACHLOROPHENOL ON THE MINERALIZATION OF ACETATE IN SOILS WITH DIFFERENT pH AND ORGANIC CARBON CONTENT

	Date	EC_{10} mg/kg	IC_{10} mg/kg	pH	% Org. C	IC_{10} mg PCP/liter	IC_{10} mg phenolate/liter	K liter/kg
Sub-S[a]	9-1-89	6	3.8	6.3	0.2	0.04	1	2
Sub-S[a]	17-5-89	750	690.0	6.4	0.1	8	400	1
Dune[a]	2-5-90	47	11.0	4.5	0.5	0.1	0.07	62
Flevo[b]	4-10-89	41	12.0	8.2	0.6	0.00	4	2
Sub-Flevo[b]	4-10-89	292	187.0	8.3	1.1	0.01	40	4
Peel[b]	12-1-90	588	176.0	3.8	2.6	0.3	0.04	460
Sub-Peel[b]	12-1-90	113	51.0	4.5	0.2	1	0.8	25
S	29-5-89	210	6.0	4.8	5.2	0.01	0.01	470
Sub-S	29-5-89	41	15.0	5.7	0.1	0.4	4	2
Sub-S	25-9-90	27	14.0	5.8	0.1	0.4	5	2
Dune	19-6-89	800	50.0	4.4	0.6	0.4	0.2	80
Dune	28-6-89	8	0.1	4.4	0.6	0.00	0.00	80
Dune	1-8-89	5	1.4	4.4	0.6	0.01	0.01	80

[a] van Beelen *et al.*, 1991.
[b] van Beelen *et al.*, 1993.

contrast the K_f value of the Freundlich equation is determined for a range of concentrations. The log transformed Freundlich equation is: $\log C_{soil} = \log K_f + (1/n) * \log C_{water}$. The measured K_f values of the Flevo, sub-Flevo, Peel, and sub-Peel soils were 2.5, 0.8, 23, and 4.4 liters/kg respectively (Van Beelen *et al.*, 1993). For the Peel soil the calculated K_p was 20 times higher than the measured K_f. For this soil $1/n = 1$ and therefore the K_p should be equal to the K_f. Therefore, the difference between the K_p and the K_f must be attributed to different properties of the organic material since the sorption curve of Lee *et al.* (1990) gives only an order of magnitude estimation of the K_p value.

Table 3 displays the IC_{10} concentrations of PCP on the mineralization of acetate in several soils. When the variation between the IC_{10} values of the various soils are caused by differences in the bioavailability of PCP, a relatively constant value of IC_{10} would result when these concentrations were expressed in mg PCP/liter. This was previously demonstrated for LC_{50} concentrations of PCP for a single species of earthworms (Van Gestel and Ma, 1990). Table 3 reflects that the IC_{10} values of PCP expressed as milligrams per liter are not constant but vary many orders of magnitude between different soils. Hence, the sensitivity of the acetate mineralizing communities differ by orders of magnitude between various soils.

The Effect of PCP in Several Soils

A statistical method can be used to determine the PCP concentration above which 5% of the most sensitive acetate-mineralizing communities in all soils are influenced. This PCP concentration will be called hazardous concentration 5% (HC_5). For this method the log-transformed IC_{10} values of the acetate-mineralizing communities of the 13 soil samples given in Table 3 are regarded as independent samples from a

logistical distribution. A statistical method to estimate the 5% most sensitive species from a limited number of single species toxicity tests was used (Van Straalen and Denneman, 1989; Aldenberg and Slob, 1991). First, all the IC_{10} values in mg PCP/kg are log-transformed giving an average value of 1.2 and, therefore, the geometrical mean $IC_{10} = 10^{1.2} = 16$ mg PCP/kg. The best estimate of the HC_5 is 0.3 mg/kg, but to be on the safe side the 95% confidence level of this HC_5 is calculated to be 25 μg/kg. Both HC_5 values were calculated from the IC_{10} concentrations from Table 3 using the ETX program of Aldenberg and Slob (1993). The direct effect of a PCP concentration above the IC_{10} in 5% of the soils would be a 10% larger half-life of acetate in these soils and the inhibition of a small but significant part of the microflora in these soils. A larger half-life of acetate would be of little consequence since there is a large variation of this half-life in different soils and subsoils. The inhibition of a significant part of the microflora, however, might have serious consequences. The degradation of a multitude of organic compounds by the soil microflora is vital for the functioning of the ecosystem. Many degradation processes are only performed by specialized microorganisms and each group has its own sensitivity for a pollutant. Hence, if a significant part of the microflora is intoxicated by a pollutant there is a real chance that a specialized group is totally inhibited and that the functioning of the soil is disturbed.

CONCLUSIONS

The 1 μg/liter acetate mineralization test is suitable to test the toxic effects of many pollutants. The sensitivity is very variable among soils and, therefore, it is advisable to test more soils in order to assess the effect of a pollutant.

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