- Polluted groundwater and sediments -

Bioremediation of groundwater polluted by a herbicide production plant

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Abstract

The feasibility of remediating the groundwater below a former herbicide production plant contaminated with various chlorinated and/or methylated phenoxyalkanoic acids and phenols was investigated. The pH value in the vicinity of the production site was alkaline and the water had a deep brown color. Microcosm experiments showed that indigenous microorganisms did not show metabolic activities for the degradation of these compounds. The inoculation of the groundwater with biodegradative strains, e.g. *Ralstonia eutropha* JMP 134, or alkaliphilic/alkalitolerant biodegradative bacteria including strains of *Comamonas acidovorans, Rhodoferax fermentans, Aureobacterium testaceum* and *Rhodococcus erythropolis* led to the almost complete degradation of the phenolic contaminants and herbicides present. The color of the water was shown by synchronous fluorescence spectroscopy to be caused by dissolved organic matter (humic substances). Both acidification and the application of trivalent cations were suitable for removing these compounds by flocculation. Thus, remediation of this site should be feasible by applying specialist inocula in an appropriate bioreactor technology, combined with flocculation treatment.

1. Introduction

Production sites used by the chemical industry are often highly polluted especially the surrounding soil, underlying groundwater and the buildings. In a feasibility study we focused on the groundwater polluted by a former phenoxy herbicide production plant which was heavily contaminated with the spectrum of products including the source compounds, intermediates and by-products. A second

source of pollutants resulted from a period of diesel fuel production at this site more than five decades ago. The groundwater below the herbicide production site itself was alkaline and exhibited a deep brown color, whereas in the more peripheral areas of the pollution plume it was slightly acidic and clear.

These pollutants must be removed in order to prevent their spreading and toxification of large areas of the environment. The aim of the investigations summarized subsequently therefore was the development of an *in situ* or a *on site* bioremediation technology for groundwater heavily polluted with herbicides and intermediates of their industrial production. The experiments were thus focused on eliminating the toxic chlorinated aromatic compounds. Microbial activities degrading various chlorinated phenols and phenoxyalkanoic acids are well known under neutral pH conditions [1-3]. As a result of our investigations of the decontamination of herbicide-polluted building materials [4, 5], we have isolated bacterial strains which also degrade these compounds under (very) alkaline pH conditions [6-8]; these were hence used. An additional problem arose from the deep brown color of the groundwater observed in certain areas. Data from synchronous fluorescence spectroscopy, which has been proven a useful tool for monitoring the distribution and changes of dissolved organic matter (humic substances) in groundwater [9, 10], have indicated that this is most probably derived from humic matter. Measures were subsequently applied for their removal (cf. [11]).

2. Results and discussion

The pollution problem as observed by annual routine measurements is summarized up in Table 1. High contamination was found immediately below the former herbicide plant (Well 45). Evidently, the main problem was caused by the chlorinated phenoxyalkanoates and phenols with AOX values as high as 6 mg/l. Pollution by hydrocarbons (mineral oils) amounted to about 5 mg/l. The identification of 2,4-dichlorophenol (DCP) and 4-chloro-2-methylphenol (MCP) was not always possible in routine HPLC analysis, as both showed similar retention times under the conditions applied. However, the concentration of MCP was higher by an order of magnitude in comparison to DCP. The analysis of the dominant pollutants within the groundwater was optimized for *on site* field monitoring purposes on the basis of solid phase extraction and subsequent GC-MS measurement. With this method, a recovery of about 100 % was obtained for DCP and MCP. In particular, MCP reached concentrations of up to 14.6 mg/l (Well 45, 10 m depth; Sept. 1996). A similar spectrum of pollution but at a lower level was found at a distance of about 1-2 km from this position (Well 40). We tested if the degradation of MCP at the polluted site was limited by the catabolic potential of the indigenous microbial community or by the availability of oxygen. To this end, we performed experiments with microcosms made up from groundwater samples of the polluted site. All microcosms were oxygenated by gentle shaking and than inoculated with *Ralstonia eutropha* JMP134 (formerly *Alcaligenes eutrophus* JMP 134), a strain able to mineralize a wide spectrum of substituted aromatics. Controls remained untreated. The survival of *R. eutropha* JMP 134 in the microcosm was followed by plate counts on selective minimal media, and the degradation of the target compounds analyzed by GC measurement as described above. In microcosms inoculated with strain JMP134 (initial density of approximately 10^7 cfu/ml), MCP and DCP were degraded within 24 hours. Final concentrations after 216 h were 0.29 mg/l (DCP) and

