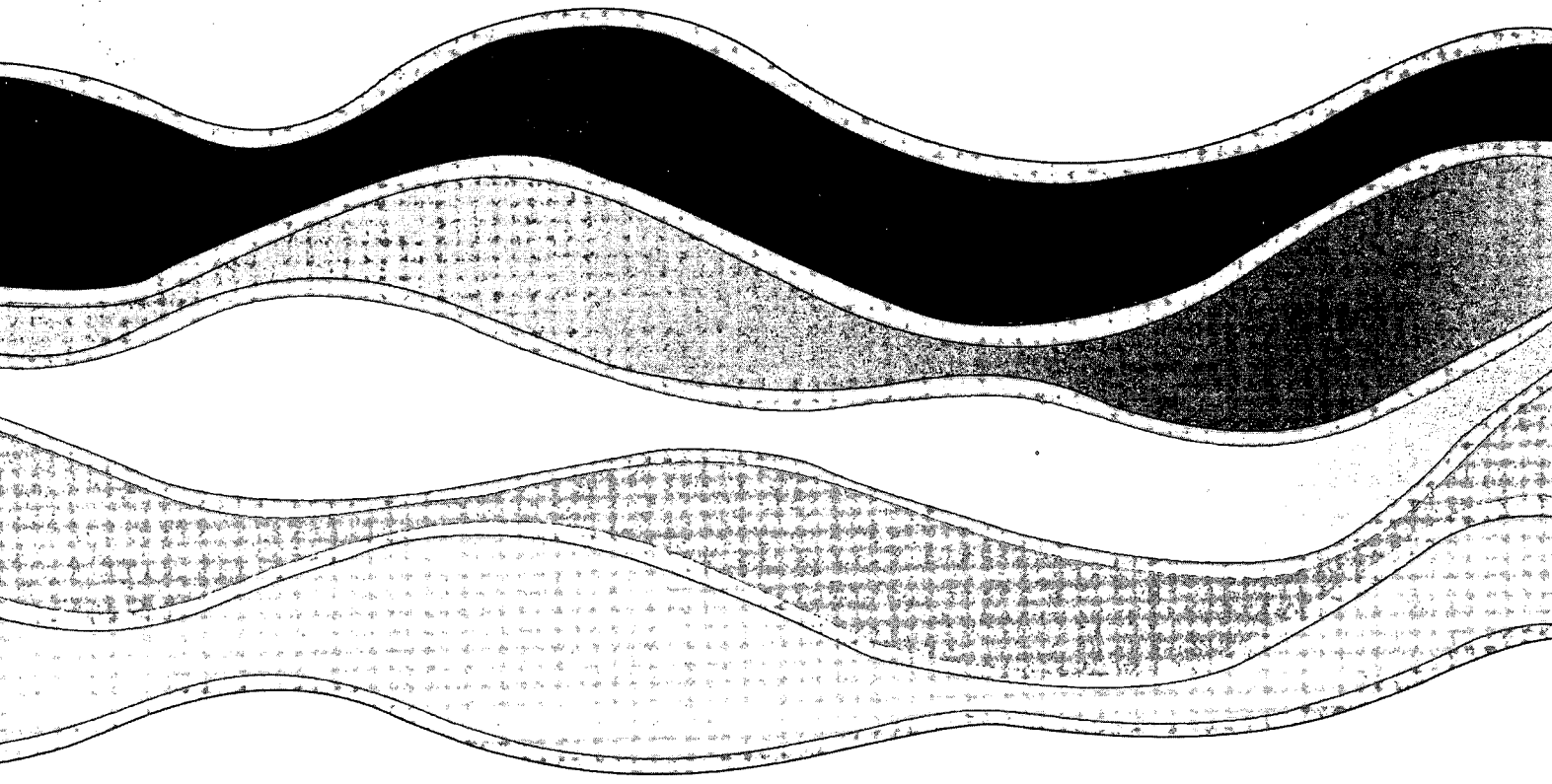
NATIONAL WATER RESEARCH INSTITUTE

INSTITUT NATIONAL de RECHERCHE sur les EAUX



CANADA-GERMANY SCIENCE & TECHNOLOGY
BILATERAL AGREEMENT PROJECT

Ecosystem Health: ENV 48 -
Chemical & Toxicological Testing

STUDY:
CHEMICAL, TOXICOLOGICAL & GENOTOXICOLOGICAL CHARACTERIZATION OF SEDIMENTS FROM THE RIVER ELBE, GERMANY

J. Kurz and R-D Wilken
S.S. Rao and J.H. Carey

NWRI Contribution No. 96-20

CANADA-GERMANY SCIENCE & TECHNOLOGY BILATERAL AGREEMENT PROJECT

Ecosystem Health: ENV 48 - Chemical & Toxicological Testing

Study:

CHEMICAL, TOXICOLOGICAL & GENOTOXICOLOGICAL CHARACTERIZATION OF SEDIMENTS FROM THE RIVER ELBE, GERMANY*

Principal Participants:

Joachim Kurz[1] and Rolf-Dieter Wilken[1]

[1]Department of Organic Trace Analysis
Institute of Physical and Chemical Analytics
GKSS Research Centre Geesthacht GmbH
Max-Planck-Str.
D-21502 Geesthacht. Germany

and

Salem S. Rao[2] and John H. Carey[2]

[2]Aquatic Ecosystem Conservation Branch
National Water Research Institute
Canada Centre for Inland Waters
867, Lakeshore Road
Burlington, Ontario, Canada L7R 4A6

**The mutagenicity & genotoxicity assays were performed at The National Water Research Institute, Burlington, Ontario, Canada during a visit by Dr. Joachim Kurz under the Germany-Canada Science & Technology Bilateral Agreement Project (Ecosystem Health: ENV 48 - Chem. Analysis and Toxicity Testing).*

Abstract

The occurrence of toxic chemicals in the sediments from river Elbe have been investigated in eight different locations from the Czech republic to the mouth of the Elbe. The method used is the bioassay-directed chemical analysis, which combines the use of chemical analysis and bioassays. The first step of the study is a sequential sediment extraction using the following solvents: water, hexane, methanol (pH=7), and methanol (pH=2). The fractions were examined for chemical analysis and for toxicity and genotoxicity testing. GC/MS with electron impact ionisation was used for the analysis of organic compounds. The acute toxicity was measured using the Microtox test (decreases in bioluminescence of the marine bacterium *photobacterium phosphoreum*). The methanol fractions (pH=7) were examined for mutagenicity using the MutaChromo-Plate assay. One fraction (OSA 68), which showed a mutagenic potential, was examined for genotoxic potential using the fish hepatic micronucleus assay in rainbow trout. The investigation showed a high toxicity in all fractions of the sediment from the river Bilina (OSA 68) and also a broad spectrum of organic pollutants. In the water fraction of this sediment the chemical responsible for the toxicity can be determined as Bisphenol A.

Introduction

The main objective of this study is to apply a combined "chemical-bioassay approach" to assess toxic and genotoxic responses of chemically characterised sediments collected from the River Elbe, Germany (Kurz, 1995). With the conventional chemical analysis of environmental samples, not all compounds can be identified, since it would be highly expensive. The majority of the analyses performed so far, have focused on the detection of PCBs (polychlorinated biphenyls), PCDDs (polychlorinated dioxins), PCDFs (polychlorinated furans), PAHs (polyaromatic hydrocarbons), chlorinated pesticides (DDT, HCHs, PCP), and P/N-pesticides. These groups of chemical compounds represent only a small part of the over 10000 existing compounds in many complex environmental mixtures. Furthermore, obtaining information on the toxicity of all these individual chemicals is highly prohibitive. With the application of the standardised bioassays, the screening of toxicity of a complex mixture sample can be achieved. However, the drawback with the bioassay approach is that no information on the specific compound/s responsible for the toxic effect can be obtained. Identification of the toxic/genotoxic substance/s using the "chemical-bioassay" directed approach, however, would enhance the basis for making the decision for control measures.

The bioassay-directed chemical analysis which is often called the Toxicity Identification Evaluation (TIE) is an interactive process and the combination of both the chemical and bioassay approach in an assessment of the toxic relevance of chemical contaminants of environmental samples. The TIE methods involve: (1) the extraction of contaminants from sediment samples using different solvents and pH, (2) the fractionation of the major toxic extracts using different analytical methods and 3) the measurement of the toxicity and mutagenicity of different sub-samples using the standardised bioassays to ascertain their relative toxic and genotoxic responses (3) and finally, (4) the identification of the components present in the toxic/genotoxic fractions using the suitable analytical methods.

Against this background, a study was undertaken to examine the chemical composition for organic compounds using GC/MS approach. In addition, the sediment extracts were also assessed for their acute toxicity using Microtox and mutagenicity and genotoxicity using the Muta-ChromoPlate mutagenicity and fish hepatic micronucleus induction assays.

Materials and Methods:

Sediment samples:

Table 1 shows the details of sample codes used, sample sites, dates of sampling, and some relevant information on the sediment samples collected from the Elbe River, Germany. The samples were stored immediately after collection in nalgene bottles at -18°C until they were used for the solvent extraction.

Table 1: Sample codes used, sample sites, dates of sampling, and further information of the examined German sediment samples.

Sample Code	Location of sample	Date of sampling	Comments
OSA 68	Bilina by Usti (Czech Republic) tributary of river Elbe Elbe-km: 337.5	22.09.1994	after the chemical factory Spol-Chemie
OSA 93	Mühlenberger Loch river Elbe after Hamburg harbour Elbe-km: 635.0	19.08.1994	after the Hamburg harbour
K1	Neufelder Sand river Elbe, Elbe-km: 705 53° 51′ 33″ N / 09° 00′ 22″ E	25.05.1994	a low contaminated sample , which is mostly sand
K2	Neufeld-Reede river Elbe, Elbe-km: 699 53° 53′ 04″ N / 09° 05′ 17″ E	25.05.1995	
K3	Neufeld-Reede river Elbe, Elbe-km: 699 53° 53′ 09″ N / 09° 05′ 27″ E	25.05.1994	
B5	Brunsbüttel, harbour North-East-Sea-Channel Channel-km: 3.6 53° 54′ 24″ N / 09° 10′ 11″ E	25.05.1994	Brunsbüttel harbour
V4	Brunsbüttel, new harbour North-East-Sea-Channel Channel-km: 0.8 53° 53′ 21″ N / 09° 08′ 13″ E	25.05.1994	new Brunsbüttel harbour
F1	Magdeburg, Fahlberg-List river Elbe, Elbe-km: 319.4	22.06.1994	after the chemical factory Fahlberg-List

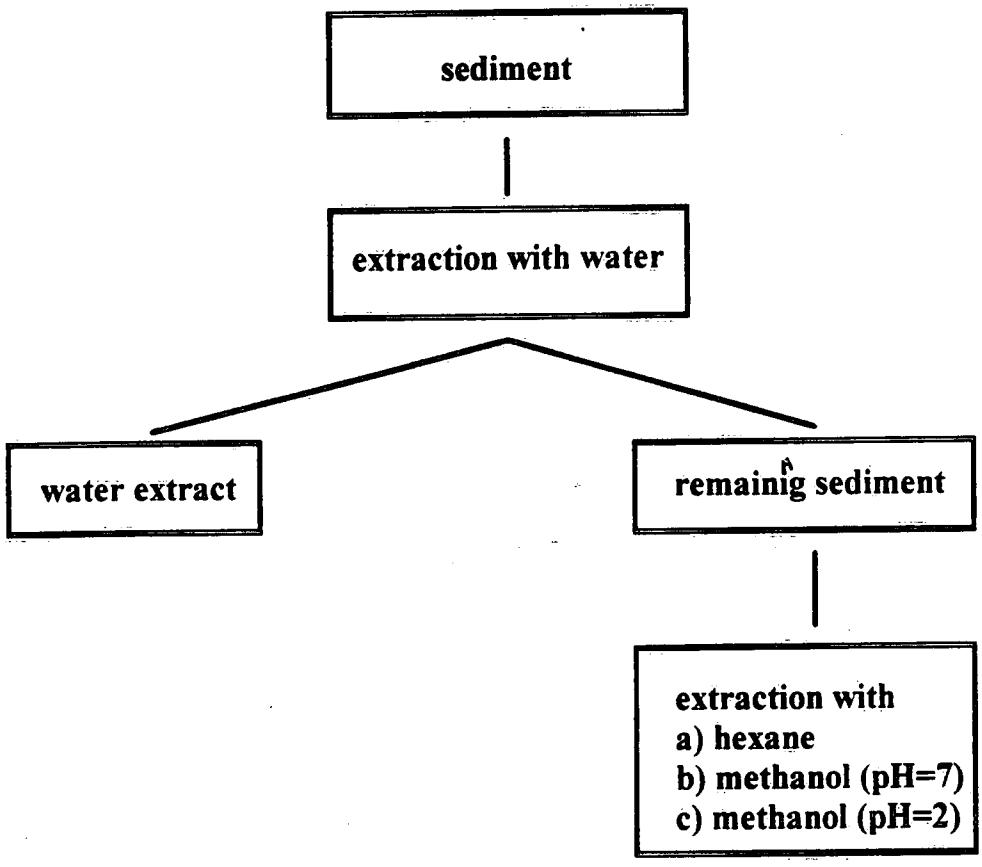
Preparation of Sediment extracts:

The sediment samples were sequentially extracted using different solvents and at different pH values to separate different fractions based on their chemical and physical properties. The scheme used in the extraction is shown in Figure 1.

For the initial sediment extraction, the following procedure was used; 15g of wet sediment was placed in an nalgene centrifuge tube and extracted with 15mL of water by shaking in a shaking

machine (Gerhardt Bonn LS, Germany) for 2 hours. The supernatant or the aqueous phase was separated by centrifugation with 10000rpm for 10 min (Heraeus Sepatech Biofuge 17 S with rotor 8570. Germany). The aqueous fraction (water extract) was collected with a Pasteur pipette. The remaining sediment was treated with 15mL hexane and extracted in the same manner as described before. This procedure was then repeated using 15mL methanol and 15mL methanol at a pH of 2.0 by adding conc. HCl (Fig. 1).

Figure 1: Extraction scheme of the sediment samples



Bioassays:

Three different bioassays were used to test toxicity and genotoxicity of the different sediment extracts: Microtox™ assay was used for determining the acute toxicity, Muta-ChromoPlate mutagenicity test Kit was used for detecting mutagenicity (Rao and Lifshitz, 1995) and Fish Hepatic Micronucleus Induction Assay (Rao et al., 1996,) for determining the genotoxicity of the potent mutagenic extract/s of the sediment.

Microtox Toxicity Assay:

The Microtox bioassay measures toxicity by recording the dose-related decreases in bioluminescence of the marine bacterium *photobacterium phosphoreum*. The general outline of the

Microtox Bioassay is the one outlined by Dutka (1989), in which each sediment extract was analysed at dilutions of 45.4%, 22.7% and 11.4% of the original sample. Results were expressed in terms of EC_{50} %.

