ECOTOXIC EFFECTS OF WET WEATHER DISCHARGES IN AN URBAN STREAM

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ABSTRACT

The urban stream Store Vejleå (Denmark), which receives discharges of urban runoff, was investigated using a combination of biological toxicity tests (biotests) and chemical analysis. The urban stormwater and road runoff did not affect the mobilization of *Daphnia magna*, whereas three of the samples gave low, but statistically significant, effects on the reproduction of the alga *Pseudokirchneriella subcapitata*. In all pre-concentrated water samples toxic effects were found with EC_{10}-values ranging from pre-concentration factors of 1.7-43 times and differences in toxicity depending on time and location of sampling were identified (e.g. inlet of road runoff was more toxic than inlet of storm water from an urban area). No toxicity was detected in an unpolluted reference sample pre-concentrated 100 times. Undiluted pore water samples from sediments collected in the stream were all toxic towards the algae and dilutions from 4-14 times were needed to reduce the growth rate inhibition to 20%. To reduce the toxicity of a pore water sample from an unpolluted stream a dilution factor of only 1.6 was required. A qualitative correlation between the toxicity of the pore water and the degree of pollution as evidenced by the metal concentration was observed, but statistically significant correlations could not be established by ranking procedures of e.g. metal content or PAH-concentrations versus the observed toxicity.

KEYWORDS: Biotests, pore-water, sediment, solid phase extraction (SPE), toxicity, urban storm-water

INTRODUCTION

In recent years the focus on environmental impacts of discharges of urban storm-water and road runoff have increased and physical, chemical, and biological problems have been addressed (Maltby *et al*., 1995a; Maltby *et al*., 1995b; Marsalek *et al*., 1999). Discharges of urban stormwater to aquatic environments have been documented to cause accumulation of heavy metals and PAHs in sediments and findings of different organic chemicals in the water phase have be described (e.g. Maltby *et al*., 1995b; Carr *et al*., 2000). Many of the compounds are known to be toxic to animals and humans and have potential for carcinogenic, mutagenic and/or allergenic effects (Ledin *et al*., 2003), but the ecotoxic potential of the chemicals present in urban runoff is not well described in the literature, though a number of studies dealing with toxicity assessment of urban wet-weather discharges have been published (review by Marsalek *et al*., 1999).

Wet-weather discharges are examples of complex environmental pollutions for which an analytical-chemical characterisation may not suffice in explaining the toxic potential (Ellis, 2000). An alternative monitoring strategy using a battery of toxicity test have been pointed out as a useful tool for screening and assessing potential receiving water impacts (Marsalek *et al*., 1999), however, only few actual studies have been carried out.

The present study focused on measuring toxicity both in the water and in the sediment phase in the stream Store Vejleå located 20 km west of Copenhagen (Denmark). The water in the stream originates primarily from discharges of urban stormwater and road runoff. As a result of previous investigations (Kjølholt *et al*., 2001; Ledin *et al*., 2003) it expected that the toxicity of stream water was low, but also that organic chemicals were present in the water phase. Therefore, two approaches for toxicity testing were applied to water samples: 1) direct testing of whole samples and 2) testing of samples fractionated and pre-concentrated by solid phase extraction (SPE) in order to quantify the toxicity of non-volatile organic chemical contaminants in the water phase. Furthermore, toxicity testing of pore water from sediments collected in Store Vejleå was included in the study.

The aim of the study was to address the order of magnitude of toxic effects of chemical constituents in water samples, not to make simulations of *in situ* toxicity. As a consequence of this, all samples were grab samples collected on different occasions (not flow-, weather-, or time-dependent sampling) and standardized toxicity tests were applied. The biotests were used as indicators for toxicity and hazard ranking of both the sediment and the water samples and two freshwater test species, that represent the primary producers and zooplankton, were used throughout the study, i.e. the alga *Pseudokirchneriella subcapitata* and the crustacean *Daphnia magna*.

MATERIALS AND METHODS

Site

The stream Store Vejleå, shown in Figure 1, is dry most of the year upstream the inlet from basin 4. Basin 4 is a detention pond, which receives runoff water from both a motorway and an urban area. About half of the water in the stream originates from basin 4, and around one third of the water flow in the stream can be attributed to the inlet from "Dybendalsgrøften", which can be characterized as urban runoff. The stream Store Vejleå has several small inlets connected to the urban rainwater system (cf. Figure 1).

