BIODEGRADATION IN 4-CHLOROPHENOL CONTAMINATED SOIL

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INTRODUCTION

A time and cost effective site characterization is a very important step toward the efficient management of contaminated land and its sustainable remediation. During the preparation of soil bioremediation, the microcosms testing of the biodegradation of the pollutant can help to select and design the best possible remedial technology, as well as the reliable methods for monitoring and assessing soil microflora and its activity. Halogenated hydrocarbons are very important group of organic soil contaminants because of their widespread utilization such as wood conservation or pest control. Their typical feature is that they are persistent, are able to sorb strongly to the solid soil matrix and are toxic to most living organisms. Since they are not easily biodegradable they persist in the soil for a long time therefore they give rise to serious environmental risk.

THE AIM OF THIS STUDY

a) To evaluate several biological soil testing methods for characterizing the biodegradation processes in 4-chlorophenol (4-CP) contaminated soil

b) To test the usefulness of a special chemical extraction method (cyclodextrin-extraction technique) for simulating the “biological extraction”, and getting by this the bioavailable fraction of the pollutant

c) To compare the applied chemical and biological test methods with each other; to find the most appropriate analytical test-battery for measuring biodegradation potential and to follow its possible enhancement in contaminated soil.

EXPERIMENTAL SETUP

Small-scale 10-week-long laboratory experiments were performed to study the bioavailability and biodegradation of 4-CP and to evaluate the parallel applied biological and chemical methodologies.

10–10 kg, three-phase garden soils were artificially contaminated in three different concentrations 10, 100 and 1000 mg/kg and placed in covered 15 dm^3 volumetric, static reactors. Microcosms were incubated under dark, aerobic conditions at 25±5 ^oC. Monitoring of the biodegradation started after two days adaptation period following spiking the soil. 10 kg uncontaminated garden soil was used as a control and handled the same way as the contaminated soils. The soils were amended with inorganic nutrients ($(NH_4)_2SO_4$, KNO_3, KH_2PO_4) every second week to reach the final C:N:P ratio of 100:10:1. Soils were stirred up before every sampling.

Six samples were taken from each microcosm between day 3 and week 10. Biodegradation and bioavailability of the contaminant and the activity of the indigenous soil microorganisms were characterized by chemical and biological methods at the 3rd day and after the 1, 3, 5, 8 and 10 weeks.

METHODS

Chemical methods

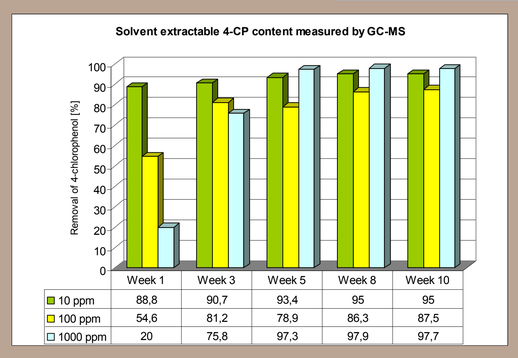
- Solvent Extractable 4-CP was measured by gas chromatography – mass spectrometry analysis (GC MS) after dichloromethane extraction
- As a chemical model for the estimation of contaminant bioavailability and biodegradability aqueous cyclodextrin solutions (10%) were applied for the ultrasonic extraction of the contaminant from soil. The cyclodextrin extracts were transferred into methanol after solid-phase extraction and analysed by
  1) Gas chromatography using flame ionisation detector (RAMEB-GC; HPBCD-GC)
  2) UV-VIS spectrophotometry (RAMEB-SP; HPBCD-SP)

Biological methods

- Contaminant degrading cell concentration (CP-CFU)
- Dehydrogenase enzyme activity (DEH)
- Soil respiration in closed bottle (RES CB)
- Glucose induced soil respiration in closed bottle (SIR)
- Soil respiration in a dynamic soil reactor (RES AER)
- Substrate utilisation of the microbial community characterised by the Biolog® System.