In the present investigation we used the pT-value for detecting the acute toxicity of the sediment extracts. The pT-value was first introduced by Krebs (1988). For the numerical designation of the toxicity the negative binary logarithm of the first non toxic dilution factor is used. Following S. P. L. Sörsensen, who introduced the pH-value as an index number to chemistry in 1909, the exponent of toxicity should be designated as pT-value. The pH-value (potentia hydrogenii = hydrogen exponent) corresponds to the negative common logarithm on the hydrogen ion concentration in a solution. The pT-value (potentia toxicologiae = toxicological exponent) is the negative binary logarithm of the first non-toxic dilution factor in a dilution series in geometric sequence with the dilution factor of two. Accordingly, the pT-value gives an indication of how many times a sample must be diluted in the ratio 1:2 to have no toxic effect. EC_{20}-value is used a non-toxic indicator of the sample. As an example, at a dilution of 1:16 the reduction of light from the Microtox bacteria was 80% (EC_{20}). In an exponential writing the dilution is $1:16 = 1:2^4 = 2^{-4}$. The negative logarithm of the basis 2 for the dilution 1:16 is 4. The sample has the pT-value 4.

The solvents used in the extraction of the samples were (as described in the extraction protocol) water, hexane, methanol, and methanol with a pH of 2. The water extract and the two methanol extracts are directly used for the Microtox assay. Because of the high toxic nature of the hexane to target bacteria in the Microtox Toxicity Assay methanol was used to replace hexane during solvent exchange process. The hexane was replaced by methanol by evaporating (1mL hexane-extract) under a N_2-flux (vapotherm, Barkley laboratory techniques, Germany) and dilution of the residue with methanol (1mL).

For the Microtox Bioassay, M500 Analyser (Microbics Corp., USA) was used. This protocol uses a software- Microtox program version 7.81d (Microbics Corp., USA) running on a AST Laptop Power Exec 4/25SL (AST Research Inc., USA). For the determination of the EC_{20}-value a serial dilution of 4 concentrations of a stock solution was used. The EC_{20}-values were measured at 5, 15, and 30 minutes. All tests were done in duplicates. Table 2 shows the results of the Microtox-Assay at different response times. Figures 2 a), b), and c) show the relative toxicity of different extracts of sediment samples 5, 15, and 30 min response time.

Table 2: Results of Microtox™-Assay

Sample Code	Extraction Solvent	Measured time (min)	Dilution factor for EC_{20}	pT-value
OSA 68	water	5	1/3735	8.54
		15	1/374.4	8.46
		30	1/340.3	8.37
	hexane	5	1/456.9	8.83
		15	1/412.3	8.65
		30	1/329.8	8.36
	methanol	5	1/4226.8	11.90
		15	1/2182.5	11.09
		30	1/2086.2	11.03
	methanol (pH=2)	5	1/14560.4	13.70
		15	1/7821.9	12.82

Table 2: Results of Microtox™-Assay (cont'd)

Sample Code	Extraction Solvent	Measured time (min)	Dilution factor for EC_{20}	pT-value
		30	1/5678.8	12.39
OSA 93	water	5		< 1.14
		10		< 1.14
		15		< 1.14
	hexane	5	1/694.4	9.44
		15	1/511.2	9.00
		30	1/295.0	8.20
	methanol	5	1/2265.3	10.88
		15	1/1153.9	10.14
		30	1/1062.3	10.02
	methanol (pH=2)	5	1/3829.4	11.85
		15	1/2676.7	11.38
		30	1/2039.3	10.99
K1	water	5		< 1.14
		15		< 1.14
		30		< 1.14
	hexane	5	1/79.4	6.31
		15	1/76.8	6.26
		30	1/75.3	6.24
	methanol	5	1/237.1	7.89
		15	1/145.6	7.19
		30	1/108.2	6.76
	methanol (pH=2)	5	1/409.9	8.65
		15	1/316.9	8.26
		30	1/305.5	8.26
K2	water	5		< 1.14
		15		< 1.14
		30		< 1.14
	hexane	5	1/381.5	8.53
		15	1/308.9	8.19
		30	1/315.6	8.30
	methanol	5	1/556.2	9.12
		15	1/472.9	8.89
		30	1/362.9	8.50
	methanol (pH=2)	5	1/833.7	9.70
		15	1/754.9	9.56
		30	1/482.2	8.91
K3	water	5		< 1.14
		10		< 1.14
		15		< 1.14
	hexane	5	1/614.0	9.26
		15	1/369.9	8.53
		30	1/240.8	7.91
	methanol	5	1/931.0	9.86

Table 2: Results of Microtox™-Assay (cont'd)

Sample Code	Extraction Solvent	Measured time (min)	Dilution factor for EC_{20}	pT-value
		15	1/683.4	9.42
		30	1/513.6	9.00
	methanol (pH=2)	5	1/1024.5	10.00
		15	1/1072.0	10.07
		30	1/956.9	9.90
B5	water	5		< 1.14
		15		< 1.14
		30		< 1.14
	hexane	5	1/223.3	7.85
		15	1/187.8	7.55
		30	1/133.8	7.06
	methanol	5	1/793.0	9.63
		15	1/655.3	9.36
		30	1/465.8	8.86
	methanol (pH=2)	5	1/1201.6	10.22
		15	1/1146.4	10.13
		30	1/905.4	9.79
V4	water	5		< 1.14
		15		< 1.14
		30		< 1.14
	hexane	5	1/286.3	8.15
		15	1/214.8	7.75
		30	1/229.7	7.84
	methanol	5	1/2782.2	10.89
		15	1/1406.7	10.46
		30	1/1074.3	10.07
	methanol (pH=2)	5	1/1023.8	10.00
		15	1/1007.4	9.97
		30	1/856.6	9.74
F1	water	5		< 1.14
		10		< 1.14
		15		< 1.14
	hexane	5	1/679.3	9.47
		15	1/584.9	9.19
		30	1/459.7	8.85
	methanol	5	1/1973.8	10.95
		15	1/1530.6	10.58
		30	1/1170.3	10.18
	methanol (pH=2)	5	1/9252.1	13.18
		15	1/9637.0	13.23
		30	1/8809.3	13.11

Figure 2a: Comparison of the Microtox Toxicity in the different sediment extracts (5 min readings)

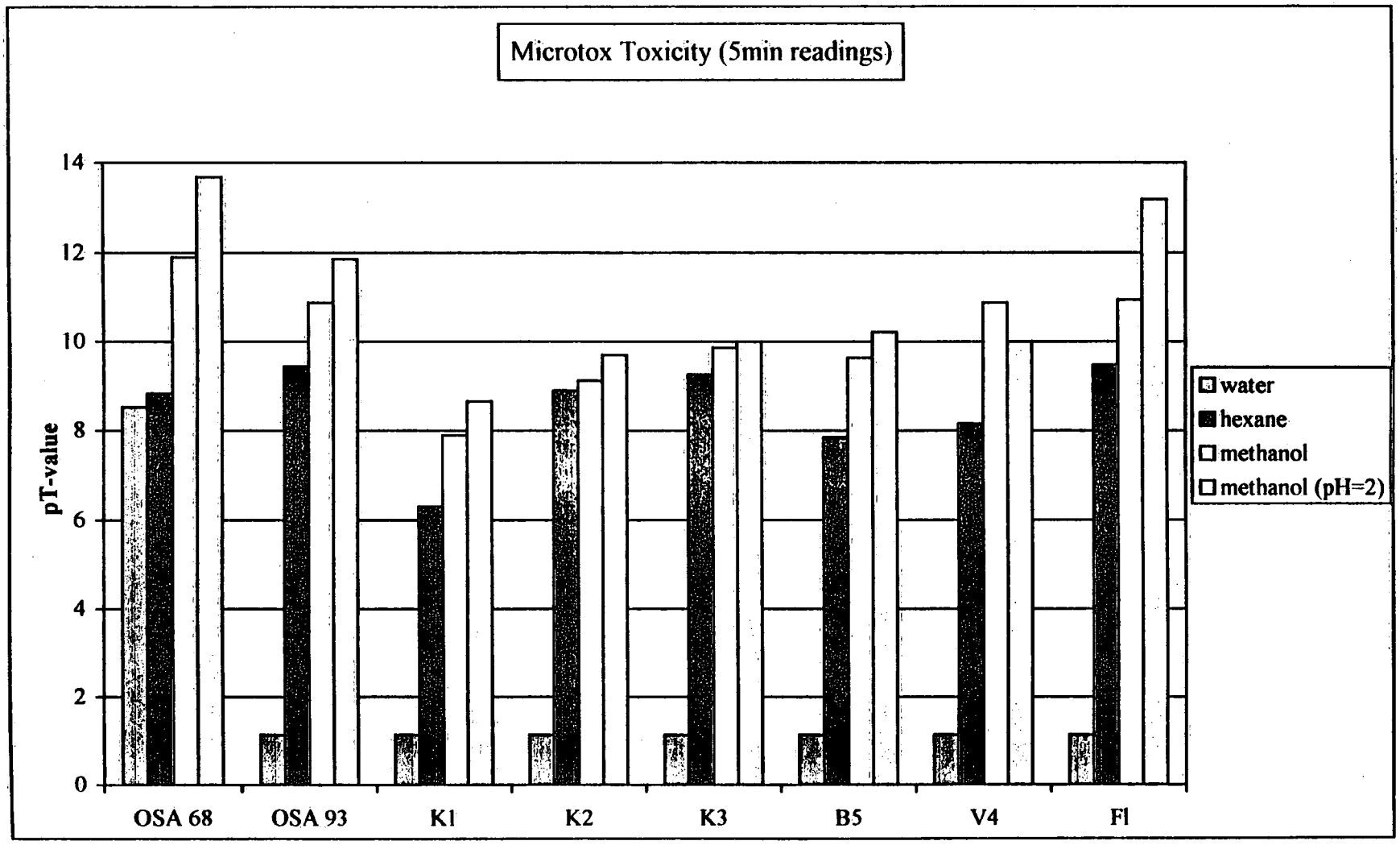


Figure 2b: Comparison of the Microtox Toxicity in the different sediment extracts (15 min readings)

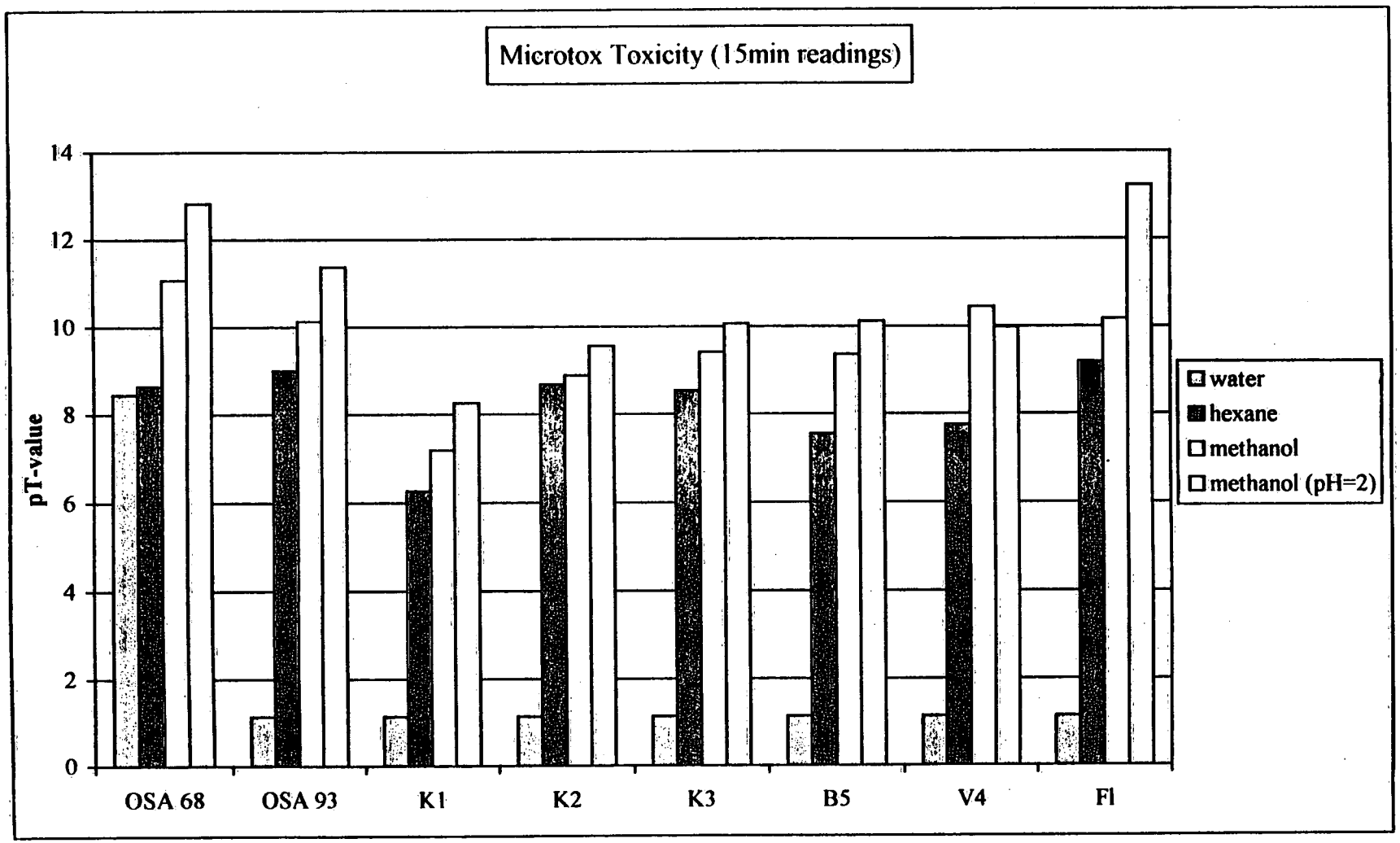
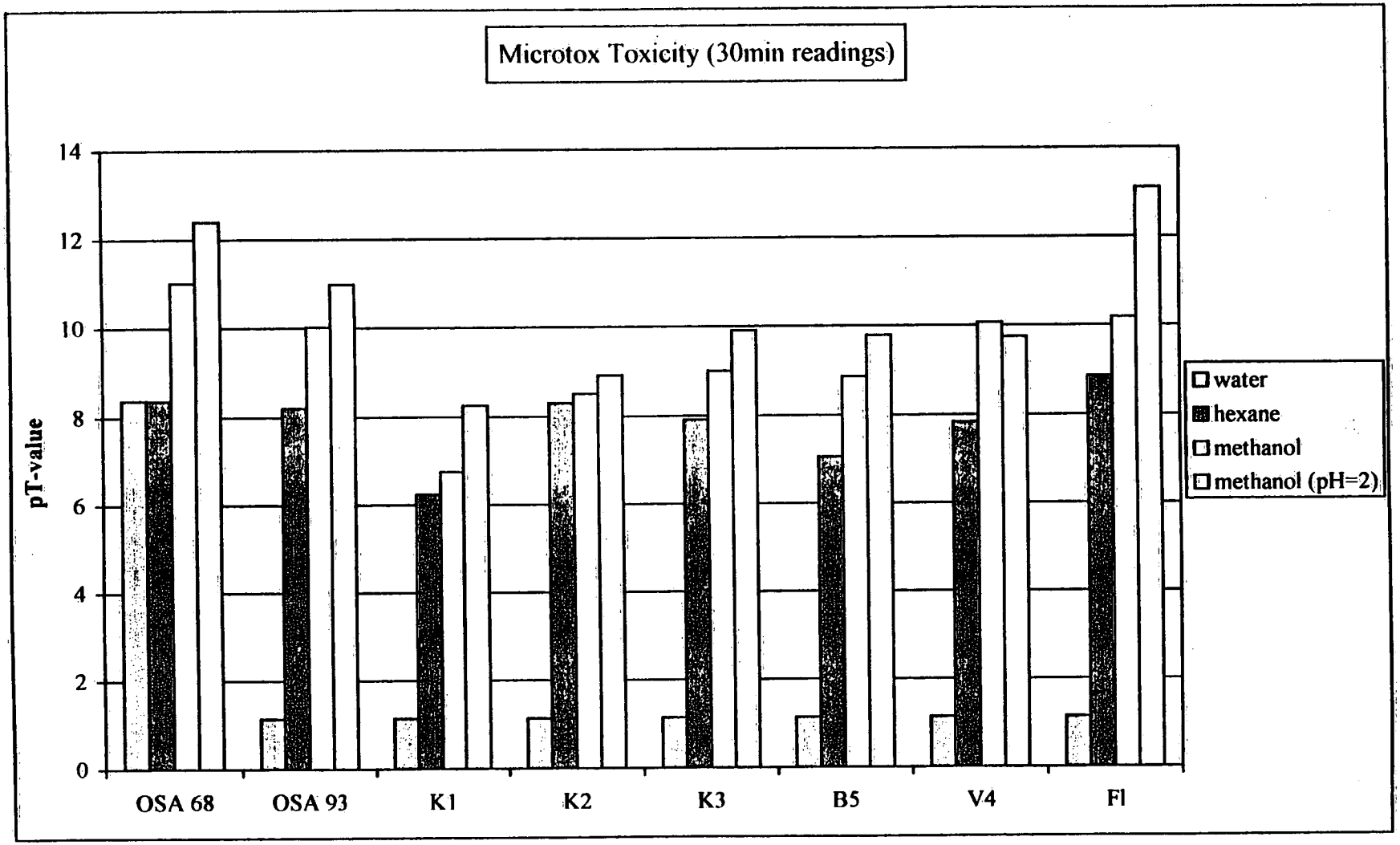