Sampling

A total of 12 water samples (grab samples on four occasions in 2001) and 9 sediment samples were collected from both the stream and the inlets to the stream (see Figure 1). Additionally, one water sample and one sediment sample were taken in an unpolluted stream (Fønstrup Bæk, Denmark). The samples were collected in acid washed bottles and kept at 4 °C. Sediment samples were sieved in the laboratory (1.5 mm) to remove larger particles. The samples were adjusted to approximately the same dry weight and shaken to ensure homogeneity before samples were taken for biotests and chemical analyses.

Chemical analyses

Water samples were analysed for general hydro-chemical parameters (pH, electric conductivity, temperature, oxygen, ammonium and chloride) and non-volatile organic carbon (NVOC). Concentrations of benzene, toluene, ethylbenzene, and xylenes (BTEX-compounds) were analysed by GC-FID. In water samples the concentrations of Pb, Cr, Cu and Zn were analysed using an AAS with graphite furnace and all sediment samples were analysed for the metals: Cd, Cr, Cu, Ni, Pb and Zn by AAS-flame. Furthermore, the concentrations of PAHs were analysed in sediment samples by GC-MS.

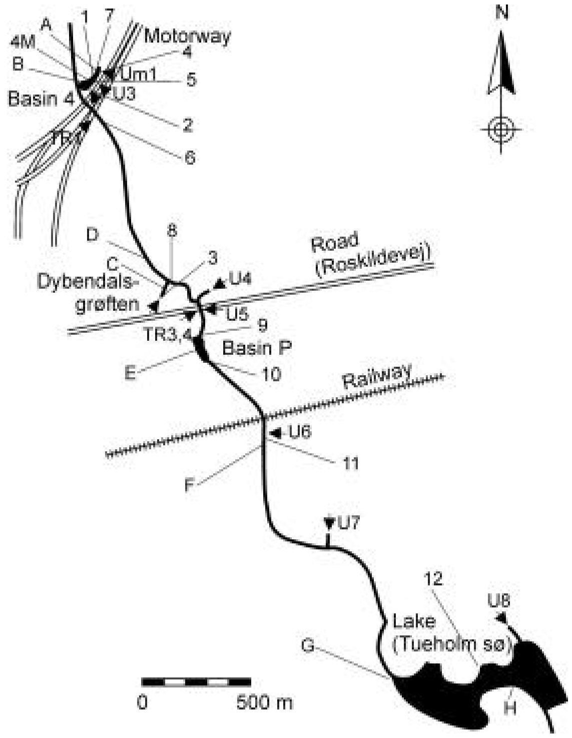

Figure 1. Map of Store Vejleå, Denmark. Inlets are illustrated with arrows, water samples with numbers (1-12) and sediment samples with letters (A-H, 4M).

The bottle was shaken for 1 hour (100 rpm) in the dark at 20 °C. The pentane was recovered by centrifugation and the extraction procedure was repeated 3-6 times with new solvent until the recovered pentane was clear. The recovered pentane was concentrated by flushing with air.

Solid phase extraction

Pre-concentration by means of solid phase extraction (SPE) at pH 7.0 followed by biotests, as described by Baun and Nyholm (1996), was used to investigate the toxicity of organic chemicals in the water phase. Isolute ENV+ columns were used as SPE resins.

Biotests

Two standardized biotests were chosen as indicators of toxicity for hazard ranking of the contaminated samples. Algal bioassays were carried out according to the ISO-standard for algal toxicity testing (ISO, 1989a) applying a mini-scale test version as described by Arensberg et al. (1995). Six untreated controls were included in all tests. The test vials were incubated on a shaker (100 rpm) in continuous white fluorescent light (80-100 $\mu E/m^2/s$) at 20 ± 2 °C. The tests were conducted at pH 8.1 ± 0.2 with typical control growth rates of 1.7-1.9 d^{-1}. Algal growth rates were determined from acetone extractions as described by Mayer et al. (1997). Concentration-response curves were described by the Weibull equation, which was fitted to data using non-linear regression applying a computer program developed by Andersen *et al.* (1998). Pore water testing was used to assess the toxic effects of sediments towards algae. The pore water was separated from the sediment by centrifugtion followed by filtration (1.2 µm). For one sample (4M) a sediment suspension was also tested. The suspension test was carried out as described by Baun *et al.* (2000). When testing the whole water samples, the pore water and the sediment suspension three replicates of each concentration were made. SPE-extracts were tested with one replicate for each concentration.