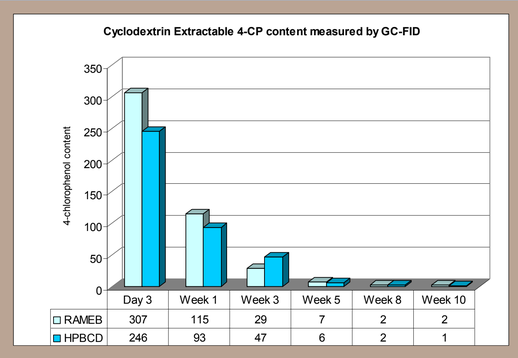
RESULTS

Evaluation of the results of the chemical analytical methods



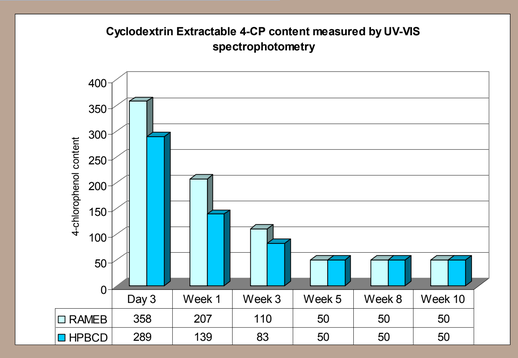
	Week 1	Week 3	Week 5	Week 8	Week 10
10 ppm	88,8	90,7	93,4	95	95
100 ppm	54,6	81,2	78,9	86,3	87,5
1000 ppm	20	75,8	97,3	97,9	97,7

Figure 1: Solvent extractable 4-CP content measured by gas chromatography-mass spectrometry analysis (GC MS)



	Day 3	Week 1	Week 3	Week 5	Week 8	Week 10
RAMEB	307	115	29	7	2	2
HPBCD	246	93	47	6	2	1

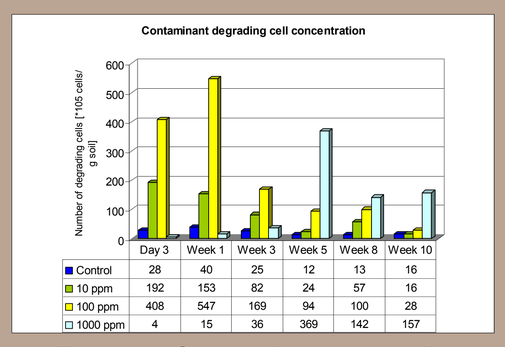
Figure 2: Cyclodextrin Extractable 4-CP content measured by gas chromatography using flame ionisation detector



	Day 3	Week 1	Week 3	Week 5	Week 8	Week 10
RAMEB	358	207	110	50	50	50
HPBCD	289	139	83	50	50	50

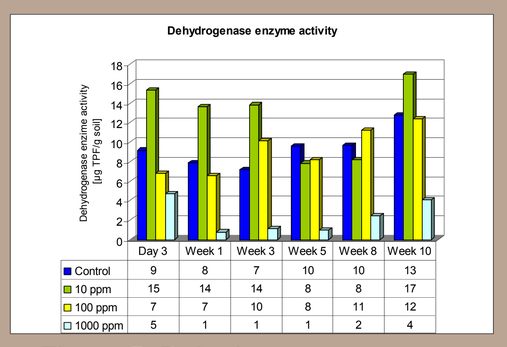
Figure 3: Cyclodextrin Extractable 4-CP content measured by UV-VIS spectrophotometry

Evaluation of the results of the biological methods



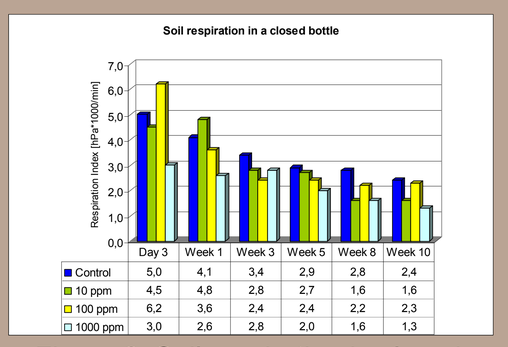
	Day 3	Week 1	Week 3	Week 5	Week 8	Week 10
Control	28	40	25	12	13	16
10 ppm	192	153	82	24	57	16
100 ppm	408	547	169	94	100	28
1000 ppm	4	15	36	369	142	157