0.077 mg/l (MCP), respectively (Figure 1). No degradation occurred in uninoculated control microcosms. Thus, biodegradation of MCP and DCP is limited by the catabolic potential of the indigenous community of the polluted aquifer. *R. eutropha* JMP134 might contribute in an inoculum to complete degradation of the herbicides present [12].

Table 1. Characteristics of the groundwater from a herbicide site

	Well 45	Well 40
Sum characteristics		
Color	dark brown	colorless
pH	8.5	5.4
TOC (mg/l)	33	4.7
AOX (mg/l)	6.1	0.25
IR Hydrocarbons (mg/l)	4.8	n.d.
Chlorinated compounds (mg/l)		
2,4-D	< 0.1	< 0.05
2,4-DP	1800	220
2,4-DB	1000	< 0.05
MCPA	290	8
MCPP	3200	120
MCPB	970	1
2,4,5-TP	60	n.d.
2-Chlorophenol	93	8.5
2,4-Dichlorophenol	52	< 0.3
2,4,6-Trichlorphenol	35	< 0.3

n.d., no data
Data provided by BASF Schwarzheide.

Besides *R. eutropha* JMP134, which is active under neutral pH conditions strains were applied which are characterized by alkaliphilic/alkalitolerant properties. These should be especially suited for treating the highly polluted alkaline groundwater with pH values up to 10 in the deepest layers of the aquifer. The degradative properties of these strains are shown in Table 2. Consequently, metabolic activity for the degradation of the main pollutants are available. Several strains and combinations of strains were applied for bioremediation purposes. The prime concern at this phase of the investigation was to establish the feasibility of bioremediation; detailed kinetic investigations are aimed at in further experiments. The application of the alkaliphilic strain *Comamonas acidovorans* P4a, for instance, resulted in the degradation of the phenoxyacetate herbicides (2,4-D and MCPA). In addition, chlorinated/methylated phenols, which are present in this groundwater and

are also intermediates in the microbial degradation of the phenoxy herbicides (e.g. DCP/MCP) were also utilized (Figure 2). *Rhodoferax fermentans* strain P230 proved to be a versatile bacterium which degraded a broad spectrum of the chloro-organic pollutants (Figure 3). Its application in the groundwater resulted in the removal of almost all of these pollutants, only leaving the phenoxybutyrates unutilized. This problem, however, might be solved by the metabolic activity of strains such as *Aureobacterium testaceum* K2-17 or *Rhodococcus erythropolis* K2-12, which were shown to attack substituted phenoxybutyrates by liberating the respective substituted phenols. These products/intermediates in turn are degradable by the activity of one of the above-mentioned bacteria. Thus, at the present state of investigation the degradation of a large spectrum of chlorophenols and phenoxyalkanoates would require the application of a consortium of two species, namely *R. fermentans* P230 and either *A. testaceum* K2-17 or *R. erythropolis* K2-12. The application of a mixture of these strains, including *C. acidovorans* P4a, indeed resulted in an almost complete elimination of the phenolic pollutants (Figure 4). The hydrocarbon contaminants arising from the previous diesel fuel production were not considered at this state of investigation. Initial inspection of the IR adsorption signal before and after the treatment of groundwater with the herbicide-degrading trinary consortium did not indicate an essential decrease of the hydrocarbon content during this treatment. Control experiments on groundwater supplied with ammonium and phosphate did not show a significant degradation of phenoxyalkanoates within this period (Figure 5).