The 48-hour immobilization tests with Daphnia magna were performed only on the whole water samples. Tests were performed according to the ISO-standard (ISO, 1989b) and the test results were analyzed by Probit analysis with maximum likelihood estimation (USEPA, 1988).

For all toxicity tests the results are expressed as EC_{10}-, EC_{20}-, and EC_{50}-values, i.e. concentrations yielding 10%, 20%, and 50% effect, respectively. These values are used as estimates of concentrations where statistically significant toxic effects start to appear.

RESULTS AND DISCUSSION

Characteristics and toxicity of whole water samples

In all samples from Store Vejleå the oxygen concentrations were > 8 mg/L and the concentrations of ammonium were <0.25 mg N/L. The conductivity and concentrations of chloride, lead, copper and zinc were much higher in samples from

Store Vejleå than for the reference sample (see Table 1). The relatively high content of NVOC in the reference sample is most likely due to litter decomposition. Concentrations of BTEXs were below the detection limit (0.2 µg/L) in all the water samples.

Three water samples collected in Basin 4 (sample 1, 2 and 7) proved to be toxic in standard laboratory test with algae with EC_{10}-values from 800-900 mL/L (Table 1). A comparison with results published by Radix *et al*. (2000) on toxicity of copper towards *P. subcapitata* (EC_{10} = 12 µg/L) shows that the toxicity of sample 1 may be due to the presence of copper (25 µg/L in sample 1, cf. Table 1) whereas copper cannot be the sole responsible for the toxicity found in sample 2 and 7. Exposure to the whole water samples did not affect the mobility of *Daphnia magna* in any of the water samples. The results obtained when testing the whole water samples indicate that the samples collected in Store Vejleå in general were not very toxic towards the two aquatic organisms tested.

Solid phase extraction (SPE) and toxicity testing

All the water samples from Store Vejleå showed at least 20% inhibition in algal tests when pre-concentrated 100 times and the estimated EC_{10}-values are shown in Table 1. For samples 2, 3, and 4 a 20 times pre-concentration resulted in a total inhibition of the algal growth rate. No significant changes in the algal growth rate were observed with the reference sample when pre-concentrated 100 times. The most toxic sample, sample 4, had to be concentrated only 1.7 times to result in 10% inhibition of the algal growth rate (Table 1).

Table 1. Hydro-chemical characteristics, metal concentrations, and algal toxicity of water samples collected in Store Vejleå (sample 1-12) and in Fønstrup Bæk (Ref.). Numbers in brackets are 95% confidence intervals.

Parameter	Unit	Sample							
		1	2	3	4	5	6	7-12	Ref.
Conductivity	mS/cm	1.1	1.3	1.3	1.3	1.1	1.3	1.0-1.4	0.6
pH		7.6	7.7	8.1	7.3	8.0	7.6	7.6-8.2	7.9
Chloride	mg/L	174	329	259	358	159	489	141-266	77
NVOC	mg/L	7.0	5.8	7.8	4.1	5.1	5.8	5.7-9.5	26
Lead	µg/L	4.7	1.7	3.6	3.1	2.0	2.7	0.6-3.5	b.d.
Chromium	µg/L	1.1	0.3	0.3	0.5	0.5	0.6	0.1-4.9	b.d.
Copper	µg/L	25	3.7	2.9	3.4	3.3	4.4	3.5-12	b.d.
Zinc	µg/L	29	15	12	17	14	20	13-23	0.8
EC_{10} (WWS)	1000 mL/L	0.9 [0.7;1.6[1)]]	0.8 [0.6;0.9]	>1.0	>1.0	>1.0	>1.0	0.8[2)]->1.0	>1.0
EC_{10} (SPE)	1000 mL/L	<16[3)]	<10[3)]	<10[3)]	1.7 [1;3]	8.5 [5;17]	11 [5.3;27]	24-43	>100

NVOC: Non-volatile organic carbon; EC_{10}: 10% effect concentration in algal tests; WWS: Whole water sample; SPE: Solid phase extraction; [1)] Extrapolated value; [2)] Sample 7, 95% confidence interval: [0.7;0.9]; [3)] >60% inhibition at the concentration; b.d.: below detection limit.