Figure 4: Contaminant degrading cell concentration (CP-CFU)



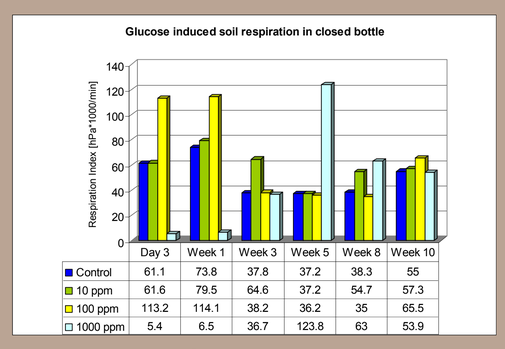
	Day 3	Week 1	Week 3	Week 5	Week 8	Week 10
Control	9	8	7	10	10	13
10 ppm	15	14	14	8	8	17
100 ppm	7	7	10	8	11	12
1000 ppm	5	1	1	1	2	4

Figure 5: Dehydrogenase enzyme activity (DEH)



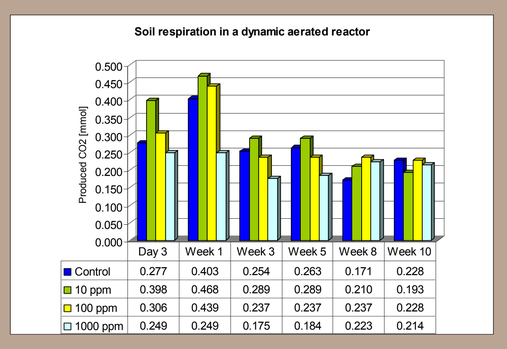
	Day 3	Week 1	Week 3	Week 5	Week 8	Week 10
Control	5,0	4,1	3,4	2,9	2,8	2,4
10 ppm	4,5	4,8	2,8	2,7	1,6	1,6
100 ppm	6,2	3,6	2,4	2,4	2,2	2,3
1000 ppm	3,0	2,6	2,8	2,0	1,6	1,3

Figure 6: Soil respiration in closed bottle (RES CB)



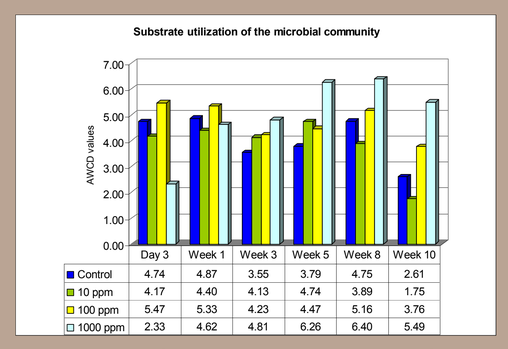
	Day 3	Week 1	Week 3	Week 5	Week 8	Week 10
Control	61.1	73.8	37.8	37.2	38.3	55
10 ppm	61.6	79.5	64.6	37.2	54.7	57.3
100 ppm	113.2	114.1	38.2	36.2	35	65.5
1000 ppm	5.4	6.5	36.7	123.8	63	53.9

Figure 7: Glucose induced soil respiration in closed bottle (SIR)



	Day 3	Week 1	Week 3	Week 5	Week 8	Week 10
Control	0.277	0.403	0.254	0.263	0.171	0.228
10 ppm	0.398	0.468	0.289	0.289	0.210	0.193
100 ppm	0.306	0.439	0.237	0.237	0.237	0.228
1000 ppm	0.249	0.249	0.175	0.184	0.223	0.214

