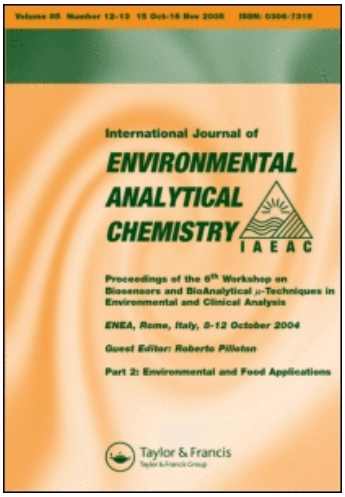

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Influence of Different Redox Conditions on the Biodegradation of the Pesticide Metabolites Phenol and Chlorophenols

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INFLUENCE OF DIFFERENT REDOX CONDITIONS ON THE BIODEGRADATION OF THE PESTICIDE METABOLITES PHENOL AND CHLOROPHENOLS

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The fate and behaviour of phenol and monochlorophenols during bankfiltration and underground passage with variable redox conditions were investigated. A model ecosystem was used consisting in laboratory filter columns filled with natural underground material and operated with natural aerobic and anaerobic groundwater to create different redox situations.

The test substances (phenol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol) were added continuously to the infiltrating water and their concentration in the filter effluents determined. Beside the redox conditions other factors known to affect microbial degradation processes like the substrate concentration and the underground material were varied stepwise.

Phenol was degraded under both, aerobic and anaerobic conditions. The presence of oxygen is more favourable to degradation; no lag phase was observed under aerobic conditions. In a sulfate reducing environment, phenol could only be degraded after microbial adaptation. The length of the lag phase was strongly influenced by the substrate concentration and the undergroundmaterial. Prior contact with phenol resulted in a shorter lag phase.

Monochlorophenols behaved almost persistent in the model system. Degradation could only be observed in a test filter that provided a more active microbial population due to prior adaptation to phenol and a more favourable underground material.

KEY WORDS: Underground passage, redox conditions, biodegradation, phenol, chlorophenols.

INTRODUCTION

During bankfiltration and underground passage, microbial degradation is one of the most important processes to reduce organic contaminants from the infiltrating surface water. Rate and extent of these reactions are affected by the physico-chemical properties of the organic compound and the environmental conditions. Especially the redox range, i.e. the presence or absence of suitable electron acceptors, strongly influences fate and behaviour of organic substances. Apart from aerobic reactions which mainly occur in the upper infiltration zone, anaerobic processes take place during underground passage using nitrate, manganese, iron and sulfate as terminal electron acceptors. The succession of biologically mediated redox reactions leads to totally different chemical and microbiological conditions, which may limit or promote microbial degradation processes.

Phenol and chlorophenols are widespread contaminants in both surface and groundwater. Their occurrence does not only result from the release of the substances itself but they are also found as pesticide metabolites, for example as degradation products of phenoxy acetic acids. Biodegradability of these compounds has already been investigated under different environmental conditions. Phenol was degraded by methanogenic, sulfate- and nitratereducing cultures[1–5]. Under natural conditions, phenol degradation has been observed in surface water and in methanogenic and sulfate reducing sediments[6–8]. Monochlorophenols have been found to be more persistent, nevertheless degradation could be observed after a lag phase under different redox conditions both in natural environments and in culture[9–13].

The scope of the investigations presented here was to investigate the fate of phenoxyacetic acids and its metabolites phenol and chlorophenols during bankfiltration and underground passage with variable redox conditions.

The results concerning the phenoxyacetic acids have already been published in detail[14]. Briefly: they were degraded aerobically after a lag phase but they were persistent in a sulfate reducing environment.

To assess the fate of possible metabolites and further the influence of the molecular structure on biodegradability, investigations then focused on phenol and monochlorophenols.

EXPERIMENTAL

Investigations were done by using a model ecosystem consisting of laboratory filter columns filled with underground material. Figure 1 shows a picture of the pilot plant.

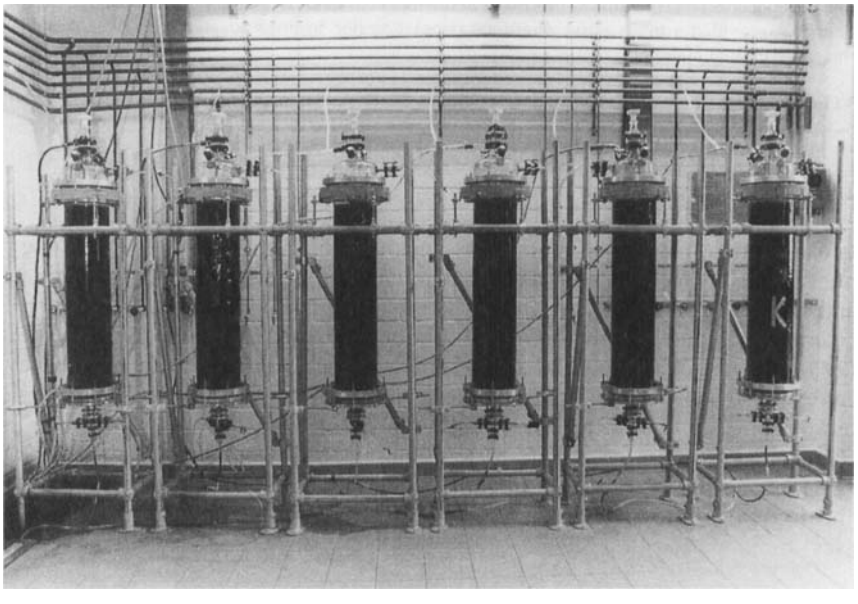

Figure 1 Photo of the pilot plant.