Figure 2c: Comparison of the Microtox Toxicity in the different sediment extracts (30 min readings)



Muta-ChromoPlate Mutagenicity Assay:

This assay was performed using a 96-micro-well procedure and was scored by recording the number of wells containing revertant cells (positive wells). With this assay, a sample was considered mutagenic when one or more doses of sediment extract induced a significant ($p<0.05$) doses-related increase in the number of positive wells relative to the negative control (Rao et al.,1994).

In this investigation two strains of ***Salmonella thyphimurium*** namely TA-98 and TA-100 were used to detect the mutagenicity of the methanol fraction of the eight (8) sediments at pH 7.0. These extracts were diluted with water to give a concentration of 0.5%. The 1mL of the 0.5% methanol extract represent a sediment content of 5mg equivalent. A negative control of 0.5% methanol was used. The positive control for TA-98 was 2-nitro-fluorene and for TA-100 was sodium azide. The mutagenicity measurements were carried out using the Muta-ChromoPlate protocol (Rao and Lifzhitz. 1995). Table 3a) and 3b) shows the results of the Muta-ChromoPlate Assay for the two bacteria strains used. Figure 3a) and 3b) shows the dose-response of the different methanol extracts of the samples. The thick lines in the graphs show the significance level for mutagenic response ($p= 0.05$)

Table 3a: Results of Muta-ChromoPlate Assay of TA98

Test-Strain: **TA 98**

Positive control: 2-nitro-fluorene
Samples: 0.5% of methanol (pH=7) extracts
Start time 31.09.1995
Reading time 05.10.1995

Results: (number of positive wells)

Negative Control: 0
Positive Control: 96
Background: 4
Significance level: 10

Extracts:

Sample	2,5mL	5,0mL	10,0mL	15,0mL
OSA 68	5	6	6	9
OSA 93	3	1	1	2
K1	2	4	3	-
K2	2	3	2	2
K3	7	4	3	2
B5	6	4	2	3
V4	6	2	2	4
F1	4	4	5	3

Table 3b: Results of Muta-ChromoPlate Assay of TA100

Test-Strain: **TA 100**

Positive control: Sodium-azide
Samples: 0.5% of methanol (pH=7) extracts
Start time 31.09.1995
Reading time 05.10.1995

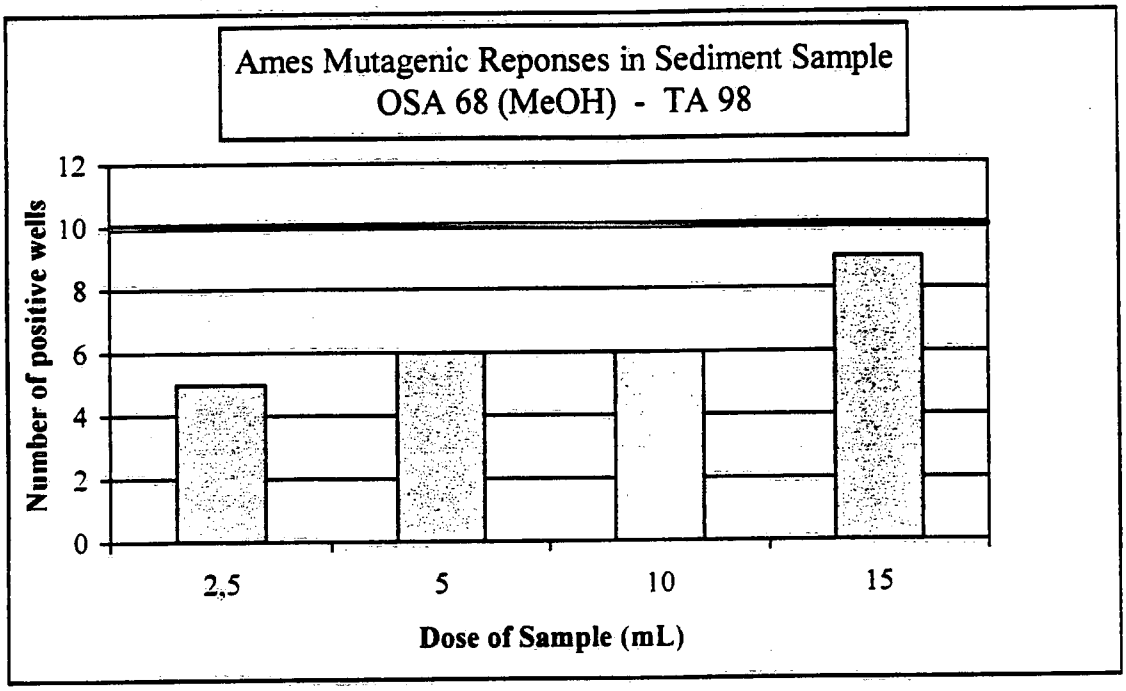
Results: (number of positive wells)

Negative Control: 0
Positive Control: 92
Background: 4
Significance level: 10

Extracts:

Sample	2,5mL	5,0mL	10,0mL	15,0mL
OSA 68	8	13	22	19
OSA 93	16	9	10	12
K1	10	10	10	10
K2	12	6	6	10
K3	4	6	7	8
B5	12	14	6	9
V4	13	8	6	10
F1	9	9	5	12

Figure 3a: Results of Muta-ChromoPlate Assay (TA 98)



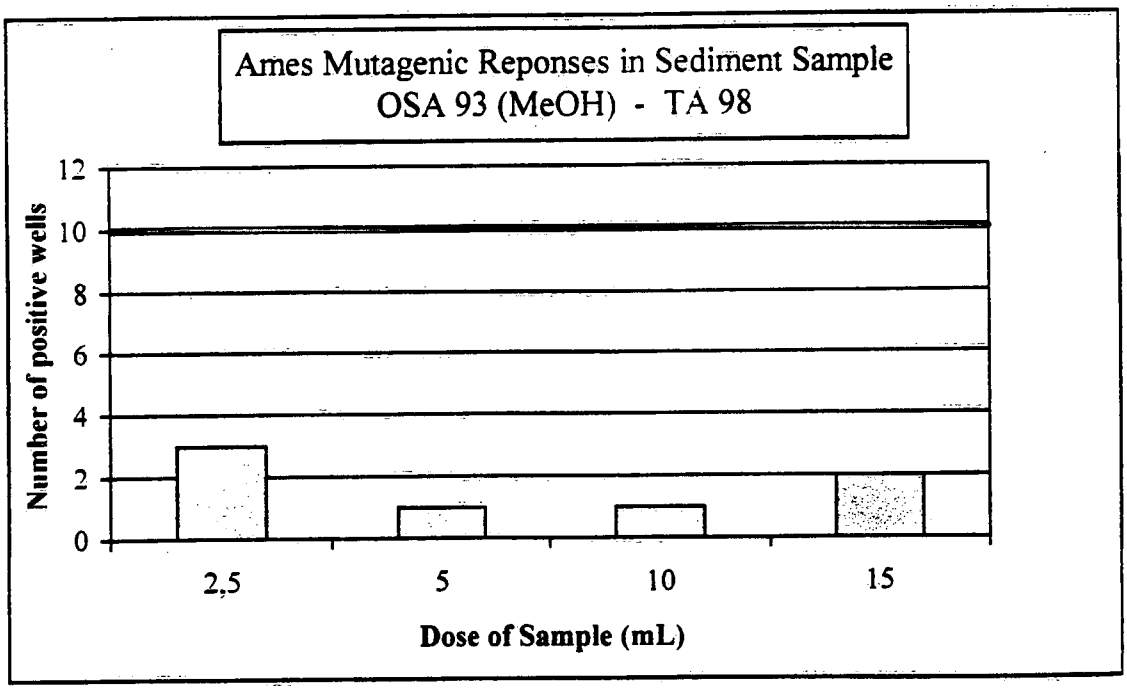
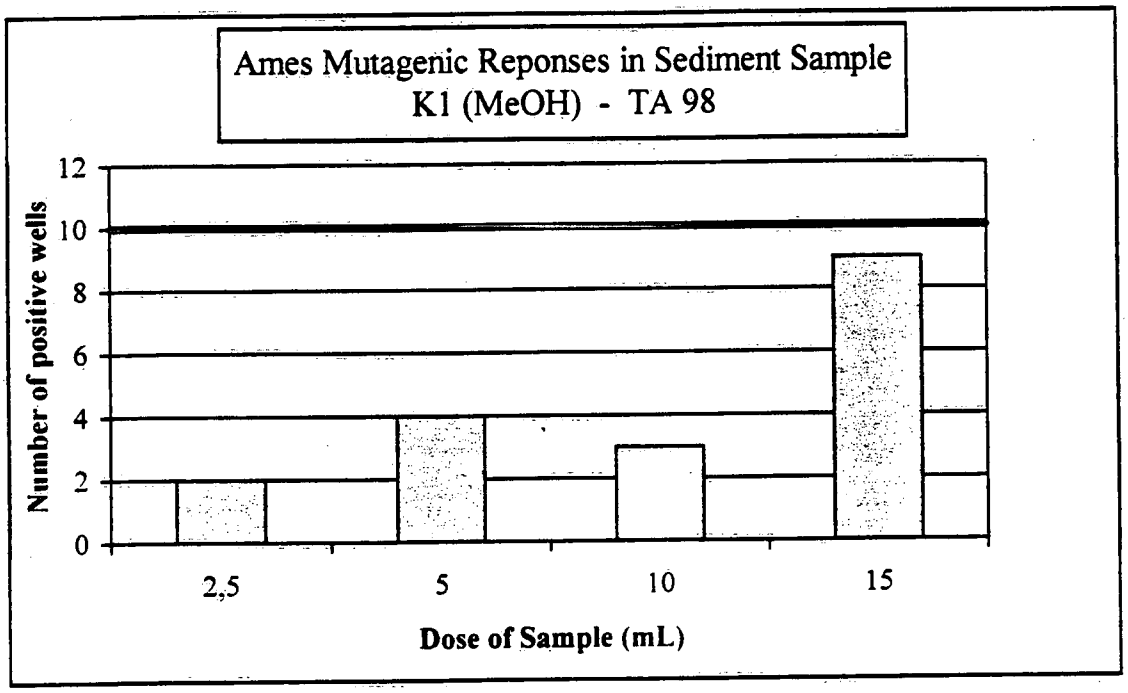


Figure 3a: Results of Muta-ChromoPlate Assay (TA 98)