Figure 8: Soil respiration in a dynamic soil reactor (RES AER)



	Day 3	Week 1	Week 3	Week 5	Week 8	Week 10
Control	4.74	4.87	3.55	3.79	4.75	2.61
10 ppm	4.17	4.40	4.13	4.74	3.89	1.75
100 ppm	5.47	5.33	4.23	4.47	5.16	3.76
1000 ppm	2.33	4.62	4.81	6.26	6.40	5.49

Figure 9: Substrate utilization of the microbial community

Correlation analysis

1000 ppm 4-CP contaminated soil	*BIOLOGICAL CHARACTERISATIONS*						*CHEMICAL CHARACTERISATIONS*				
	CP CFU	*DEH*	*RES CB*	*RES AER*	*SIR*	*AWCD*	*GC MS*	*RAMEB SP*	*HPBCD SP*	*RAMEB GC*	*HPBCD GC*
CP CFU	1.0000										
	p= ---										
DEH	-.2919	1.0000									
	p=.575	p= ---									
RES CB	-.5909	-.0230	1.0000								
	p=.217	p=.966	p= ---								
RES AER	-.5412	.5223	.1742	1.0000							
	p=.267	p=.288	p=.741	p= ---							
SIR	***.9769***	-.3678	-.5746	-.6709	1.0000						
	p=.001	p=.473	p=.233	p=.145	p= ---						
AWCD	.7067	-.5880	-.7673	-.5284	.7665	1.0000					
	p=.116	p=.220	p=.075	p=.281	p=.075	p= ---					
GC MS	-.7057	.3449	.7915	.7172	-.7956	-.8944	1.0000				
	p=.117	p=.503	p=.061	p=.109	p=.058	p=.016	p= ---				
RAMEB SP	-.6694	.5078	.7870	.6670	-.7478	***-.9570***	***.9690***	1.0000			
	p=.146	p=.304	p=.063	p=.148	p=.087	***p=.003***	***p=.001***	p= ---			
HPBCD SP	-.6139	.5973	.7418	.6458	-.6913	***-.9584***	.9270	***.9903***	1.0000		
	p=.195	p=.211	p=.091	p=.166	p=.128	***p=.003***	p=.008	***p=.000***	p= ---		
RAMEB GC	-.5808	.6097	.7149	.6664	-.6670	-.9451	.9229	***.9862***	***.9984***	1.0000	
	p=.227	p=.199	p=.110	p=.148	p=.148	p=.004	p=.009	***p=.000***	***p=.000***	p= ---	
HPBCD GC	-.6215	.5753	.7698	.6137	-.6916	***-.9642***	.9260	***.9905***	***.9988***	***.9950***	1.0000
	p=.188	p=.232	p=.073	p=.195	p=.128	***p=.002***	p=.008	***p=.000***	***p=.000***	***p=.000***	p= ---
Cum. Corr. Coeff.	***8.8729***	***5.8887***	***8.4295***	***7.4747***	***9.5558***	***10.6800***	***10.5226***	***10.7590***	***10.5522***	***10.4188***	***10.5650***

Figure 10: Correlation analysis of chemical and biological methods

CONCLUSION

The applied methodology resulted remarkable outcome. There wasn’t a noticeable selection pressure in soil contaminated with 10 ppm 4-CP, therefore the process of adaptation didn’t rise significantly the biochemical or the genetic potential of the microflora. At the same time, the activity of microbes increased in the 100 ppm contaminated soil as a result of the additional energy source. At the beginning of the experiment this concentration caused a moderate toxic effect but later there was a serious metagenomic activity. In the 1000 ppm contaminated soil the mechanism of multilevel adaptation has been followed by biological testing methods. The ability of tolerance and the utilization of the contaminant could evolve during 5 weeks adaptation period. Until this time the soil microflora went through complex genetic changes and adaptation processes and as a result, the 1000 ppm 4-CP contaminated soil microcosm became the most intensively degrading one compared to the control and less contaminated ones.

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ACKNOWLEDGEMENTS

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