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Microbial Activity of Soil Contaminated with Chlorinated Phenol Derivatives

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ABSTRACT. Chlorinated phenol derivatives were found to display an effect on soil microorganisms and their physiological and biochemical activity — nitrification, ammonization of proteins, total metabolic activity detected by the production of CO_2, and total cellulolytic activity. The effect of chlorinated phenol derivatives increases with the degree of chlorination.

Chlorinated organic compounds penetrate into the biosphere; some of them are used as pesticides in agriculture, *e.g.* the herbicides 2-chlorophenol, 2,4-dichlorophenol and 2,4,5-trichlorophenol or the fungicides pentachlorophenol and 4-chlorophenol. Other significant sources are contaminated waters and the pulp and paper industry which produces a large amount of organically bound chlorine (*e.g.* chlorophenols, chloroguaiacols, chloropyrocatechols and chlorosyringols (Knuutinen *et al.* 1983; Lindström and Schubert 1984). Sludges from the effluent treatment plants also contain chlorinated organic compounds. When used as fertilizers, they attack soil microorganisms and affect the soil-forming process. Large amounts of organic chlorinated compounds arise in combustion. Their toxic effect increases with the number of chlorinated atoms in the molecule (Voss *et al.* 1980) and decreases in the presence of methoxy groups on the benzene ring (Paasivirta *et al.* 1981; Čerňáková 1994).

MATERIALS AND METHODS

Compounds tested. Phenol (PHE), pentachlorophenol (PCP), pyrocatechol (PCA) and tetrachloropyrocatechol (TCC).

Intensity of microbial processes in soil was determined *via* CO_2 production according to Bernát and Seifert (1995). The experiments in a 1 L jar with 50 g of soil were carried out with: (*1*) sand, (*2*) soil, (*3*) soil + $(NH_4)_2SO_4$, (*4*) soil + urea, (*5*) soil + tested samples, (*6*) soil + $(NH_4)_2SO_4$ + tested samples, (*7*) soil + urea + tested samples.

The soil was used without and with nourishment. The source of nitrogen was present at 60 mg per kg soil. Soil humidity was 60 % of the full water capacity. In each jar, a crucible with 30 mL of 0.1 mol/L KOH was placed on the soil, in the control with sand 15 mL of 0.1 mol/L KOH. The vessels were closed and the cultivation carried out for 1, 2, 12 and 20 d at 28 °C. The CO_2 production was estimated by titration. In the control with sand the titration determined the atmospheric CO_2 contained in jars.

The production (P) of CO_2 (mg/kg soil per time) was calculated according to the following formula:

$$P = (x - y) \times 2.2 \times 20$$

$$x = a - b \qquad y = a - c$$

x = amount of 0.1 mol/L KOH bound with CO_2 from the sample
y = amount of 0.1 mol/L KOH bound from the control (sterile sand)
a = 0.1 mol/L KOH in mL in a control dish before the experiment
b = consumption of 0.1 mol/L HCl in sample titration
c = consumption of 0.1 mol/L HCl in control titration
2.2 = mg CO_2 required for the neutralization of 1 mL of 0.1 mol/L KOH.

The soil samples tested were collected at a plot of the cooperative farm at Bratislava-Rača in July 1996. The soil characteristics are given in Table I.

Effect of chlorinated phenol derivatives on ammonization of proteins in a liquid cultivation medium was estimated (*a*) in the medium containing the nutrient broth with 2 % peptone, (*b*) in a mineral medium with 2 % urea.

The medium was inoculated with the soil in an amount of 0.1 g per 10 mL medium. The incubation lasted 7 d at 28 °C. The amount of ammonia was estimated by titration according to Kopčanová *et al.* (1979). The controls were (*a*) without inoculation and (*b*) without the substance tested. The medium was a nutrient broth with 2 % peptone. The content of produced ammonia was given in mg per 100 mL and calculated according to the formula:

$$\text{mg } NH_4^+\text{-N} = (T \cdot N \cdot A_m \times 100)/v$$

where $T = a - b$

a = consumption of 50 mmol/L H_2SO_4 in the sample

b = consumption of 50 mmol/L H_2SO_4 in the control

N = normality of H_2SO_4

A_m = atomic mass N = 14.008

v = volume of the titrated nutrient medium.