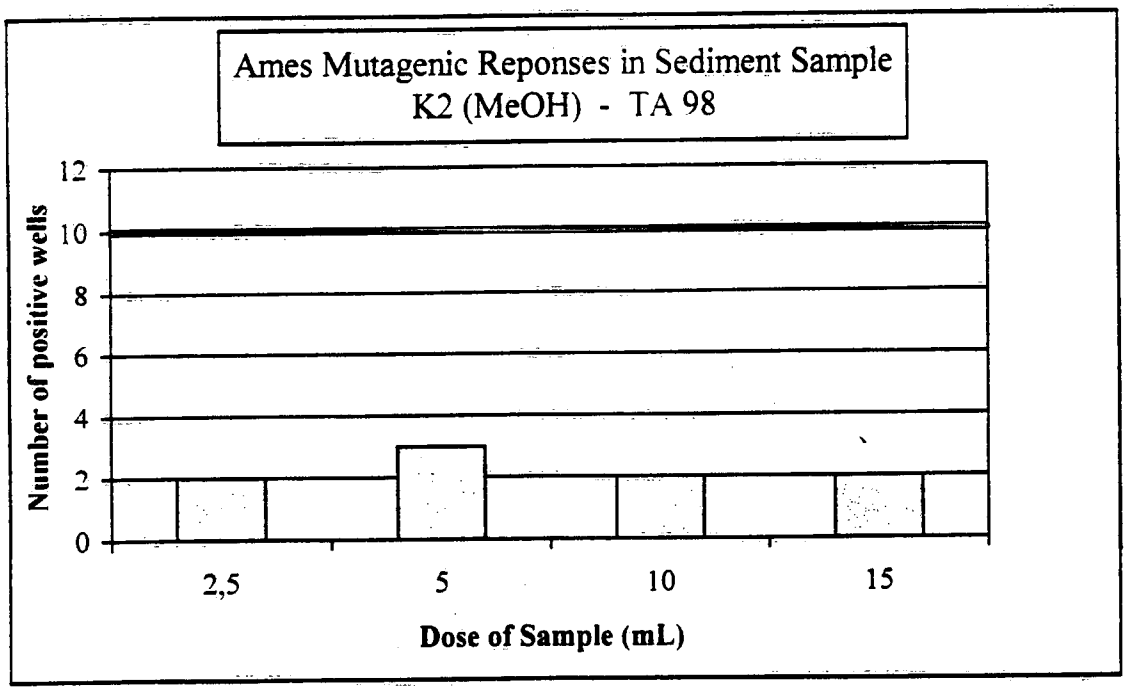
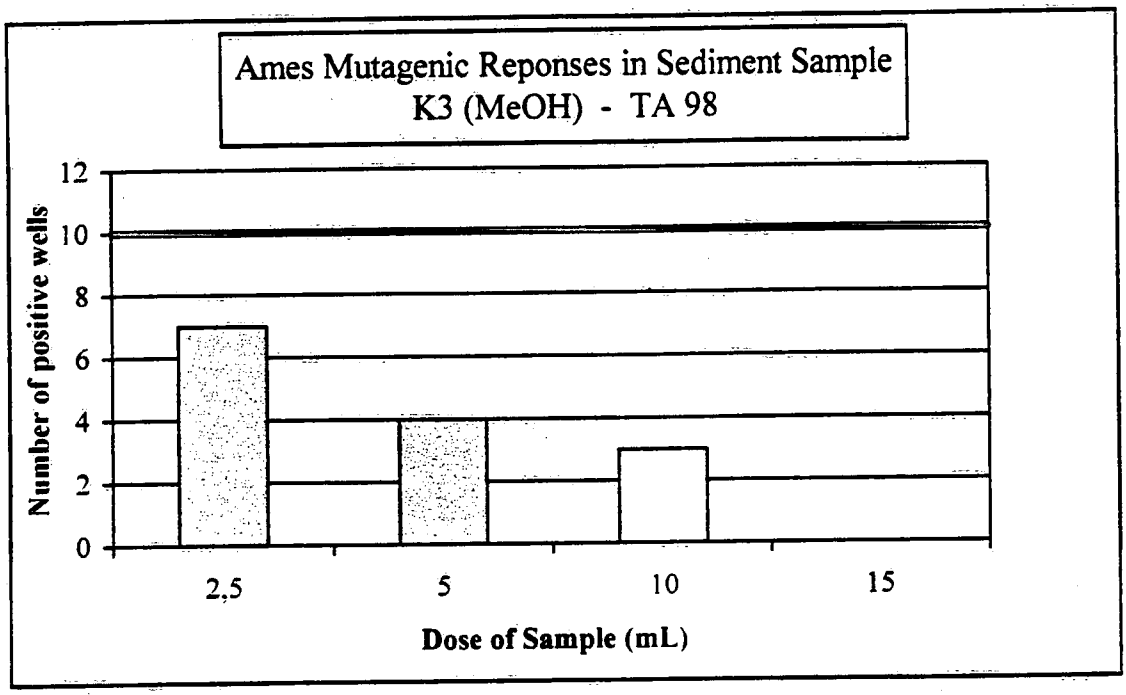


Figure 3a: Results of Muta-ChromoPlate Assay (TA 98)



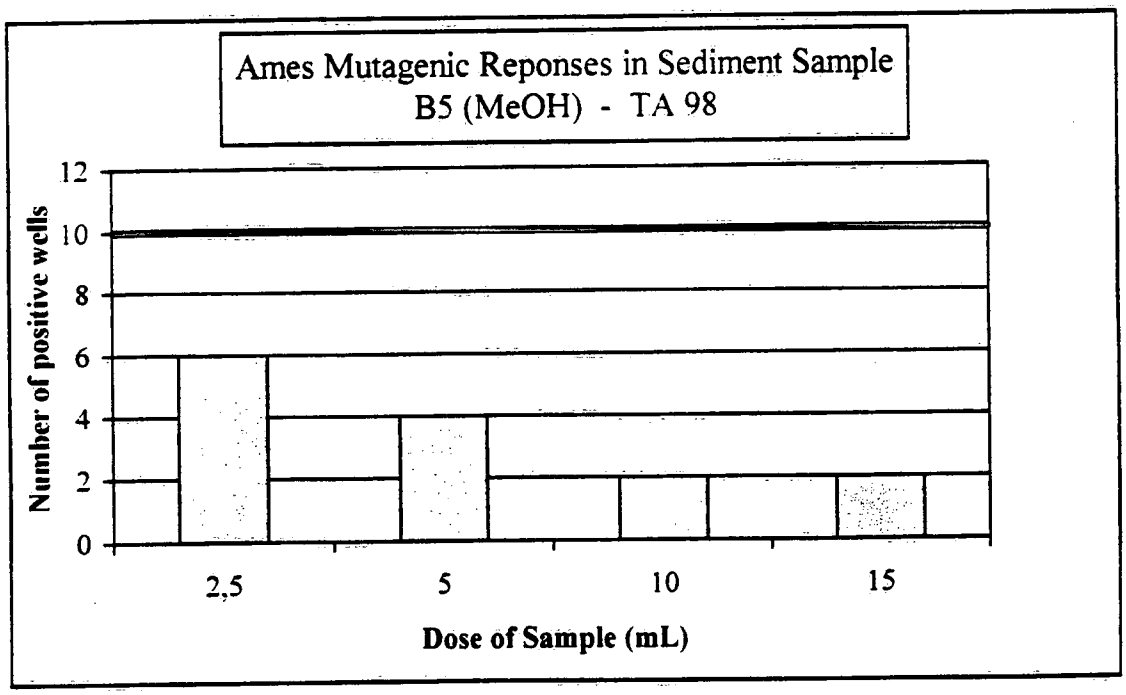
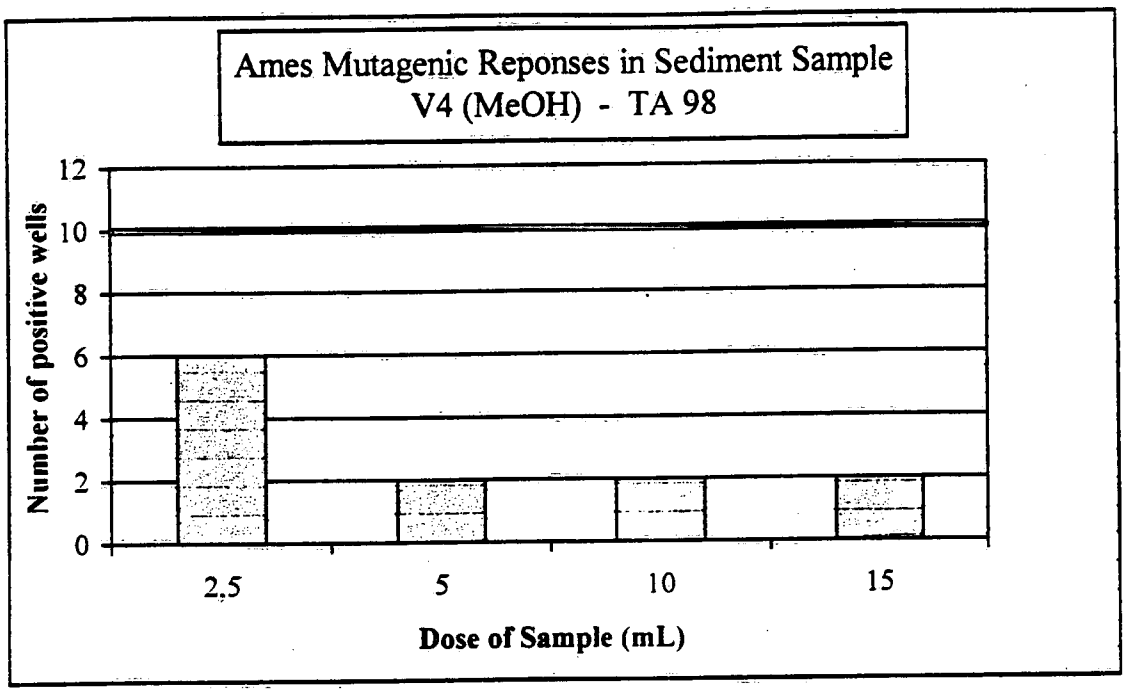


Figure 3a: Results of Muta-ChromoPlate Assay (TA 98)



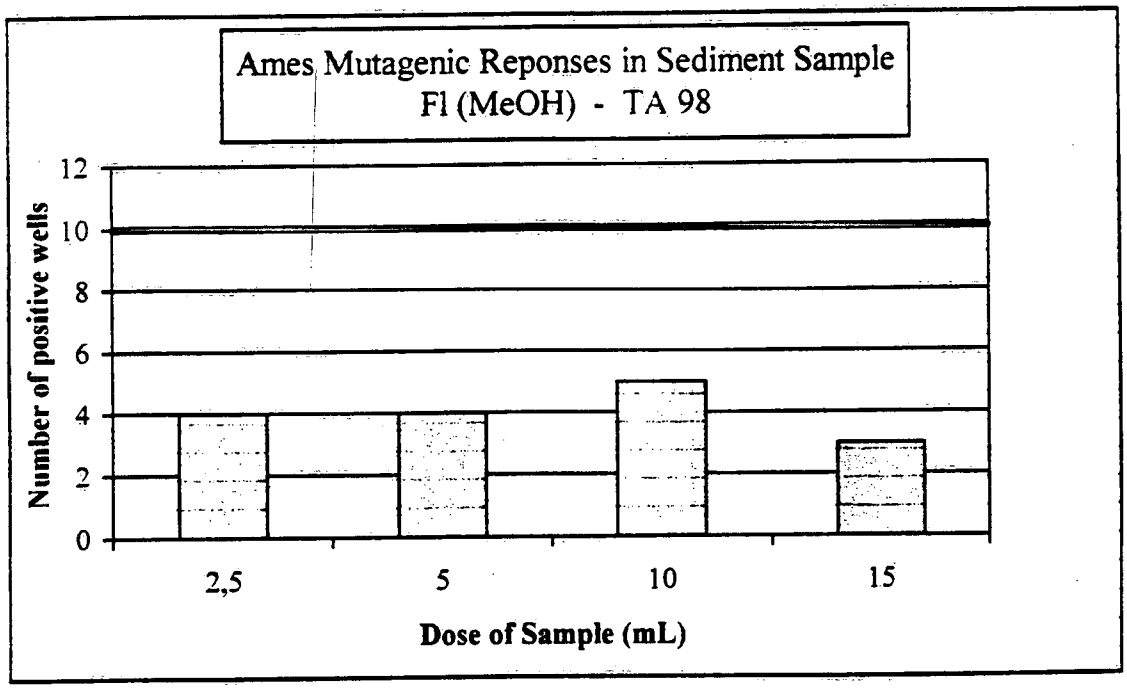
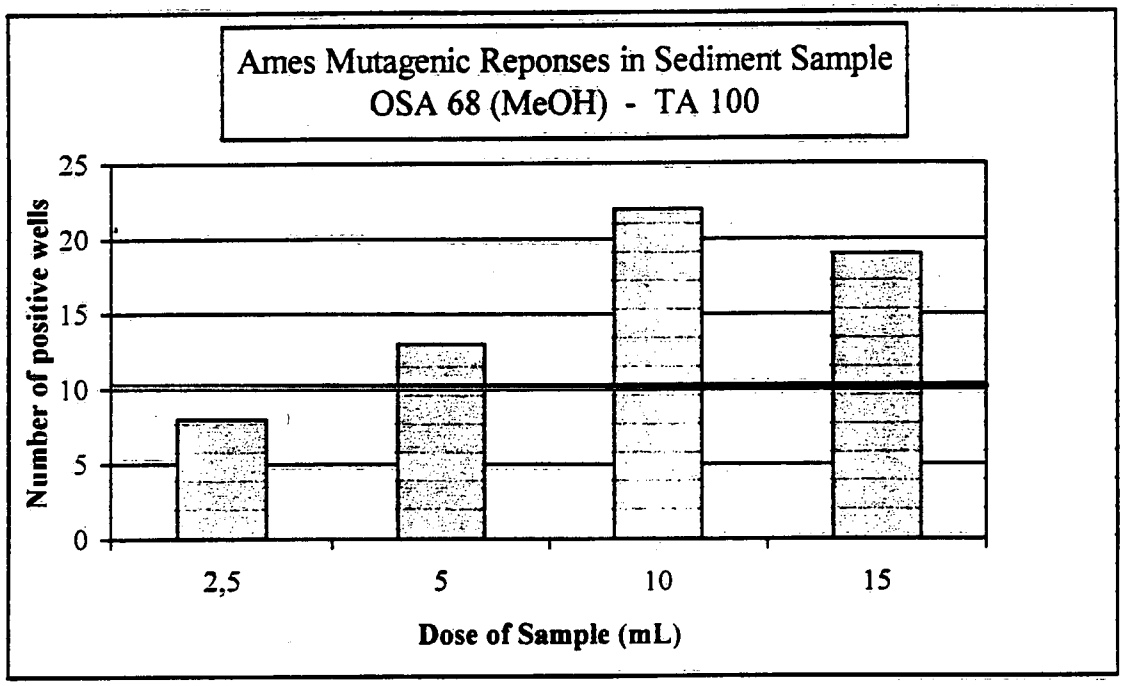


Figure 3b: Results of Muta-ChromoPlate Assay (TA 100)