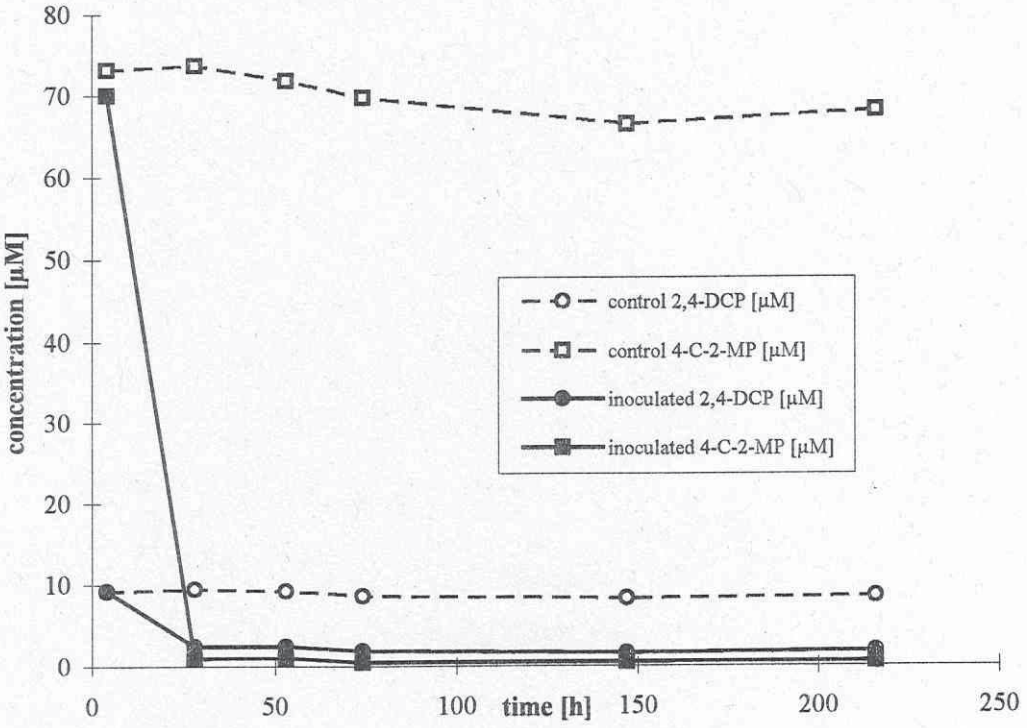

Fig. 1. Degradation of 2,4-dichlorophenol and 4-chloro-2-methylphenol in groundwater microcosms inoculated with *Ralstonia eutropha* JMP 134
Circle, DCP; Square MCP; Open symbols, control; Filled symbols, inoculated