The percentage of mineralized nitrogen was calculated. The nitrogen content in the peptone was approximately 16 %, *i.e.* 320 mg organic nitrogen in 100 mL medium. The percentage of mineralized nitrogen was converted to this basis. During the ammonization of urea, the amount of mineralized nitrogen was calculated from the per cent the nitrogen content in urea and from the nitrogen concentration in the medium. Thus for 100 mL medium containing 2 g urea with 46.6 % nitrogen (= 466 mg N/g) contained the total of 932 mg of organically bound nitrogen.

Nitrate content in soil samples. The 1 L jars with 50 g soil contained as controls (*1*) soil, (*2*) soil + $(NH_4)_2SO_4$, (*3*) soil + urea. Experimental variants contained (*4*) soil + tested samples, (*5*) soil + $(NH_4)_2SO_4$ + tested samples, (*6*) soil + $CO(NH_2)_2$ + tested samples.

Table I. Soil characteristics

Soil type	brown loamy soil	
$CaCO_3$ content, %	6.0	
Soil reaction, pH/KCl	neutral (pH 6.9)	
Need for limiting; CaO, g/hm^2	0	
Content of accessible nutrients,		
mg per kg soil, ppm P	160	
K	263	
Grain size distribution, μm,	10	95.2
and content frequency, %	1	16.2
	1–10	19.0
	10–50	49.1
	50–250	14.3
	250–2000	1.4
Organic carbon, %	1.24	
Total nitrogen, %	0.115	
Humus, %	2.14	
Specific conductivity, μS/cm	180	

The variants with oxygen were enriched with 3.5 mg N (approximately 300 kg N/hm^2). The soil humidity was adjusted to 60 % FWC and the cultivation lasted 14 d at 28 °C.

After the cultivation, soil extract was obtained by shaking with 1 % K_2SO_4 in a horizontal separating funnel for 1 h. The suspension was left to settle down and filtrated through a folded filter. The nitrate content in the filtrate was determined spectrophotometrically.

Total cellulase activity (C_x) was tested on hydroxyethylcellulose cross-linked by 2-chloromethyloxirane and inoculated with the soil. One-week cultivation was done at 28 °C in the presence of chlorinated phenol derivatives (Čerňáková *et al.* 1990). The reaction caused liquefying of the hydroxyethylcellulose.

All experiments were done in three parallel runs.

The results were evaluated statistically by the Lord test (Čakrt *et al.* 1989).

RESULTS AND DISCUSSION

Phenol, pentachlorophenol, pyrocatechol and tetrachloropyrocatechol (0.08–0.32 mmol per kg of soil) did not show any remarkable influence on the total metabolic activity (CO_2 production) of the soil microorganisms (Table II) but had negative effects on the ammonization of proteins in soil and on the total cellulase activity and nitrification intensity reflected in a lowered amount of mineralized nitrogen (Table III). The chlorinated phenol derivatives also suppressed the activity of the nitrification bac-

teria which are very sensitive to these substances (Table IV). The highest inhibition effect (75–100 % inhibition) was found with the total cellulase activity (C_x). Pentachlorophenol and tetrachlopyrocatechol were effective even at 0.16 mmol/L (Table V).

Table II. Effect of chlorinated phenol derivatives (CPs) on the intensity of microbial processes expressed by CO_2 production (mmol per kg soil) after 1, 2, 12 and 20 d[a]