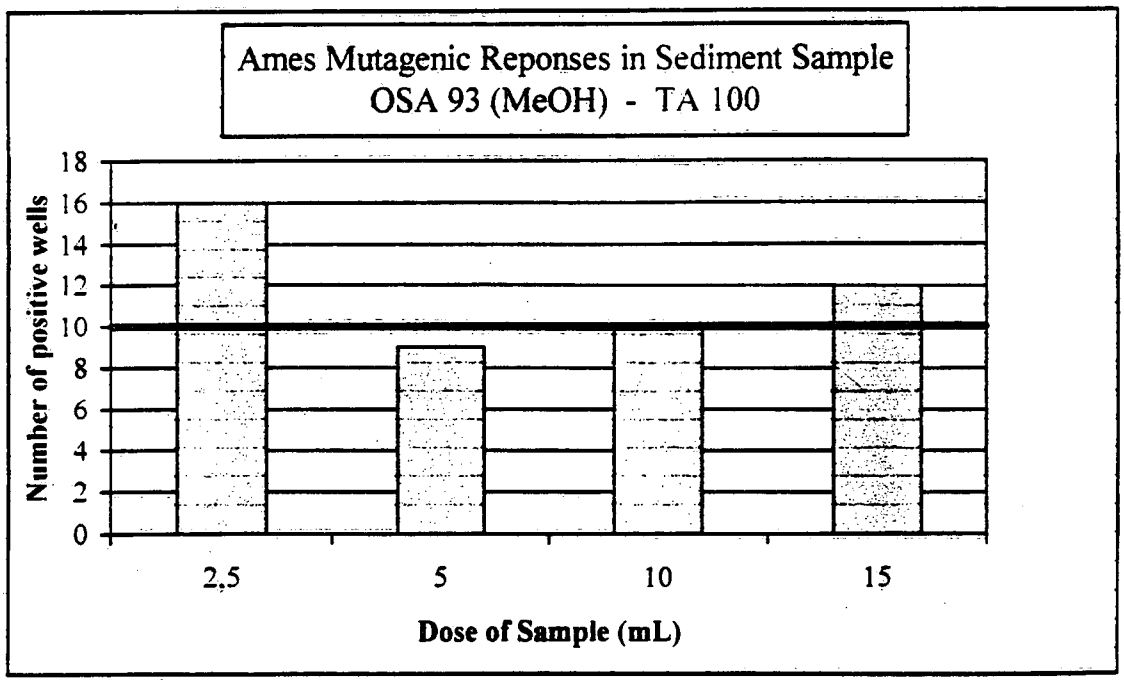
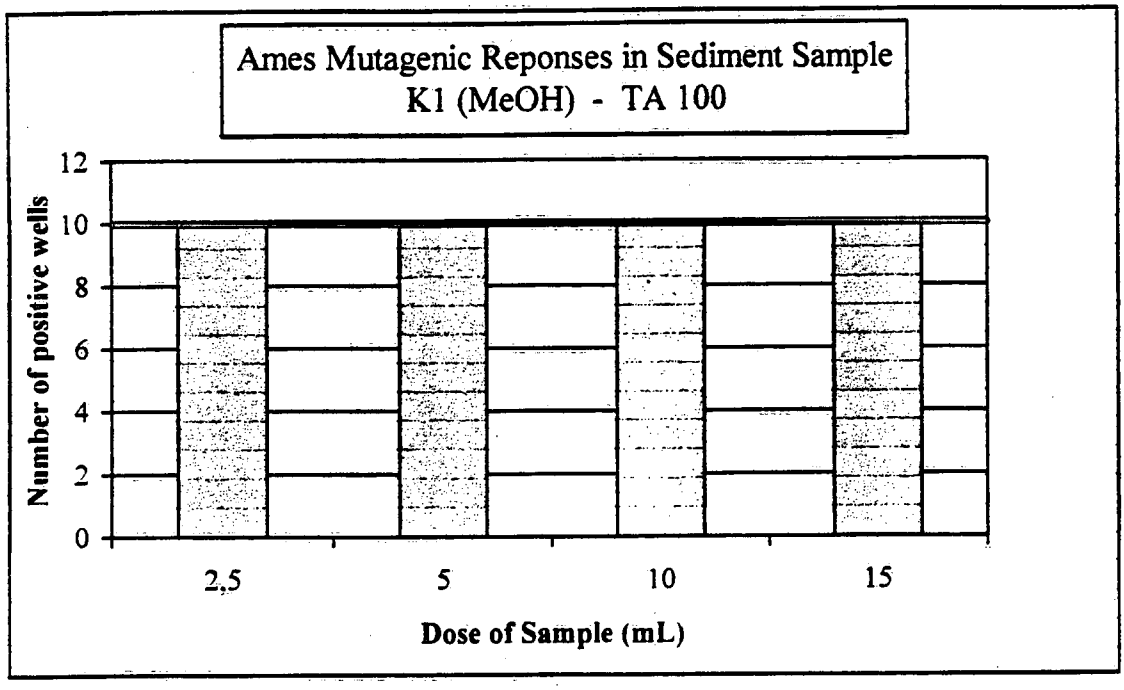


Figure 3b: Results of Muta-ChromoPlate Assay (TA 100)



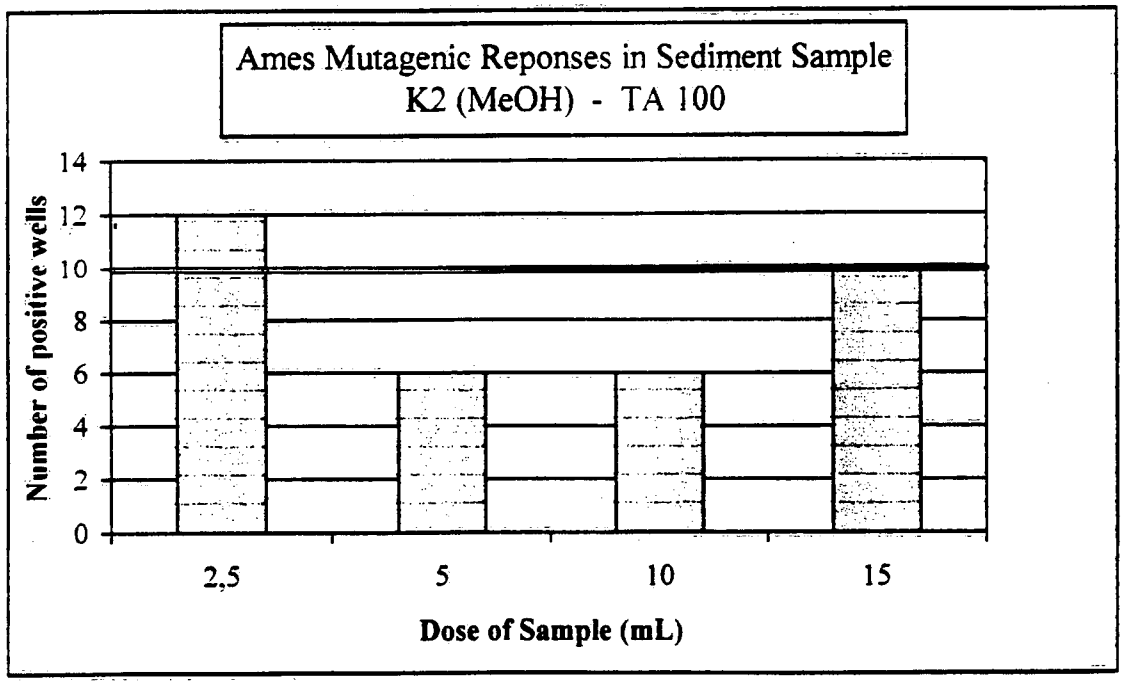
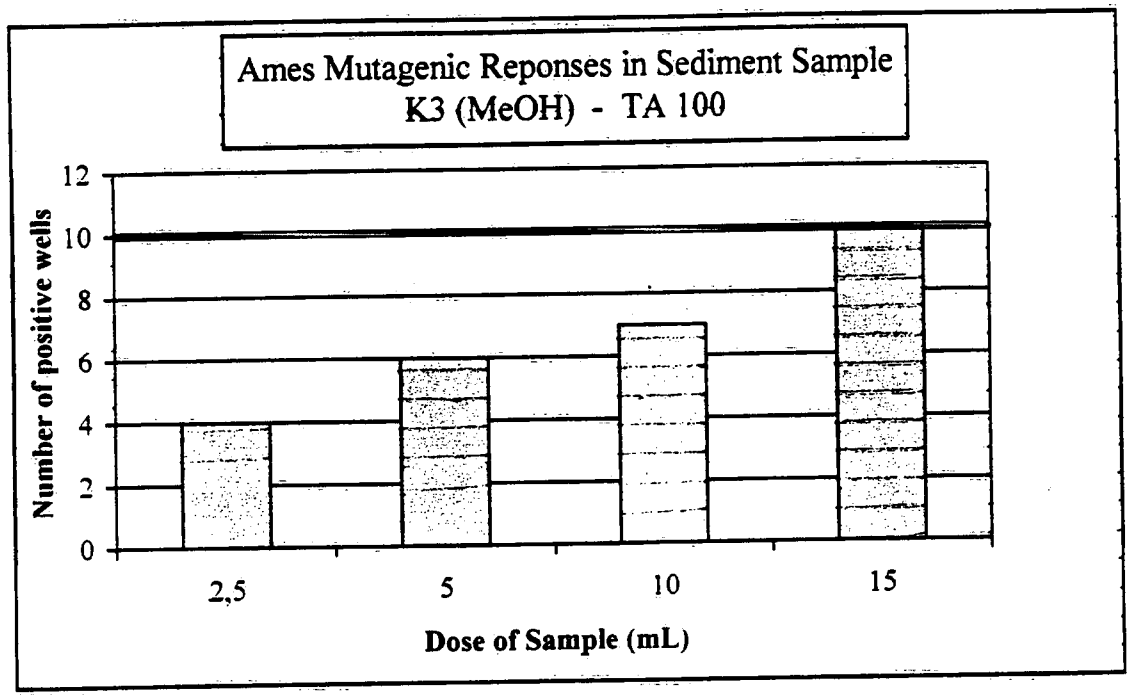


Figure 3b: Results of Muta-ChromoPlate Assay (TA 100)



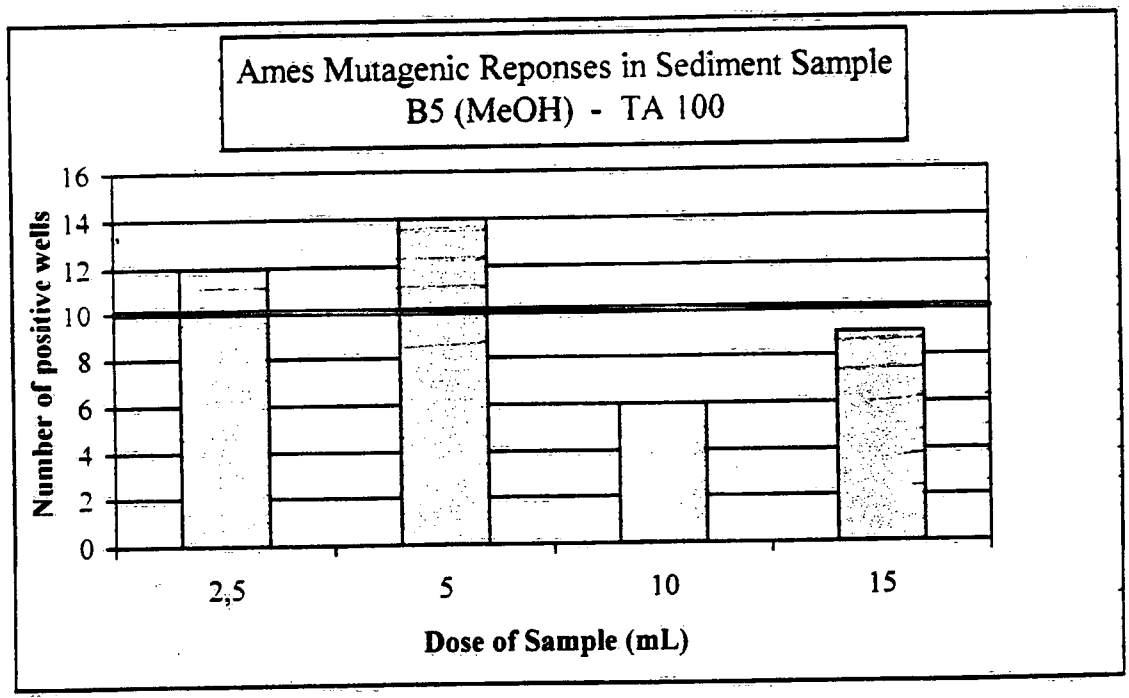
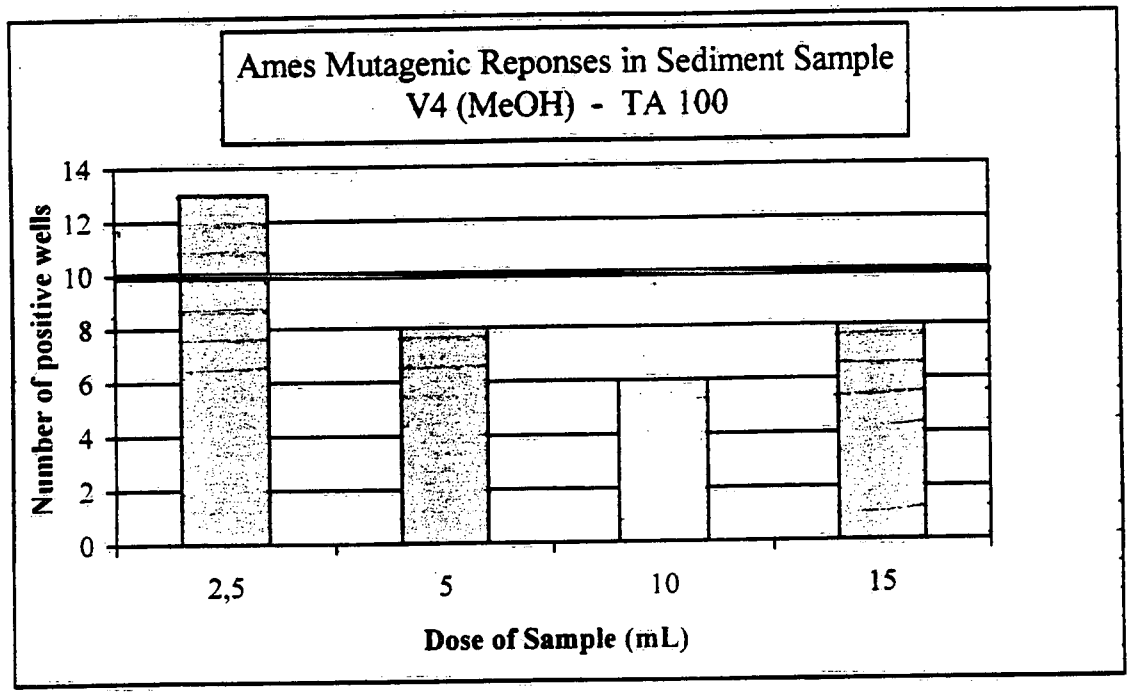
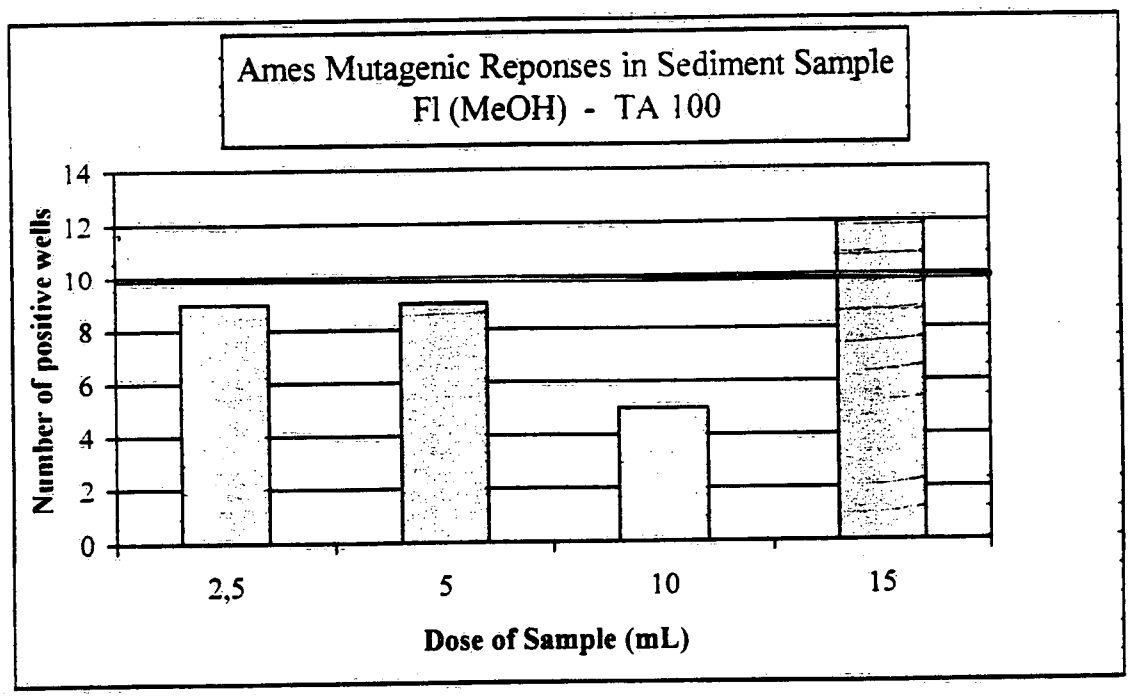