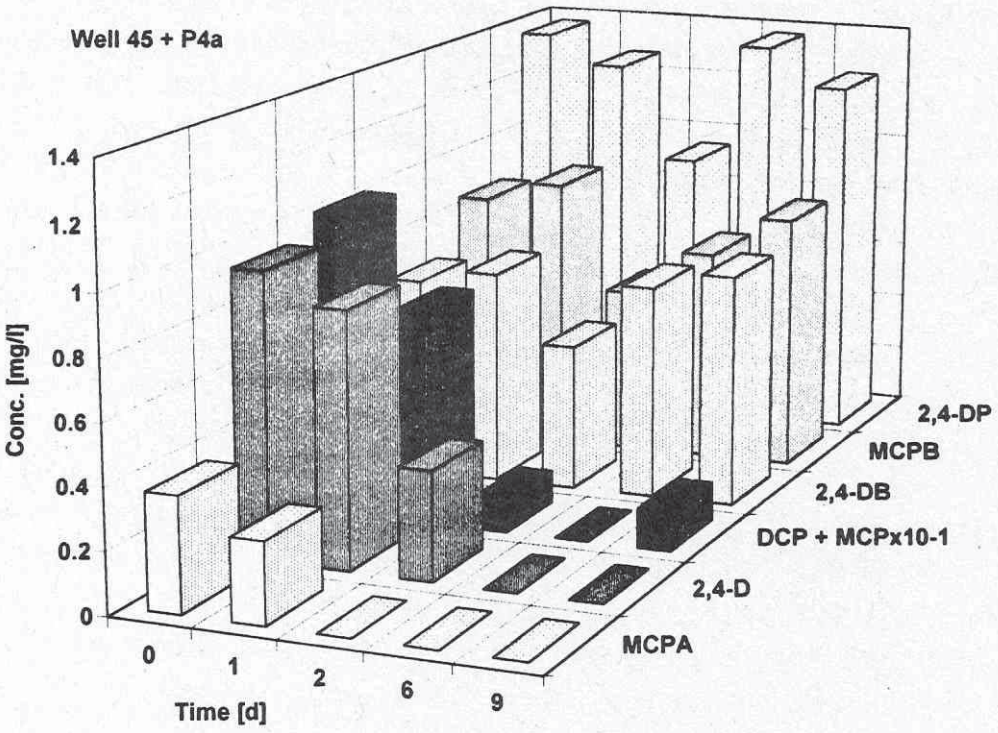

Fig. 2. Degradation patterns of the phenolic contaminants after application of *Comamonas acidovorans* P4a

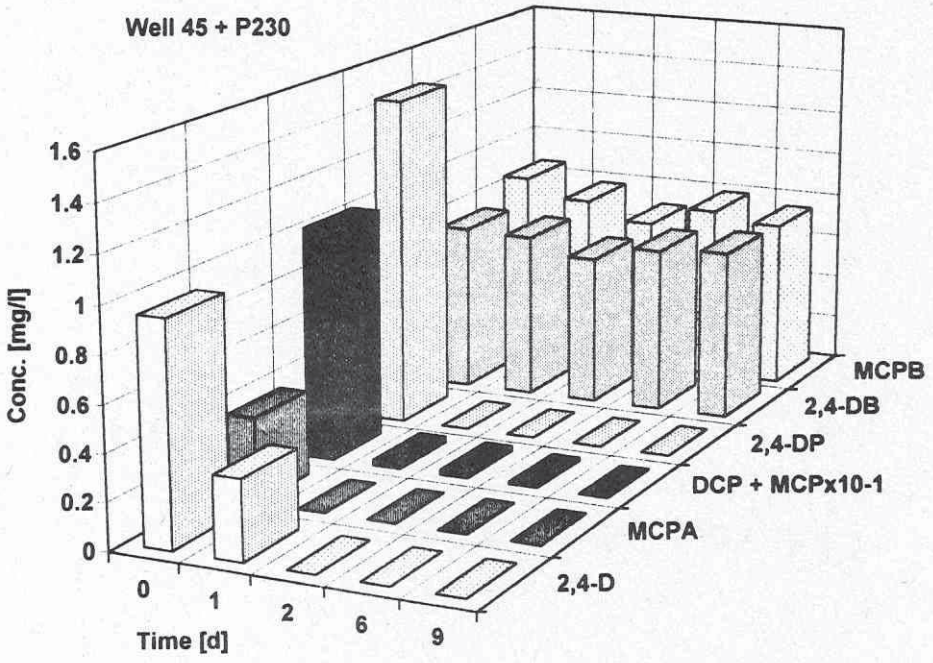

Fig. 3. Degradation patterns of the phenolic contaminants after application of *Rhodoferax fermentans* P230