Different redox conditions could be created by operating the filters with natural aerobic and anaerobic groundwater. The anaerobic groundwater comes from a well which is situated in a sulfate reducing site of bankfiltration in a water catchment area. Typical for its quality is a high amount of iron, manganese, ammonium and very low sulfate concentrations. Nitrate and nitrite are normally beyond the detection limit.

With a Rhine sand that is used for slow sand filters and gravel from the river Ruhr with a fraction < 2 mm of 10 % as filter-materials, two quite different underground situations were included.

To create a stable chemical, biological and hydraulical situation, the filters were operated with anaerobic resp. aerobic groundwater for several weeks before the experiments started. Phenol, 2-chlorophenol, 3-chlorophenol or 4-chlorophenol were then added continuously for up to four weeks to the infiltrating groundwater. Every test-substance was spiked individually. Substrate concentration ranged from 100 to 500 µg/l in different experiments. Normally filters were used which before had been operated with groundwater only. In some cases, filters to which phenol had already been dosed were utilised.

Table 1 shows the different experimental conditions.

During the experiments, samples were taken from the filter influents and effluents and analysed for the tested compounds normally every day. Redox environment was controlled by measuring nitrate, sulfate, ammonium, manganese and iron every two days.

Determination of phenol and 4-chlorophenol

Phenol and 4-chlorophenol were analysed according to German Standard Methods[15]. This method is based on the coupling of phenolic substances with 4-aminoantipyrine. The coloured product is extracted and its concentration determined photometrically. This rather old fashioned method had its advantages because it is fast, easy to handle and very precise in the chosen concentration range. With this method, all substances which react

Table 1 Experimental conditions.

Test-substance	*Concentration (µg/l)*	*Filter-material*		*Redox-conditions*		*Time (weeks)*	*Adapted*
		Sand	*Gravel*	*anaerobic*	*aerobic*		
Phenol	100	•		•		2	
Phenol	100	•		•		2	•
Phenol	100		•	•		2	•
Phenol	200	•		•		2	•
Phenol	400	•		•		2	•
Phenol	400		•	•		3	
Phenol	100	•			•	2	
Phenol	100		•		•	2	
2-Chlorophenol	500	•		•		2	
2-Chlorophenol	500		•	•		2	
3-Chlorophenol	100	•		•		4	
3-Chlorophenol	100		•	•		4	
3-Chlorophenol	100	•		•		4	•
3-Chlorophenol	100		•	•		4	•
4-Chlorophenol	100	•		•		3	
4-Chlorophenol	100		•	•		3	

with 4-aminoantipyrine are determined so it was necessary to subtract the background of these substances in the un-spiked groundwater.

Determination of 2-chlorophenol and 3-chlorophenol

2-Chlorophenol and 3-chlorophenol could nòt be determined with this method because they react insufficiently with the coupling agent. After enrichment on a solid phase, determination of 2-chlorophenol was done by HPLC and 3-Chlorophenol was measured with GC-MS.

To 200 ml of the acidified sample (pH < 2) 200 g NaCl was added and dissolved. Chlorphenols were enriched on bakerbond SPE cyclohexyl cartridge (Bakerbond, Philippsburg, USA). The cartridge was eluated twice with 0,5 ml of acetonitrile. The eluate was brought to 1 ml with acetonitrile.

HPLC

Hewlett Packard HP 1090 HPLC with diode array detector
Column: Chromspher 5 PAH Reversed Phase (250*4,6 mm i.d.) (Chrompack, Middelburg, The Netherlands)
Flow rate: 1 ml/min
Injection volume: 20 µl
Temperature: 25°C
Eluent A: 0,1% (v/v) ortho phosphoric acid (Fluka, Buchs, Switzerland), Eluent B: Acetonitril G CHROMASOLV (Riedel de Häen, Seelze, Germany)
Gradient: 75%A:25%B to 30%A:70%B
Wavelength: 230 and 280 nm

Gas chromatography

Hewlett Packard HP 5890 gas chromatograph Series II
Column: DB5 (30 m, 0,25 mm i.d., 0,25 pm film) (J&W, Köln, Germany)
Carrier gas: Helium 5.0
Injection volume: 5 µl
Injector temperature: 270°C
Detector temperature: 320°C
Temperature program: 70°C for 1 min, from 70°C to 182°C with 8°C/min, 182°C for 34 min
Detector: Mass selective detector Hewlett Packard HP 5971 A, Selected Ion Modus for chlorophenols with the mass of 128

RESULTS

The main processes to reduce organic substances added to the filters are adsorption and microbial degradation. Phenol and monochlorophenols as rather water soluble substances behaved very mobile in the system and sorption did not lead to a relevant reduction during

filter passage. Concerning degradation* behaviour they showed significant differences in the test system.