Chlorinated phenol derivatives[b]	A		B		C	
	$\bar{x}$ ± SD	I, %	$\bar{x}$ ± SD	I, %	$\bar{x}$ ± SD	I, %
1 day						
Phenol (PHE)	90 ± 1.3*	–	116 ± 2.6*	–	124 ± 1.3*	–
	90 ± 1.3*	–	115 ± 3.9*	1	123 ± 2.6*	1
Pentachloro-phenol (PCP)	89 ± 2.6*	1	114 ± 2.6*	2	120 ± 3.9**	3
	86 ± 2.6*	4	106 ± 5.2**	11	118 ± 5.2**	5
Pyrocatechol (PCA)	90 ± 1.3*	–	116 ± 1.3*	–	124 ± 1.3*	–
	90 ± 2.6*	–	116 ± 2.6*	–	123 ± 1.3*	1
Tetrachloro-pyrocatechol (TCC)	89 ± 2.6*	1	116 ± 1.3*	–	122 ± 2.6*	2
	87 ± 3.9*	3	108 ± 3.9**	7	120 ± 3.9**	3
Control	90 ± 2.6		116 ± 1.3		124 ± 2.6	
2 days						
PHE	118 ± 0.6*	–	176 ± 1.3*	–	156 ± 2.6*	–
	117 ± 1.3*	1	174 ± 1.3**	1	154 ± 2.6*	1
PCP	116 ± 0.6***	2	171 ± 1.1***	3	152 ± 1.3*	3
	116 ± 0.5***	2	168 ± 1.1***	5	144 ± 2.6*	8
PCA	118 ± 0.9*	–	176 ± 1.3*	–	156 ± 2.6*	–
	118 ± 1.3*	–	177 ± 2.6*	+	155 ± 1.3*	1
TCC	118 ± 1.3*	–	174 ± 2.6*	1	154 ± 2.6*	1
	116 ± 1.3**	2	170 ± 3.9**	3	150 ± 2.6**	4
Control	118 ± 1.3		176 ± 1.3		156 ± 2.6	
12 days						
PHE	428 ± 1.3*	–	410 ± 1.3*	–	428 ± 1.3*	–
	427 ± 1.3*	0.2	406 ± 1.3***	1.0	420 ± 1.3***	2.0
PCP	418 ± 3.9***	2.0	400 ± 2.6***	2.0	420 ± 2.6***	2.0
	414 ± 2.6***	3.0	391 ± 3.9***	5.0	408 ± 3.9***	5.0
PCA	428 ± 1.3*	–	410 ± 1.3*	–	428 ± 1.3*	0.5
	428 ± 1.3*	–	409 ± 1.3*	0.2	426 ± 1.3**	1.0
TCC	422 ± 6.5*	1.0	408 ± 2.6*	0.5	424 ± 2.6**	1.0
	418 ± 7.8**	2.0	398 ± 3.9***	3.0	418 ± 5.2**	2.0
Control	428 ± 3.9		410 ± 3.9		428 ± 3.9	

continued

	20 days					
PHE	514 ± 2.6*	0.4	464 ± 1.3*	–	484 ± 1.3*	–
	512 ± 1.3***	1.0	458 ± 1.3***	1.0	482 ± 2.6	0.4
PCP	492 ± 2.6***	5.0	454 ± 2.6***	2.0	478 ± 2.6**	1.0
	476 ± 2.6***	8.0	438 ± 3.9***	6.0	474 ± 3.9***	2.0
PCA	516 ± 3.9*	–	464 ± 2.6*	–	484 ± 1.3*	–
	515 ± 2.6*	0.2	462 ± 2.6*	0.4	483 ± 1.3*	0.2
TCC	515 ± 1.3*	0.2	458 ± 3.9**	1.0	480 ± 2.6**	1.0
	498 ± 2.6***	3.0	443 ± 5.2***	5.0	471 ± 3.9***	3.0
Control	516 ± 2.6		464 ± 2.6		484 ± 2.6	

[a]A – soil without nutrients, B – soil with $(NH_4)_2SO_4$ as a nitrogen source, C – soil with urea as a nitrogen source.
+ = stimulation, – = without inhibition; I = inhibition, %.
* – not significant, ** – significant difference, *** – very significant difference.
[b]*First lines*: 80 μmol/kg soil, *second lines*: 320 μmol/kg soil.