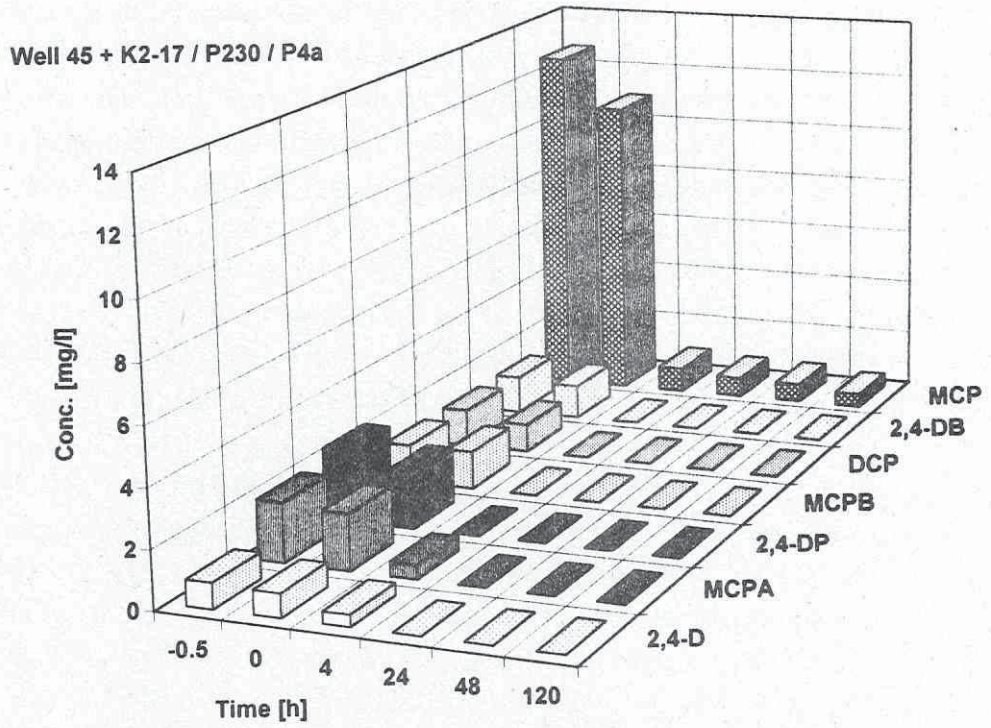

Fig. 4. Degradation patterns of the phenolic contaminants after application of a bacterial mixed culture consisting of *Comamonas acidovorans* P4a, *Rhodoferax fermentans* P230 and *Aureobecterium testaceum* K2-17

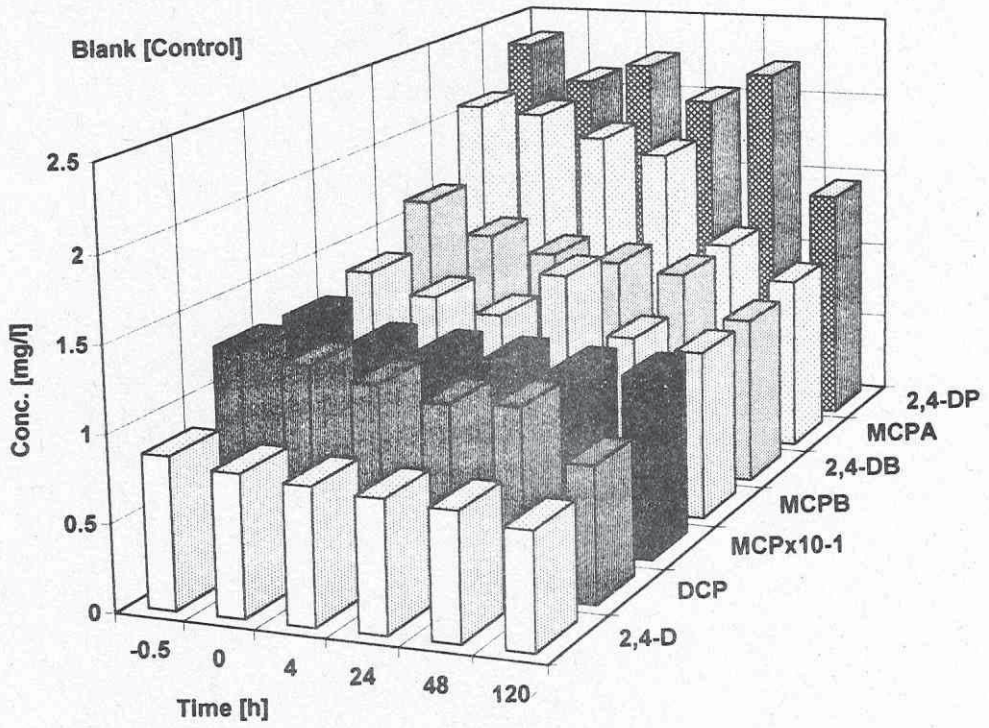

Fig. 5. Concentration profile of the phenolic compounds during aerobic treatment of groundwater supplemented with ammonium and phosphate without inoculation