Phenol was metabolised under all experimental conditions after a lag phase. Lag phases were generally shorter when the filters had already been used in previous experiments and so already were adapted to phenol degradation. In detail the time required for microbial adaptation was influenced by the redox conditions, the filter material and the substrate concentration.

In the presence of molecular oxygen, the lag phase required for the degradation of 100 µg/l phenol was significantly reduced in comparison with sulfate reducing filters (Table 2). In the aerobic sand filter, phenol degradation was complete after 2 days while it took 12 days in the sulfate reducing one. Aerobic processes were in all cases faster than anaerobic ones.

Comparing the two underground materials, gravel filled filters showed a higher microbial activity and as a result shorter lag phases. Though the contact time calculated by tracer experiments# is shorter than in the sand filled filters, structure and composition of the gravel seem to be more favourable for the development of a suitable microbial population. In addition precipitation of iron- and manganese-oxides and hydroxides from the anaerobic groundwater was observed in the gravel which provides surfaces that can easily be colonised by microorganisms.

Lag phases observed for sand and gravel filters dosed with 100 µg/l phenol were notably different (Table 2). Both filters were operated anaerobically and had already been in contact with phenol in a previous experiment. Degradation started almost at once in the gravel filter. The sand filter showed a lag phase of 5 days, which was still significant shorter than for the sand filter, which had been operated before with anaerobic groundwater only.

Also the substrate concentration did influence the lag phase. When the input concentration was raised from 100 µg/l phenol to 200 µg/l and than to 400 µg/l, microorganisms were able to degrade a part of the infiltrating phenol, but complete

Table 2 Effects of experimental conditions on lag phases.

Test-substance	*Concentration (µg/l)*	*Filter-material*		*Redox-conditions*		*Adapted*	*Lag phase*
		Sand	*Gravel*	*anaerobic*	*aerobic*		
Phenol	100	•		•			12
Phenol	100	•			•		2
Phenol	100		•	•		•	5
Phenol	100	•		•		•	< 1
Phenol	100	•		•		•	5
Phenol	200	•		•			6
Phenol	400	•		•			11
3-Chlorophenol	100	•		•			> 30
3-Chlorophenol	100		•	•			> 30
3-Chlorophenol	100	•		•		•	> 30
3-Chlorophenol	100		•	•		•	7

* Degradation in this case means the disapperance of the test substance. Possible metabolites have not been determined.

Breakthrough curves of chloride as a substance that does not adsorb and moves with the water front were registered from which hydraulic parameters like flow velocity, effective porosity and contact times can be derived.

degradation was only observed after a lag phase of 6 resp. 11 days (Table 2). After adaptation, even the high concentrations were degraded efficiently.

The monochlorophenols showed a totally different behaviour. No degradation could be observed in anaerobic gravel and sand filters, which before had been operated exclusively with anaerobic groundwater.

Degradation of a monochlorphenol could only be observed in one experiment, where 3-chlorphenol was addedd at a concentration of 100 µg/l to a filter that provided conditions that had already been observed to favour microbial degradation in these experiments: the filter-material was gravel and the filter was already adapted to phenol degradation. This led to the development of a more active microbial population in the filter that was able to metabolise 3-chlorophenol after 7 days (Table 2).

This experiment demonstrated that although the substitution with chlorine renders the molecule less accessible to degrading processes, under favourable conditions with an active microbial population, monochlorophenols can be degraded in a sulfate reducing environment.

DISCUSSION

Persistence or degradation of the test compounds are not an inherent substance specific property but depend on the environmental conditions. While the rather simple aromatic compound phenol was degraded in all experimental settings, degradation of monochlorophenol could only be observed under very favourable conditions.

Including the results from the experiments with phenoxyacetic acids[14], which were degraded only under aerobic conditions, influence of the redox conditions, the filter-material and the molecular structure on degradability can be assessed.

- Filters operated with anaerobic groundwater in general showed lesser microbial activity and adaptation processes are more time consuming than in aerobic ones. After adaptation degradation is as efficient as under aerobic conditions.
- The gravel used in these experiments provides an environment which is more favourable for the development of a suitable microbial population than the sand. Lag phases are significantly shorter in this material.
- Chlorinated compounds proved to be less accessible for microbial attack. Dehalogenation which has been postulated as the first step in the metabolism of chlorinated aromatic compounds[16] apparently requires a longer adaptation period.

Nevertheless, the investigations demonstrated that a reduction of the tested compounds is possible even in anaerobic parts of the underground. But also if other factors are favourable for degrading processes, long lag phases have to be taken into account.

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