Figure 3b: Results of Muta-ChromoPlate Assay (TA 100)





Fish Hepatic Micronucleus Assay:

For our investigation rainbow trouts, *Oncorhynchus mykiss* (20-65g), were used. After anaesthesia with MS-222 (tricane methyl sulphonate), five to seven rainbow trouts (for each treatment) were injected inter-peritoneal (i.p.) with either 2μL/g fish of diethylnitrosamine (DEN) (35% DEN in corn oil) (positive control), 0.2μL/g fish of 0.5% methanol in water (negative control), and 0.2μL/g fish of 0.5%, 0.25% and 0.125% dilution of the methanol extract from sediment OSA 68 in water. In a previous experiment two fish were injected with a 0.5% methanol solution in water to test the toxicity of the methanol to fish. The fish in this experiment overlived one week and showed that the methanol was non-toxic to fish at this dose. The fishes were then placed in 16-20 L aquaria containing 10 L of continuously aerated, dechlorinated Burlington, Ontario city water, and the water in each tank was replaced every 24 hours. After 48 hours, the fish were re-injected a second time with the same volume of extract and placed back in the appropriate aquaria. At 48 hours following the final injection, the fish were injected with 0.1μL/g fish of allyl formate (AF) to induce hepatocyte proliferation. AF induces liver necrosis and subsequent regenerative proliferation of hepatocytes in the liver (Williams and Metcalfe, 1992). Table 4 shows the dates of the i.p. injection protocol. During the experiment, mortalities occurred among the treated fish; possibly because of the trauma of the experimental procedure. Ninety-six hours after the allyl formate (AF) injection, the remaining fish were sacrificed with an over dose of MS-222.

Table 4: Treatment protocol of rainbow trouts for the hepatic micronucleus assay

a) Negative control: 0.5% methanol, 0.2μL/g rainbow trout

weight of fish (g)	dose of sample (μL)	injection of sample 05.Sept.95	injection of sample 07.Sept.95	dose of allyl formate (μL)	injection of allyl formate 09.Sept.95	final number of fish
17.6	4	+	+	2	+	1
34.6	6	+	+	3	+	2
43.8	8	+	+	4	+	3
43.3	8	+	+	4	+	4
30.2	6	+	dead			
31.1	6	+	dead			
27.8	6	+	+	2	+	5

b) Positive control: 35% DEN in corn oil, 0.2μL/g rainbow trout

weight of fish (g)	dose of sample (μL)	injection of sample 05.Sept.95	injection of sample 07.Sept.95	dose of allyl formate (μL)	injection of allyl formate 09.Sept.95	final number of fish
25.6	6	+	+	2	+	6
35.6	8	+	+	2	+	7
21.7	4	+	+	1	+	8
41.5	8	+	+	dead		
32.4	6	+	+	2	+	9
41.8	8	+	+	dead		

Table 4: Treatment protocol of rainbow trouts for the hepatic micronucleus assay (cont'd)

c) OSA 68 (MeOH, pH=7): 0.125% methanol extract, 0.2µL/g rainbow trout

weight of fish (g)	dose of sample (µL)	injection of sample 05.Sept.95	injection of sample 07.Sept.95	dose of allyl formate (µL)	injection of allyl formate 09.Sept.95	final number of fish
36.5	8	+	+	2	+	10
37.6	8	+	+	2	+	11
40.8	8	+	+	2	+	12
49.8	10	+	dead			
30.7	6	+	dead			
30.6	6	+	+	2	+	13

d) OSA 68 (MeOH, pH=7): 0.25% methanol extract, 0.2µL/g rainbow trout

weight of fish (g)	dose of sample (µL)	injection of sample 05.Sept.95	injection of sample 07.Sept.95	dose of allyl formate (µL)	injection of allyl formate 09.Sept.95	final number of fish
65.9	12	+	+	3	+	14
33.4	6	+	+	2	+	15
27.7	6	+	+	2	+	16
54.2	10	+	+	3	+	17
36.8	8	+	dead			
35.7	8	+	+	2	+	18

e) OSA 68 (MeOH, pH=7): 0.5% methanol extract. 0.2µL/g rainbow trout

weight of fish (g)	dose of sample (µL)	injection of sample 05.Sept.95	injection of sample 07.Sept.95	dose of allyl formate (µL)	injection of allyl formate 09.Sept.95	final number of fish
52.3	10	+	+	3	+	19
21.3	4	+	+	1	+	20
28.8	6	+	dead			
19.8	4	+	+	dead		
22.6	4	+	+	1	+	21
38.6	4	+	+	2	+	22

Hepatocyte suspensions were prepared from trout livers as described by Williams and Metcalfe. 1992). Briefly, the livers were macerated and rinsed with a 1% citrate solution, and then placed in 8ml of 0.1% collagenase and vortexed approximately 8 times in a 30 min period. The hepatocyte suspension produced by this treatment was centrifuged for 2 min by approximately 650G, and the hepatocyte pellet was resuspended in 2-3 drops of citrate solution. The remaining solution was then

centrifuged for a second time for 3 min by approximately 1250G, and the hepatocyte pellet was treated as described before. The suspensions were applied to microscope slide with a Pasteur pipette as described by Williams and Metcalfe (1992) and air dried. The slides were placed for 1h in 5N HCl and afterwards for 10min in 1N HCl to break the covalent bindings of the DNA. The slides were placed in Carnoy's fixative for 1 h, and then allowed to dry. The slides were stained with Schiff's reagent and counter-stained with 2% light green in ethanol. Afterwards the slides were examined by a light microscope with an oil immersion lens (1000x) and micronuclei were identified as Schiff-positive microbodies in the cytoplasm. All slides were coded and scored blind and 1000 hepatocytes were scored per fish. Differences between the mean incidence of micronuclei (per 1000 hepatocytes) in each treatment were tested statistically using non-parametric methods.

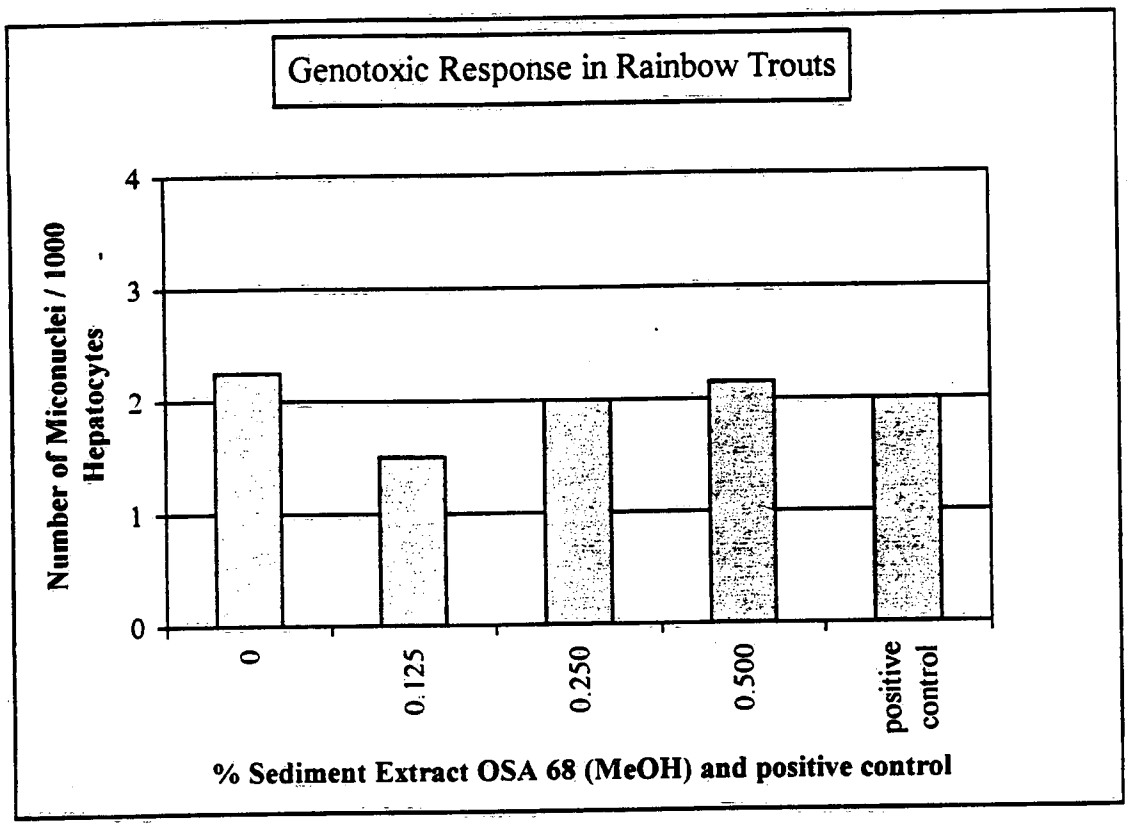
From the large amount of prepared slides 11 were scored for micronuclei. Table 5 showed the number of the examined fish, the treatment procedures of these fishes and the number of the counted micronuclei. In the fourth column the calculated average numbers of micronuclei/1000 hepatocytes at the different treatment levels are shown.

Table 5: Genotoxic responses in rainbow trouts

Fish number	Treatment	Micronuclei / 1000 Hepatocytes	Average micronuclei / 1000 Hepatocytes
1	negative control (0.5% methanol)	0	2.25
2		0	
4		6	
4		3	
12	0.125% OSA 68 (MeOH)	1	1.50
13		2	
16	0.250% OSA 68 (MeOH)	0	2.00
17		4	
20	0.500% OSA 68 (MeOH)	6.45 (4/620)	2.15
21		0	
21		0	

Figure 4 shows the average number micronuclei/1000 hepatocytes at the different treatment levels. The numbers of micronuclei from the positive control (35% DEN in corn oil) have an average number of 2 micronuclei/1000 hepatocytes.

Figure 4: Average number of micronuclei/1000 hepatocyctes in extract OSA 68 (MeOH)



Chemical analysis of the sediment extracts:

In the analysis of the sediment extracts we were confronted with two problems: 1) the extracts were polluted with many unknown organic compounds; 2) these compounds belong to many different categories or classes of chemicals. Therefore, it was necessary to apply an analytical method that can separate and identify these different substances. The best approach was to resolve this problem was to apply the high resolution gas chromatography with mass selective detection (HRGC/MS). The high resolution gas chromatography with capillary column is one of the best separation technique and from the resultant mass spectra the structure of the compounds can be determined.

For our investigation we used a HP 5890 series II gas chromatograph with a HP 5989A mass spectrometer (Hewlett Packard, Waldbronn, Germany). For the separation a DB 5-625 (methyl-/ 5% phenylpolysiloxane phase) column (J&W Scientific, USA) was used. The column parameters were 30m length, 0.32 inner diameter and a film thickness of 0.25mm. Helium was used as carrier gas with a flow of 30cm/sec (column head pressure 41psi). The used chromatographic temperature program was 40°C (3min) --> 10K/min --> 100°C --> 3K/min --> 260°C (10min). The injection was carried with a HP 7673 on-column auto sampler (Hewlett Packard, Waldbronn, Germany) and the following injection temperature program: 40°C (10min) --> 5K/min --> 120°C. The injected volume was 2.5mL.

The mass spectrometer conditions were as followed: scan region 50-500amu, EI-(electron impact) ionisation, ionisation energy 70eV, ion source temperature 200°C, quadruple temperature 100°C.

and transfer line temperature 280°C. The obtained mass spectra were compared with spectra from a NIST library (>120000 compounds) in a HP apollo series 400 UNIX computer. A spectrum will be identified, when the comparability is over 90%.

For the GC/MS analysis a non-polar solvent was needed. In this case we employed the water and the methanol extracts in a more convenient solvent. Also the levels of the components were too low for the detection limit in the GC/MS system. In order to increase the levels by a factor of five, 5mL of the water extracts were extracted with 1mL dichloromethane (DCM) and dried with Na_2SO_4. The hexane extracts were dried with Na_2SO_4 and concentrated by a factor of five under a N_2 flow. 2mL of the methanolic extracts (pH=7 and pH =2) were extracted with 0.4mL hexane and dried with Na_2SO_4.

Water extracts

The water extracts of the sediment samples were less contaminated with organic compounds except the sample OSA 68, which contained 4,4′-bisphenol-dimethyl-methane (Bisphenol A). Bisphenol A is an industrial chemical produced during the synthesis of epoxy resins. Besides this chemical (99%) following compounds were found in minor levels: p-hydroxy-toluene, 2-hydroxy-naphthalene, and 2,4′-bisphenol-dimethyl-methan. The other samples showed no organic contaminants in their water extracts.