An additional problem concerns the deep brown color of the highly polluted groundwater which remains unchanged after microbiological treatment. Synchronous fluorescence spectra reveal mayor peaks at 350, 395 and 470 nm indicating the presence of dissolved organic material (humic matter) [9, 10]. Although these data are not suitable for chemical identification, they are useful for pattern recognition. This is shown for the groundwater from the herbicide site in comparison to the solution of aquatic humic acid standards (Swannee River humic acid, IHSS International Humic Substances Society). Apparently, the two almost coincide (Fig. 6). Comparing these data to a soil humic acid standard from the IHSS which is characterized by a higher content of high-molecular weight structures, reflected in the high intensity of the peak at 470 nm [15] indicates that the content of low and high molecular weight humic matter is more balanced in the 1996 water samples. Surprisingly, samples taken in October 1997 exhibited a very different picture. A peak at 275 nm became dominant in the fluorescence spectra corresponding to a high content of low moleculare weight structures (Fig. 7) [15]. The pH of the groundwater had dropped to below 7 and the content of humic matter was very low. The data of this water sample resemble the figures found in a lake of wastewater from low temperature carbonization (Schwelvollert) after treatment (precipitation of humic matter). The reasons for the change in the hydrochemical characteristics are actually unknown but may result from a stratification of the aquifer. The highly polluted water (1996 sample) was treated by chemical measures in order to remove these humic compounds. Acidification by HCl, for instance, was successful. At a pH of about 3.5 almost all of these compounds were removed by flocculation. This rather strong acidification is unsuitable for technical application. Flocculation was also caused by the addition of trivalent cations [11]. With Al^{3+}, for instance, an equivalent of these ions as few as 2-2.5 mM led to the almost complete elimination of the precipitable material in the aqueous phase while only decreasing the pH to about 6.7 (Table 3). This decrease in the load of humic matter is reflected by the fluorescence spectrum (Fig. 7).

To summarize, preliminary microbial investigations have shown that strains are available and applicable which are active in eliminating the main and most toxic compounds in this groundwater under the resident conditions. The problem arising from a high content of humic matter might subsequently be solved by the application of conventional technical measures.

Table 3. Treatment of the groundwater by $Al_2(SO_4)_3$

Concentration (g/l)	pH	Extinction at 352 nm
0	8.7	1.95
0.05	8.24	1.93
0.1	7.7	1.83
0.2	7.28	1.54
0.4	6.85	0.152
0.5	6.58	0.111
0.6	6.37	0.085
0.8	5.98	0.066