Table III. Effect of chlorinated phenol derivatives on ammonization of proteins (% of mineralized nitrogen) in the soil after 7 d[a]

Chlorinated phenol derivatives	mmol/L[b]	From peptone		From urea	
		$\bar{x}$ ± SD	I, %	$\bar{x}$ ± SD	I, %
Phenol	0.08	90 ± 6.5*	–	82 ± 5.2*	6
	0.16	86 ± 5.2*	4	80 ± 3.9**	8
	0.32	85 ± 5.2*	6	76 ± 2.6***	13
Pentachlorophenol	0.08	80 ± 6.5**	11	77 ± 2.6***	11
	0.16	78 ± 9.1**	13	65 ± 1.3***	25
	0.32	72 ± 3.9***	20	57 ± 1.3***	34
Pyrocatechol	0.08	90 ± 7.8*	–	85 ± 3.9*	2
	0.16	87 ± 9.1*	3	80 ± 5.2**	8
	0.32	86 ± 6.5*	4	70 ± 2.6***	20
Tetrachloro-pyrocatechol	0.08	84 ± 3.9***	7	80 ± 2.6***	8
	0.16	80 ± 2.6***	11	75 ± 1.3***	14
	0.32	76 ± 5.2***	16	68 ± 2.6***	22
Control	0	90 ± 0.6		87 ± 1.3	

[a]– = without inhibition; I = inhibition, %.
* – not significant, ** – significant difference, *** – very significant difference.
[b]Of nutrient solution.

Phenols were found to be more toxic for soil microorganisms than pyrocatechols. The toxicity of chlorinated phenol derivatives is influenced by the degree of their chlorination, *i.e.* the number of chlorine substituents on the benzene ring. The toxicity of the tested substances was found to increase in the sequence pyrocatechol < phenol < tetrachloropyrocatechol < pentachlorophenol.

Table IV. Effect of chlorinated phenol derivatives on nitrification intensity (nitrates after cultivation, mg per kg soil) in the soil after 14 d[a]

Chlorinated phenol derivatives	mmol/kg soil	Soil without added nitrogen source		Soil with $(NH_4)_2SO_4$[b]		Soil with urea[b]	
		$\bar{x}$ ± SD	I, %	$\bar{x}$ ± SD	I, %	$\bar{x}$ ± SD	I, %
Phenol	0.08	64 ± 1.3***	43	72 ± 6.5***	61	86 ± 1.3***	57
	0.16	62 ± 1.3***	45	71 ± 5.2***	61	85 ± 1.3***	58
	0.32	61 ± 2.6***	46	68 ± 2.6***	63	82 ± 2.6***	59
Pentachloro-phenol	0.08	60 ± 1.3***	46	67 ± 2.6***	63	81 ± 3.9***	60
	0.16	58 ± 2.6***	48	65 ± 3.9***	64	80 ± 2.6***	60
	0.32	55 ± 3.9***	51	63 ± 2.6***	66	75 ± 5.2***	63
Pyrocatechol	0.08	64 ± 3.9***	43	76 ± 6.5***	58	87 ± 1.3***	57
	0.16	64 ± 5.2***	43	73 ± 3.9***	60	86 ± 1.3***	57
	0.32	55 ± 3.9***	51	63 ± 2.6***	66	75 ± 5.2***	63
Tetrachloro-pyrocatechol	0.08	62 ± 6.5***	45	71 ± 6.5***	61	86 ± 3.9***	57
	0.16	61 ± 5.2***	46	67 ± 3.9***	63	83 ± 2.6***	59
	0.32	53 ± 6.5***	53	66 ± 2.6***	64	81 ± 1.3***	60
Control	0	112 ± 2.6		183 ± 3.9		201 ± 3.9	

[a]Nitrates before cultivation represented 32 mg per kg soil.
[b]As a nitrogen source.

Table V. Effect of chlorinated phenol derivatives on the cellulolytic activity C_x in the soil after 7 d

Chlorinated phenol derivatives	mmol per L of nutrient solution	C_x[a]
None (control)	0	3
Phenol	0.08	1
	0.16	1
	0.32	0
Pentachlorophenol	0.08	0
	0.16	0
	0.32	0
Pyrocatechol	0.08	2
	0.16	1
	0.32	1
Tetrachloropyrocatechol	0.08	1
	0.16	0
	0.32	0

[a]0 — negative result, 1 — very weak liquefying of gel, 2 — partial liquefying of gel, 3 — total liquefying of gel.

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