Hexane extracts

The hexane extracts of the different sediments were also less polluted with organic compounds except of the sample OSA 68. In this extract following component groups were found: methylated toluenes (1,3-dimethyl-toluene, 1,2,3-trimethyl-toluene, and 1,3,5-trimethyl-toluene), chlorinated ethers (bis(1,3-dichloroprop-2-yl)ether, bis(2,3-dichloropropyl)ether), alkyl-cyanides (hexadecane-nitrile, octadecane-nitrile), and alkanes (tetracosane, docosane, tricosane). Also small amounts of naphthalene, hexachloro-1,3-butadiene, N-phenyl-2-naphthalenamine, 2,4′-DDD, bisnaphthyl-sulfone, and different cholesterol derivates were found. The majority of the these compounds eluted in the methanol extract as discussed below. Also sulphur can be found in this extract. The other sediments show only sulphur in the hexane extracts. The low levels of organic components in this type of extracts can also occur from the concentration step by evaporating the hexane under a nitrogen flow. In this step there can be a loss of organics, when the evaporation was not done carefully. For this purpose the extracts have to be checked with further analysis.

Methanol extracts (pH=7)

The methanol extracts of the sediments are highly polluted with organic chemicals. Over 90% of these compounds could be identified. Table 6 shows the identified substances in the different sediment samples. The compounds are subdivided in different classes of chemicals like alkanes and alkenes, alkylated benzenes, naphthalene and derivates, polycyclic aromates (PAH) and derivates, chlorinated compounds, carbon acid methyl esters, phthalic acid esters, nitriles, cholesterol derivates and various compounds.

Table 6: Organic compounds identified in the methanol fractions of German sediments (xxx: dominating compounds, xx: medium levels, and x: trace amounts)

	OSA 68	OSA 93	K1	K2	K3	B5	V4	Fl
alkanes and alkenes								
nonane	x							
3-methyl-nonane	x							
decane	x	x	xx	xx	x	x	x	x
undecane	x	x		x				x
dodecane	x							x
tridecane	x							x
tetradecane								x
1.13-tetradecadiene								x
pentadecane								x
2.6.10.14-tetramethyl-hexadecane	x							
1-hexadecene								x
heptadecane		x						x
eicosane	x							
5-eicosene (E)		x						
1-nonadecene							x	
2.6.10.15.19.23-hexamethyl-tetracosane	x							
2.6.10.14-tetramethyl-hexadecane	x							
cyclohexadecane	x	x						
2.6.10.14,18,22-hexamethyl-2.6.10.14.18.22-tetracosahexane	x							
cyclotetracosane		x			x			
9-tricosene (Z)								x
alkylated benzenes								
1.2-dimethyl-benzene	x							
1-methyl-ethyl-benzene	x							
propyl-benzene	x							
1-ethyl-2-methyl-benzene	xx							
1-ethyl-4-methyl-benzene	x							
1.2.3-trimethyl-benzene	xxx							
1.2.4-trimethyl-benzene	x							
1.3.5-trimethyl-benzene	xx							
1.2-diethyl-benzene	x							
1.3-diethyl-benzene	x							
1-methyl-4-(1-methylethyl)-benzene	x							
1-ethyl-2.4-dimethyl-benzene	x							
1-methyl-3-(1-methylethyl)-benzene	x							
(1-butylhexyl)-benzene	x							
(1-propylheptyl)-benzene	x							
(1-ethyloctyl)-benzene	x							
(1-pentylhexyl)-benzene	x							
(1-butylheptyl)-benzene	x							

Table 6: Organic compounds identified in the methanol fractions of German sediments (cont'd)

	OSA 68	OSA 93	K1	K2	K3	B5	V4	Fl
(1-propyloctyl)-benzene	x							
(1-ethylnonyl)-benzene	x							
(1-pentyl-heptyl)-benzene	x							x
(1-butyloctyl)-benzene	x							
(1-pentyloctyl)-benzene	x							
(1-butylnonyl)-benzene	x							
(1-propyldecyl)-benzene	x							
(1-ethylundecyl)-benzene	x							
naphthalene and derivates								
naphthalene	x							
1-methyl-naphthalene	x							
2-methyl-naphthalene	x							
2-ethyl-naphthalene	x							
1.8-dimethyl-naphthalene	x							
2.3-dimethyl-naphthalene	x							
2.4-dimethyl-naphthalene	x							
2.6-dimethyl-naphthalene	x							
2.3.6-trimethyl-naphthalene	x							
1.2.3.4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene	x							
polycyclic aromates (PAH) and derivates								
phenanthrene	xx							
anthracene	x							
3-methyl-phenanthrene	x							
4H-cyclopenta(def)phenathrene	x							
fluoranthrene	xx							
1.2.3.4.4a.9.10.10a-octahydro-3,3,9-trimethyl-7-(1-methylethyl)-phenanthrene	x							
pyrene	x							
1-methyl-pyrene	x							
chrysene	x							
benz(a)anthracene	x							
chlorinated compounds								
hexachloro-1.3-butadiene	x							
pentachlorobenzene (QCB)	x							
hexachlorobenzene (HCB)	xx							
1.1.1-trichloro-2.2-bis(p-chloro-phenyl)ethane(DDT)	x							

Table 6: Organic compounds identified in the methanol fractions of German sediments (cont'd)

	OSA 68	OSA 93	K1	K2	K3	B5	V4	Fl
carbon acid methyl esters								
decanoic acid, methyl ester		x						
dodecanoic acid, methyl ester								x
tridecanoic acid, methyl ester		x						
tetradecanoic acid, methyl ester		xx				x	x	xx
12-methyl-tetradecanoic acid, methyl ester								x
pentadecanoic acid, methyl ester		x				x		x
heneicosanoic acid, methyl ester	x							
11-hexadecenoic acid, methyl ester	x	xx				xx		xxx
9-hexadecenoic acid (Z), methyl ester							xx	x
hexadecanoic acid, methyl ester	xx	xx			x	xx	xx	xxx
hexadecatrienoic acid, methyl ester								x
9,12-octadecadienoic acid (Z,Z), methyl ester	x							x
8-octadecenoic acid (Z), methyl ester							x	xx
9-octadecenoic acid (Z), methyl ester	x	x				x		xx
10-octadecenoic acid (Z), methyl ester						x		
13-octadecenoic acid, methyl ester							x	
octadecanoic acid, methyl ester	x	x			x		x	xx
methyl arachidonate								x
phthalic acid esters								
butyl-, 2-ethylhexyl-phthalic acid ester	x							
bis(2-ethylhexyl)-phthalic acid ester	xx	xx		x	xx	x	xx	xxx
nitriles								
hexadecanenitrile	x							
octadecanenitrile	x							
cholesterol derivates								
dihydrocholesterol	xxx	xx			x	xx	xx	xxx
cholest-5-en-3-ol-(3β)....	xxx	xxx		x	xx	xx	xx	xxx
cholestan-3-ol.(3α,5β)-....	xx	xxx						xx
cholestan-3-ethoxy,(3α,5β)-...							x	
methyl ergostan-3-ol.(3β,5α)-...	xx	x						
ergost-5-en-3-ol. (3β)...		xx						
(24R,25R)-24,26-dimetylcholesta-5,22...		x						
(24R,25R)-5,6-dihydro-5α-aplystero...		xx			x			
stigmast-5-en-3-ol. (3β,24R)-...		xx			xx			
cholest-4-en-3-one	x							
stigmasta-5,23-dien-3β-ol	x							
24,11-etylcholest-5-en-3β-ol						xx		
D-friedoolean-14-en-3-one					x			

Table 6: Organic compounds identified in the methanol fractions of German sediments (cont'd)

	OSA 68	OSA 93	K1	K2	K3	B5	V4	Fl
various compounds								
decahydro-1,5,5,8a-1,2,4-methenoazulene	x							
1,1'-biphenyl	x							
junipene	xx							
β-patchouleane	x							
1,2-dihydro-acenaphthylene	xx							
dibenzofurane	x							
9H-fluorene	x							
2-methyl-1,1'-biphenyl	x							
1,2,3,4,5,6,6-heptamethyl-cyclohexadienyl	x			xx				
sulphur	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xx
2,2'-((1-methylethylidene)bis(4-phenoldimethyl)methane)-oxirane	x							
1-docosanol, formate		x						
2,4-bis(1-methylethyl)-phenol				x			x	
1-(4-(hydroxy-1-methylethyl)-phenylethanone					x			
10-methoxybenz(a)azulen-1,4-dione					x			
3.7,11,15-tetramethyl-2-hexadecen-1-ol						x	x	xxx

The examined sediments are contaminated with a broad range of different organic compounds. The highest polluted sediment was OSA 68 with 89 identified compounds. In sample K1 only two chemicals (decane and sulphur) were observed. The other sediments showed a low to medium contamination (OSA 93: 25 chemicals; K2: 7; K3: 13; B5: 13; V4: 15; and Fl: 30). The groups of alkylated benzenes, naphthalene and ist derivates, polycyclic aromates and derivates, chlorinated compounds, nitriles, and some other compounds could be only found in the methanol extract of OSA 68. In comparison with the other samples, sediment OSA 68 has a local point source input of organic pollutants. One possible source is the SPOL-Chemie, a chemical factory located before the sample site at the river Bilina. For example, it is known that this company produces a large amount of epoxy resins. In the synthesis of these resins two chemicals were needed: Bisphenol A and 3-chloro-propane-epoxyde. Bisphenol A could be identified in the water extract. Chlorinated ethers (bis(1,3-dichloroprop-2-yl)ether, bis(2,3-dichloropropyl)ether), which were by-products of 3-chloropropane-epoxyde, could be found in the hexane extracts. The first condensation product (2,2'-((1-methylethylidene)bis(4-phenol-dimethyl)methane))-oxirane) was also identified in the methanol extract. The other groups of chemical, which are only presented in OSA 68, are also characteristic as pollutants of an industrial origin. For example, following sources of these compounds can be found: alkylated benzenes are by- and decomposition products of detergents, naphthalenes and PAH are formed during combustion processes, and the represented chlorinated compounds are typical industrial products and also used in agriculture. The other sediments contained an average spectrum of organic chemicals like phthalic acid esters and alkanes, which are ubiquitous in the environment.

Methanol extracts (pH=2)

The methanol extracts (pH=2) of the sediments are also high polluted with organic chemicals. Table 7 shows the identified substances in the different sediment samples. The compounds are subdivided in different classes of chemicals in the same manner as described above.

Table 7: Organic compounds identified in the methanol (pH=2) fractions of German sediments (xxx: dominating compounds, xx: medium levels, and x: trace amounts)

	OSA 68	OSA 93	K1	K2	K3	B5	V4	Fl
alkanes and alkenes								
nonane	x							
decane	x	xx	xxx	xx	xx	xx	xx	x
undecane	x	x						x
tridecane								x
tetradecane								x
5-tetradecene (E)								x
1.13-tetradecadiene		xx						
pentadecane								x
2.6.10.14-tetramethyl-hexadecane								x
1-hexadecene								x
heptadecane								x
eicosane								x
9-eicosane								x
2.6.10.15.19.23-hexamethyl-tetracosane								x
docosane								x
tricosane								x
pentacosane		x						
heptacosane		x						
alkylated benzenes								
1.3-dimethyl-benzene	x							
1-methyl-ethyl-benzene	xx							
propyl-benzene	xx							
1-ethyl-2-methyl-benzene	xx							
1-ethyl-4-methyl-benzene	xxx							
1.2.3-trimethyl-benzene	xx							
1.2.4-trimethyl-benzene	xxx							
1.3.5-trimethyl-benzene	xxx							
1.2.3.5-tetramethyl-benzene	x							
1.2-diethyl-benzene	x							
1.3-diethyl-benzene	xx							
1-methyl-4-(1-methylethyl)-benzene	x							
1-ethyl-3.5-dimethyl-benzene	xx							
2-ethyl-1.3-dimethyl-benzene	xx							
(1-butyloctyl)-benzene								x

Table 7: Organic compounds identified in the methanol fractions (pH=2) of German sediments (cont'd)

	OSA 68	OSA 93	K1	K2	K3	B5	V4	Fl
naphthalene and derivates								
naphthalene	xx							
1-methyl-naphthalene	xx							
2-methyl-naphthalene	xx							
2-ethenyl-naphthalene	xx							
2-ethyl-naphthalene	x							
1,2-dimethyl-naphthalene	x							
1,7-dimethyl-naphthalene	x							
1,8-dimethyl-naphthalene	x							
2,3-dimethyl-naphthalene	x							
2-phenyl-naphthalene	x							
polycyclic aromates (PAH) and derivates								
phenanthrene	xxx							
anthracene	xx							
3-methyl-phenanthrene	x							
9-methyl-anthracene	x							
fluoranthene	xxx							
pyrene	xx							
1-methyl-pyrene	x							
benz(a)anthracene	x							
triphenylene	x							
benzo(a)pyrene	x							
perylene	x							
chlorinated compounds								
hexachlorobenzene	x							
carbon acid methyl esters								
dodecanoic acid, methyl ester		x						x
10-methyl-docecanoic acid, methyl ester		x						
12-methyl-tridecanoic acid, methyl ester		x				x		
tetradecanoic acid, methyl ester	x	xx					x	xx
pentadecanoic acid, methyl ester	x	x						x
heneicosanoic acid, methyl ester	x							
11-hexadecenoic acid, methyl ester					x	xx	xx	xxx
9-hexadecenoic acid (Z), methyl ester		xxx						x
hexadecanoic acid, methyl ester	xx	xxx		x	x	xx	xx	xxx
heptadecanoic acid, methyl ester		x						x
16-methyl-heptadecanoic acid, methyl ester		x						

Table 7: Organic compounds identified in the methanol fractions (pH=2) of German sediments (cont'd)

	OSA 68	OSA 93	K1	K2	K3	B5	V4	Fl
9,12-octadecadienoic acid (Z,Z), methyl ester	xx	x						xx
9,12,15-octadecatrienoic acid, methyl ester		x						
6-octadecenoic acid (Z), methyl ester							x	
9-octadecenoic acid (Z), methyl ester	x	xx			x	x	x	xxx
11-octadecenoic acid (Z), methyl ester		xx				x		xx
octadecanoic acid, methyl ester	xx	x	x		x	x	x	xx
octadecanoic acid, butyl ester				x				
eicosanoic acid, methyl ester		x						x
docosanoic acid, methyl ester	x	x						x
tricosanoic acid, methyl ester								x
tetracosanoic acid, methyl ester		x		x				xx
hexacosanoic acid, methyl ester		x		x				x
phthalic acid esters								
bis(2-etylhexyl)-phthalic acid ester	xxx	x				x	x	xx
cholesterol derivates								
cholesta-3,5-diene		x						
dihydrocholesterol	xxx	xx				x	x	xxx
cholest-5-en-3-ol-(3β)....	xx	xx				x	x	xx
cholestan-3-ol,(3α,5β)-....		xx						
3,3'-dimethoxy-5α-cholestane	x							xx
triterpane	x							
cholestan-3-ethoxy,(3α,5β)-...								xx
methyl ergostan-3-ol,(3β,5α)-...								x
ergost-22-en-3-one, (5β,22E)...						x		
stigmast-7-en-3-ol, (3β,5α,24R...	x							
stigmast-5-en-3-ol, (3β,24S)...						x		xx
cholest-4-en-3-one								x
cholestan-3-one								x
stigmasta-5,23-dien-3β-ol								x
(23,Z)-23-ethylcholestanol		x						
stigmast-8(14)-en-3β-ol		x						
β-sitosterin							x	
various compounds								
patchoulene	x							
1-tetradecanol		x						
2-tetradecanol						x		
1-hexadecanol		xx						