Sample 4, 5 and 6 were taken immediately after a period of rain, therefore the water can be characterized as road runoff, urban runoff and as a mixture of both, respectively. The road runoff was significantly more toxic than runoff from the urban area. Sample 2 and 3 were taken in a period with almost no precipitation. Both samples had to be concentrated only 10 times to result in more than 60% inhibition of the algal growth rate. The cause for this is probably, that a period with low precipitation gives rise to higher concentrations of chemicals in the runoff. In the period before the samples 7-12 were taken there had been much precipitation and these samples were much less toxic than the samples 2-6. This supports the assumption that dilution has lead to lower concentrations of toxic compounds in the water phase.

Sediment samples: PAH, metals and toxicity

As shown in Figure 2 higher concentrations of metals and PAHs were found in the sediment samples from the urban stream compared to the reference sample collected in an unpolluted stream. The sediments collected in Basin 4 (samples A, B, 4M) showed the highest contents of metals and PAH with total concentrations up to 930 mg/kg and 4.4 mg/kg, respectively. Zinc is the metal found in the highest concentrations with a maximum concentration of 560 mg/kg, but significant concentrations of copper (max. 120 mg/kg), lead (max. 200 mg/kg), and nickel (max. 160 mg/kg) are also found in a number of samples.

The results of toxicity tests of sediment pore water samples are also shown in Figure 2. The results are expressed in terms of toxicity units (TU) for the graphical comparison as a higher TU-value means higher toxicity. TU_{20}-values can be interpreted as the number of times the sample needed to be diluted in order to go from total inhibition to 20% effect. It is observed that all pore water samples were toxic towards the algae and that samples from Store Vejleå were more toxic than the sample from the reference stream. The pore water samples from Store Vejleå had to be diluted from 4.4 $[3.6\text{-}6.3]_{95\%}$ to 13 $[8.5;24]_{95\%}$ times to reduce the toxicity to 20% inhibition, whereas the reference sample had to be diluted only 1.6 $[0.2\text{-}2.1]_{95\%}$ times. A statistical ranking analysis, the Spearman ranking procedure, was carried out in order to investigate if a correlation between the “chemical rank” and the “toxicity rank” could be established.

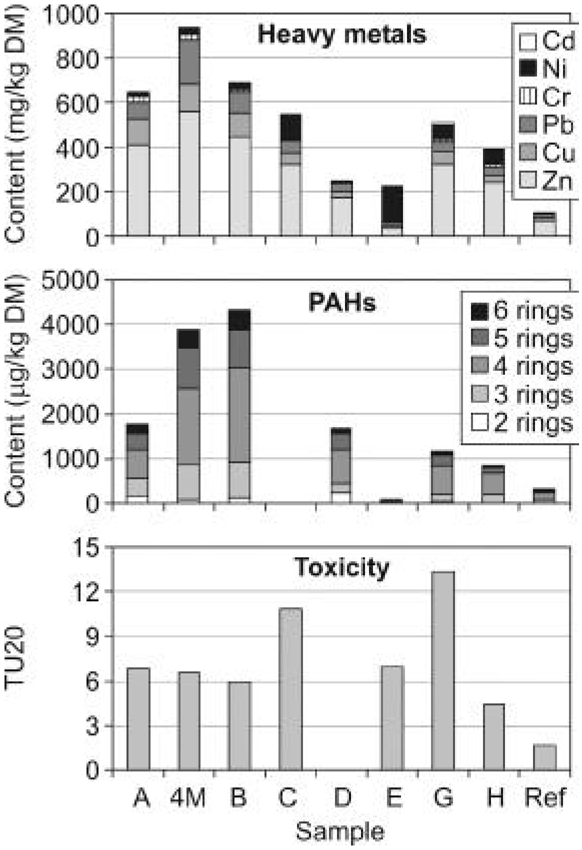

Figure 2. Concentrations of metals and PAHs in sediment samples and algal toxicity of sediment pore water. No PAH results for sample C and no toxicity results for sample D. TU20 corresponds to 1000/EC_{20} in g/kg dry matter (DM).