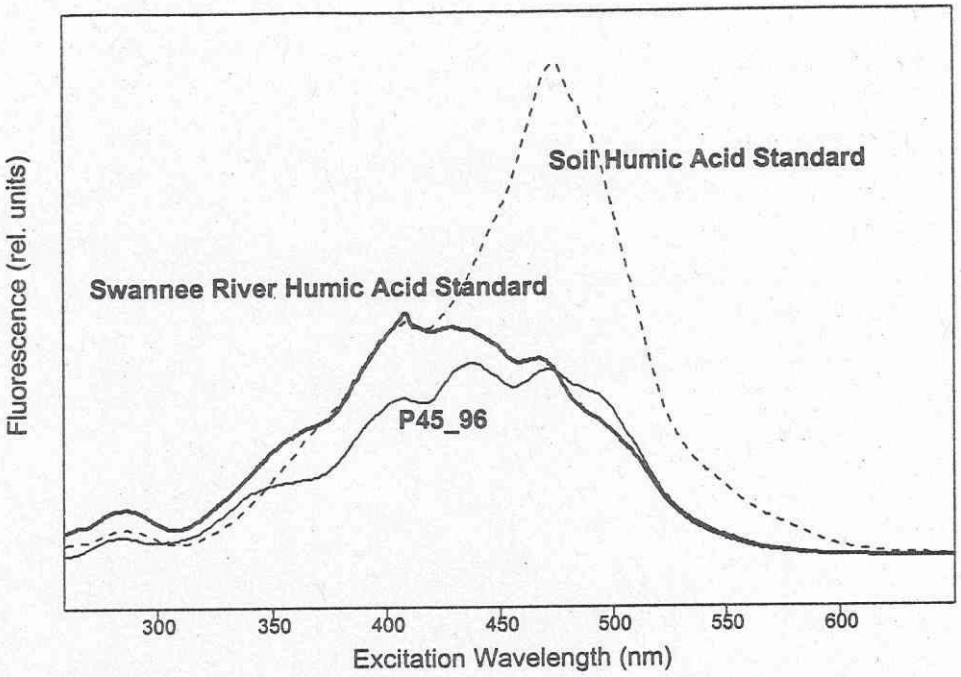

Fig. 6. Synchronous fluorescence spectra of groundwater from a herbicide site (September 1996) in comparison to solutions of humic acid standards from the IHSS (pathlength of the cuvette 5 mm, $\Delta\lambda = 18$ nm)

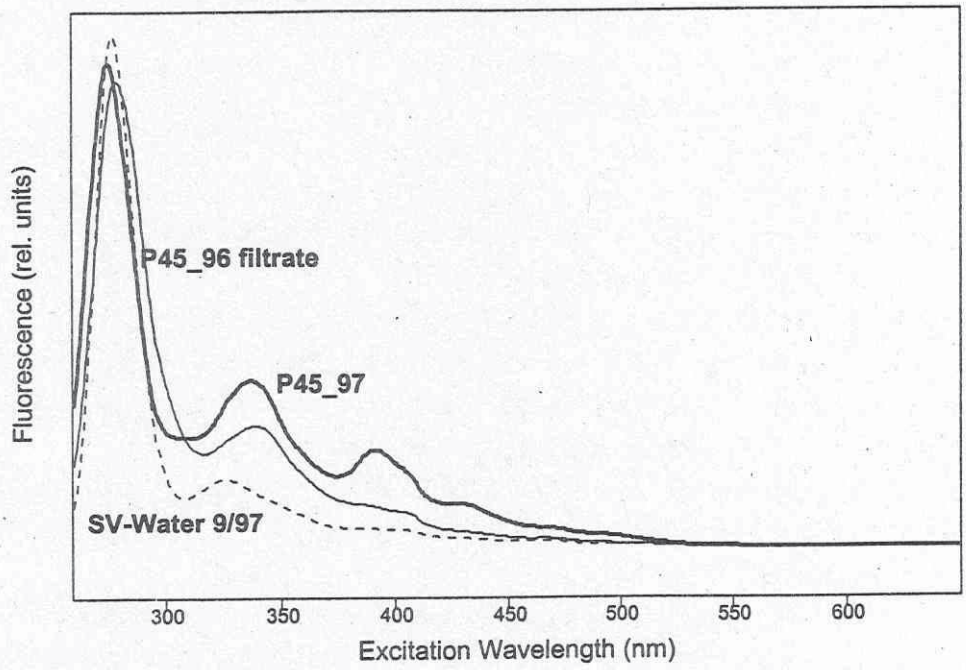

Fig. 7. Synchronous fluorescence spectra of groundwater after treatment by Al^{3+} and from the herbicide site in October 1997. The data were compared to the spectrum of a carbonization waste water (Schwelvollert) after flocculation (conditions see Fig. 6)

Abbreviations

DCP - 2,4-Dichlorophenol
2,4-D - 2,4-Dichlorophenoxyacetate
2,4-DB - 2,4-Dichlorophenoxybutyrate
2,4-DP - 2,4-Dichlorophenoxypropionate
MCP - 4-Chloro-2-methylphenol
MCPA - 4-Chloro-2-methylphenoxyacetate
MCPB - 4-Chloro-2-methylphenoxybytyrate
MCPP - 4-Chloro-2-methylphenoxypropionate

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