Table 7: Organic compounds identified in the methanol fractions (pH=2) of German sediments (cont'd)

	OSA 68	OSA 93	K1	K2	K3	B5	V4	Fl
(Z)-9-octadecen-1-ol		x						
3,7,11,15-tetramethyl-2-hexadecen-1-ol		xxx				xx	xx	
1-tetracosanol								x
1,2-dihydro-acenaphthylene	xxx							
2-methyl-1,1'-biphenyl	x							
4-methyl-1,1'-biphenyl	x							
1,1-diphenyl-2-propanone	x							
dibenzofurane	xx							
4-methyl-dibenzofurane	x							
9H-fluorene	xx							
4-methyl-9H-fluorene	x							
sulphur	xxx	xxx	xx	xxx	xxx	xxx	xxx	xxx
1-(2-hydroxy-5-methylphenyl)-2-hepten-1-one			x					
hexadecanoic acid	xx							
octadecanoic acid	x							

The methanol fractions (pH=2) of the examined sediments showed almost similar contamination levels as the methanol fractions (pH=7). As a result of the low pH, more acid and polar compounds like alcohols (1-/2-tetradecanol, 1-hexadecanol, (Z)-9-octadecen-1-ol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, and 1-tetracosanol) and carbon acids (hexadecanoic acid and octadecanoic acid) could be extracted. In the extract of sediment OSA 68 some high molecular PAHs like triphenylene and perylene could be determined.

Results and Discussion

Acute Toxicity / Mutagenicity / Genotoxicity

As indicated in the experimental section, the acute toxicity was determined in the water, hexane, and methanol (pH=7 and pH=2) extracts, while the mutagenicity was assessed only in the methanol (pH=7) extracts of the eight sediment samples collected from the river Elbe. The genotoxicity was examined in only one extract, namely the methanol (pH=7) extract of the sediment OSA 68. The chemical characterization by examination with GC/MS was done with all extracts of the different sediments. The bioassays will discussed individually and one chapter about the bioassay-directed chemical analysis will be follow.

Acute toxicity of the sediment extracts

Microtox toxicity was detected in all hexane and methanol (pH=7 and pH=2) extracts. The water extracts, except sediment OSA 68, showed no Microtox acute toxicity. Among the three measurement times (5, 15, and 30 min) no significant differences were observed. The water extract

of sediment OSA 68 indicates a high toxic response, which was comparable with the toxicity of the other extracts. Comparing the different extraction solvents. the water extracts have the lowest toxicity. The highest toxicity occurs from the methanol extracts at a pH=2. The toxicity of the different solvent can be arranged in the following sequence:

water << hexane < methanol (pH=7) < methanol (pH=2)

Comparing the different sediment samples the following toxicity sequence can be estimated:

K1 << K2 ≡ B5 < K3 < V4 < OSA 93 < Fl << OSA 68

The Microtox bioassay is useful for comparing the acute toxicity of sediment extracts. The advantages of this bioassay, in comparison to others, are the low costs and the relative short testing time (30min).

Mutagenicity of the sediment extracts (MeOH)

Muta-ChromoPlate-Mutagenicity protocol allows the incorporation of sample volumes as large as 15mL into the assay medium. A sample was considered mutagenic in the Muta-ChromoPlate mutagenicity assay when one or more effluent doses (volumes) induced a significant ($p< 0.05$) dose related increase in the number of positive wells relative to the negative control (Gilbert, 1980).

As shown in Tables 3a and 3b and Figures 3a and 3b none of the eight methanol extracts indicated mutagenic responses when tested with *Salmonella typhimurium TA-98* without S-9 activation. (Information using S-9 activation was not collected). Of the eight methanol extracts, mutagenic activity was detected in only one of the eight methanol extracts (OSA 68 - MeOH) when tested with *Salmonella typhimurium TA-100* without S-9 activation suggesting, that the majority of the mutagenicity was associated with this fraction. This extract at volumes of 5 - 10mL showed sufficient levels of mutagenic responswes. Furthermore. since this extract showed significant mutagenicity when tested with *Salmonella typhimurium TA-100* without the use of exogenous activation with S-9, the mutagenic compounds consisted of direct acting mutagens (Rao et al..1994). As the levels of mutagenic components increased. a dose related type of response was seen (Figure 3b). This result was used to estimate the mutagenic potency of the sample. Mutagenic potency is defined here as the minimum volume of the extract in which an increase in mutagenic response was first detected compared to the background. Accordingly, ~ 5.0mL of the extract exhibited mutagenic potency (Figure 3b). This would represent 25mg of the river sediment. At this stage it is difficult to generalise that this extract contained no other types of mutagenic materials. Using exogenous activation with S-9 would determine whether the extract contains pro-mutagenic substances.

Genotoxicity of the mutagenic sediment extract (OSA 68-MeOH)

As seen in Figure 4 there was no indication of the induction of micronucleus in the hepatocytes in the experimental rainbow trout when injected with MeOH extract of the sediment OSA 68. A mean micronucleus incidence of 2,15 micronucleii per 1000 hepatocytes was observed at the maximum dose of 0.5% of the methanol extract of the sediment. It is possible that either the mutagenic constituents in the test extract are non-genotoxic or it could be that the experimental exposure duration was inadequate to observe such genotoxic induction. This preliminary study, therefore,

needs to be performed to examine the effects of extended exposure period of fish to different concentrations and different doses of the mutagenic extract. Furthermore, the study should also examine the effects of exogenous activation system (S-9) to ascertain if these bacterial mutagenic constituents are pro-mutagens.

Bioassay-directed Chemical Analysis

The main objective of this investigation was the identification of toxic chemicals in river Elbe sediments. The approach to solve this problem was by using the bioassay-directed chemical analysis. The first step was a sequential extraction method. From the results of the chemical characterization it was obvious that the extraction scheme employed had to be optimised. The identified chemicals emerged mostly in two different fractions. For dividing the compound classes totally in different extracts a more efficient extraction procedure during the sequential extraction was needed. For this purpose following extraction conditions will be tested in future studies: repeated extraction, amount of extraction solvent, and drying of the remaining sediment between the different extraction steps.

In one case, the toxicity of an extract could be related directly to an identified compound. The high toxicity of the water extract of sample OSA 68 resulted from the compound Bisphenol A, which was the only identified compound in this fraction. The hexane and methanol extracts contained too many chemicals to compare and establish single chemicals responsible for the toxicity. In these cases a second separation technique has to be used. An additional separation step distributes the different classes of compounds in sub-fractions, which can be examined individual with toxicological and chemical methods. A possible analytical method for the separation is high performance liquid chromatography in normal (NP-HPLC) or reversed-phase (RP-HPLC) modus. Another technique, which can be used, is the gel permeation chromatography (GPC). This method separates the different compounds based on their molecular weight. First investigations with RP-HPLC on a octadecyl-phase showed reliable results.

For the final determination and identification of organic chemicals in the different extracts and fractions additional methods should be introduced. In the particular case of polar compounds the GC/MS technique is not a good method. Therefore, high performance liquid chromatography with mass selective detection (HPLC-MS) will be used in future.

Conclusions

This report presents the first step of a Toxicity Identification Evaluation approach (TIE) for identifying toxic organic pollutants in sediment samples from the river Elbe. The results indicate that this method is usable for this purpose. Different technical steps had to be optimised for a better interpretation of the results. In one case a direct relation between an identified compound and a high toxicity of a sediment extract could be observed. In sample OSA 68 the toxic chemical is Bisphenol A, an industrial product. This allows decision making authorities to impose on a decreased input of this chemical to the river Elbe. In conventional analytical monitoring programs this compound is not examined and therefore not noticed. The normal use of bioassay monitoring detects a high toxicity, but the responsible compound will not be known. The bioassay-directed chemical analysis is a link between the chemical analysis and the use of bioassay as an environmental monitoring.

Acknowledgements

The authors would like to express their sincere thanks to Mrs. Tannis Neheli for her technical assistance during this study. Financial assistance to one of the authors (J. Kurz) from the Germany-Canada Bilateral Agreement project is greatly appreciated. Special thanks are given to Mr. Bianchi from the International Bureau at the GKSS, Prof. Dr. Hansen from the Technical University of Berlin and Dr. Carey from the NWRI for their help in making the visit of Dr. J. Kurz to the NWRI, Burlinton, Ontario, Canada.

References

Dutka B.J. (1989) Methods for Microbiological and Toxicological Analysis of Waters, Wastewaters and Sediments. I.W.D., Dept. of Environment, National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ontario, Canada

Gilbert R.J. (1980) The analysis of fluctuation test. Mutation Research 74: 283-289

Krebs F. (1988) Der pT-Wert, Ein gewässertoxikologischer Klassifizierungsmaßstab. GIT Fachzeitschrift für das Laboratorium 32: 293-296

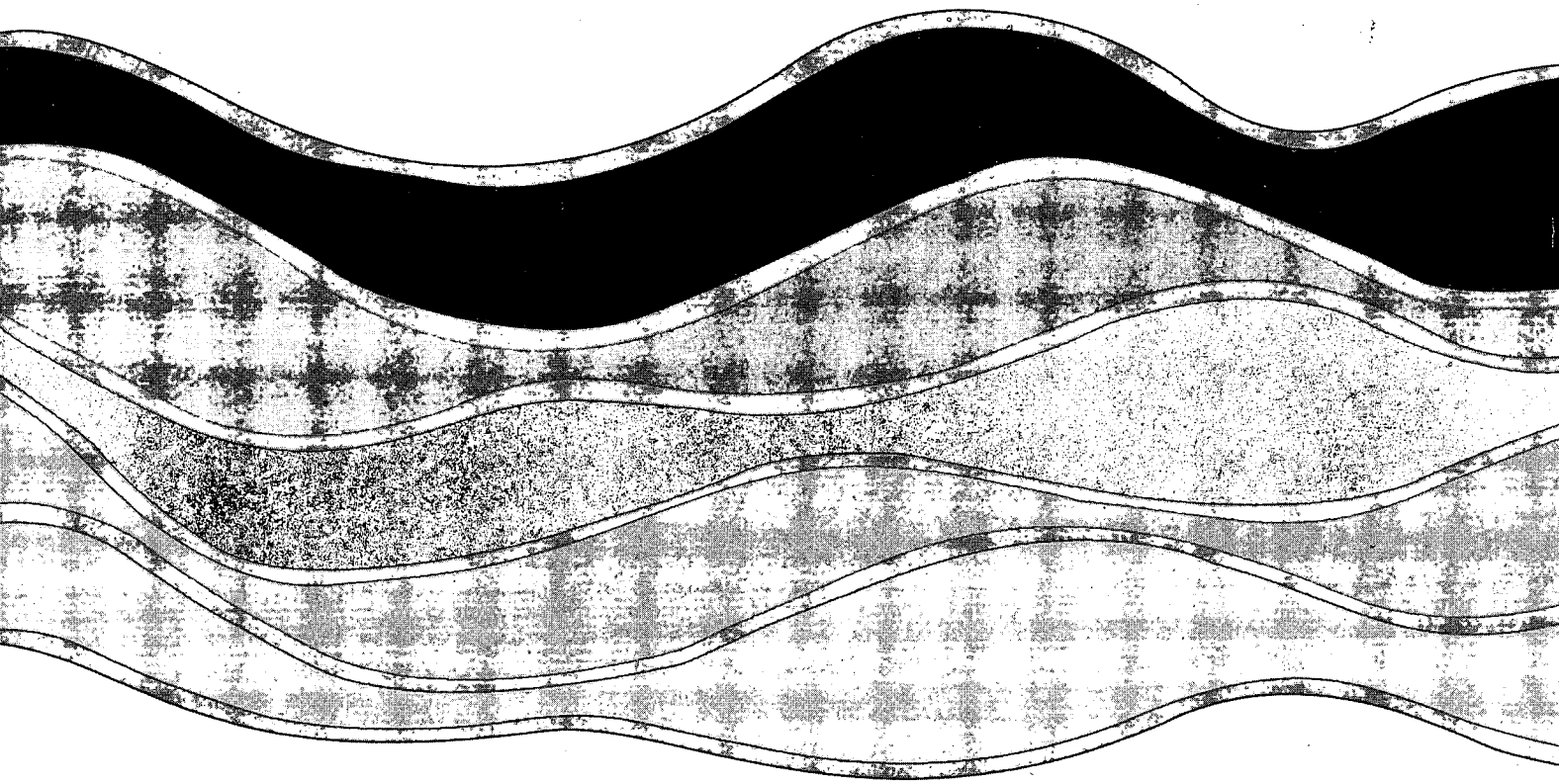
Kurz J. (1995) Combined Bioassay-chemical Fractionation Scheme for the Determination of Toxic Organic Chemicals in River Sediments, Poster presented at the 5th SETAC Europe Congress. 24.-28. June 1995, Copenhagen

Rao S.S., B.K. Burnison, D.A. Rokosh, and Taylor C.M. (1994) Mutagenicity and Toxicity Assessment of Pulp Mill Effluent. Chemosphere 28: 1859-1870

Rao S.S., and Lifshitz R. (1995) The Muta-Chromoplate Method for Measuring Mutagenicity of Environmental Samples and Pure Chemicals. Environmental Toxicology and Water Quality 10: 307-313

Rao S.S., Quinn B.A., Burnison B.K., Hayes M.A., and Metcalfe C.D. (1996) Assessment of Genotoxic Potential of Pulp Mill Effluent using Bacterial, Fish and Mammalian Assays. Chemosphere 31: 3553-3566

Williams R.C., and Metcalfe C.D. (1992) Development of an in vivo Hepatic Micronucleus Assay with rainbow trout. Aquatic Toxicology 23: 193-202



NATIONAL WATER RESEARCH INSTITUTE
P.O. BOX 5050, BURLINGTON, ONTARIO L7R 4A6

Environment Canada Environnement Canada

Canada

INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX
C.P. 5050, BURLINGTON (ONTARIO) L7R 4A6

Think Recycling!

Pensez à recycler !