Table 2. Toxicity of sediment sample 4M in 48 hours algal tests (*P. subcapitata*) tested as suspension and pore water.

Test phase	EC_{10} (g DM/L)	EC_{50} (g DM/L)
Pore water	84 [44;125]$_{95\%}$	441 [286;1720]$_{95\%}$
Suspension	1.5 [0.78;2.5]$_{95\%}$	9.9 [6.1;52]$_{95\%}$

DM: Dry matter

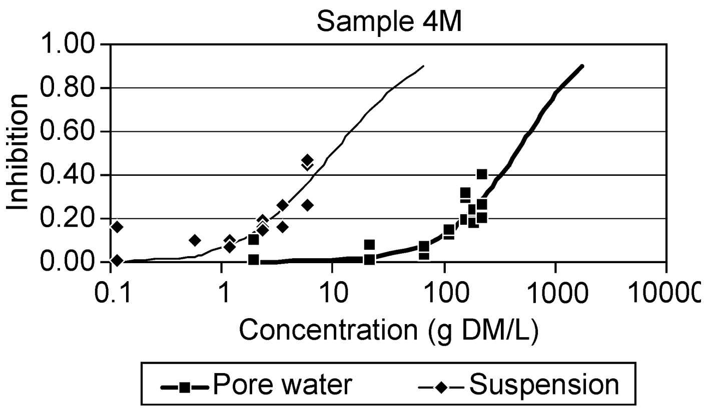

Figure 3: Toxicity of sediment from basin 4 tested as pore water and sediment suspension in 48 hours algal test.

The analysis comprised ranking of the following parameters Cd, Cr, Cu, Ni, Pb, Zn, total metals, individual PAHs, total PAHs and 12 PAHs against the toxicity ranking based on EC_{20}- and EC_{50}-values obtained in tests of porewater in algal tests. In none of the case statistically significant correlations were found at a 5% significance level ($p > 0.025$). Thus, the compounds found in the chemical analyses cannot explain the toxicity found in pore water. The sediment sample 4M, collected in the retention basin (Basin 4), was tested both as pore water and suspension in the algal test. Table 2 shows EC-values obtained in the two tests and Figure 3 illustrates the concentration-response curves. It is clearly shown that the suspension test resulted in lower EC-values, i.e. higher toxicity, and that the differences between the two tests are statistically significant. In fact the suspension was about 44-56 times more toxic than the pore water as judged from EC_{50}- and EC_{10}-values (Table 2).This difference in sensitivity between pore water tests and suspension tests has previously been described by Baun *et al.* (2002) for contaminated soils. The reason for this difference might be that a direct contact between the particles and the organisms mimics the bioavailable toxicity to a larger extent than testing of pore water. This is of great importance for risk assessments of urban wet weather discharges as the sediments deposited in e.g. rainwater retention ponds are frequently washed out into receiving waters during rain events. The findings of this study show that sediments may contribute significantly to toxic effects in receiving waters due to release of pore water and/or suspension of particles.

CONCLUSION

Using standardized bioassays with algae and crustaceans only low toxicity was detected in whole water samples from the stream Store Vejleå and in its inlets. However, pre-concentration by SPE resulted in full concentration-response relationships in the toxicity tests with pre-concentration factors up to 100 times. The overall findings indicate that biotests applied on pre-concentrated water samples can be a useful tool for toxicity characterization and hazard ranking of recipient water receiving urban runoff. The results from the pre-concentrated samples show that it is necessary to dilute several of the inlets to the Store Vejleå stream in order to protect aquatic organisms in the stream. Especially the road runoff appears to be more toxic than runoff from the urban area.

The chemical analyses showed that the sediments were contaminated with both heavy metals and PAHs. Pore water from the retention basin sediments and the stream sediments were toxic to algae and had to be diluted from 4-13 times to reduce

the toxicity to 20%, in comparison the background toxicity in the reference sample required a dilution factor of 1.6. A sediment sample, tested directly as a suspension, was 44-56 times more toxic to algae than pore water from the same sediment sample, indicating that suspension of sediment particles may cause toxic effects in the receiving waters.
The study underlines that a toxicity assessment is needed for risk assessment of sediments related to wet weather discharges. The toxicity assessment should be based on both chemical and biological methods as sediment toxicity was found to be high and could not be predicted directly from chemical analyses covering metals and PAH-compounds.

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