Institutionen för vattenförsörjnings- och avloppsteknik

Chalmers University of Technology
Department of Sanitary Engineering

Address:
Department of Sanitary Engineering
Chalmers University of Technology
S-412 96 Göteborg, Sweden

ISSN 0280-4034

Keywords: dehydrogenase, Microtox, urban sediments, metals, pollutant fractionation

Investigation of the Ecological and Toxicological Effects of Urban Runoff

Chen Wei

Publication 3:91

Licentiate thesis

Contents i

Abstract iii

List of tables iv

List of figures v

Glossary of abbreviations vi

Publications appended vii

Acknowledgements viii

1. Introduction 1

2. Objectives 3

3. Theoretical aspects of the chosen methods and their applicability 3

4. Experimental methodology 4
4.1 Study sites and sampling 4
4.2 Sediment processing 5
4.3 Microbial activity assays 6
4.4 Metal analysis 7
4.5 COD and pH 7
4.6 Data analysis 7

5. Results and discussion 8
5.1 COD and metals in urban rivers 8
5.2 Variations in toxicity 9
5.3 Toxicity test selection 13
5.4 Variations along urban rivers 14
5.4.1 Kvillebäcken 14
5.4.2 Mölndalsån 16
5.4.3 Balltorpsbäcken 17
5.5 Inhibition of the toxic fraction on microorganisms in the sediments 17

6. Conclusion 19

7. Recommendations and future work 19

References 21

Abstract

The effect of pollutants due to combined sewer overflow (CSO), separate stormwater runoff (SSR) and industrial discharges in three urban rivers on sediment enzyme activity and *Photobacterium phosphoreum* has been investigated. A comparison of enzyme activities show that, dehydrogenase activity assay is easier, more sensitive, and more reliable than others. Enzyme activity was higher in Kvillebäcken which receives only CSO and SSR than in Mölndalsån and Balltorpsbäcken which receive not only CSO and SSR, but also industrial discharges. Variation profiles along each river were obtained based on the enzyme activity, concentrations of chemical oxygen demand and metals. Enzyme activity and Microtox EC_{50} reflected the influences of CSO, SSR and industrial discharges.

Dehydrogenase activity assay and Microtox test were chosen to identify the inhibition of pollutants on the ecology of urban river sediments. Evidence has shown that besides the effect of metals, significant effect of polyaromatic hydrocarbons or chloroform hydrocarbons on the urban river sediments existed in Mölndalsån and Balltorpsbäcken. Inhibition of these pollutants to Microtox EC_{50} can be greater than 474 %. Extraction of the metal and ethanol-extractable fractions reduced inhibition of dehydrogenase activity in the urban sediments.

Keywords: dehydrogenase; Microtox; urban sediments; metals; pollutant fractionation

List of tables

Table 1.	Concentrations of pollutants in urban runoff and comparison with freshwater toxicity criteria	1
Table 2.	Description of urban rivers and quality	5
Table 3.	Chemical analysis results from Kvillebäcken	8
Table 4.	Chemical analysis results from Mölndalsån	8
Table 5.	Chemical analysis results from Balltorpsbäcken	9
Table 6.	Bioassay results from Kvillebäcken	10
Table 7.	Bioassay results from Mölndalsån	10
Table 8.	Bioassay results from Balltorpsbäcken	10
Table 9.	The standard deviation (±SD%) for dehydrogenase activity	11
Table 10.	Effect of chemical extraction on Microtox test in the sediments	18
Table 11.	Effect of physico-chemical extraction on DHA in the sediments	18

List of figures

Figure 1.	Location of sampling sites in Göteborg	5
Figure 2.	Enzyme activity in Kvillebäcken	12
Figure 3.	Enzyme activity in Mölndalsån	12
Figure 4.	Enzyme activity in Balltorpsbäcken	13
Figure 5.	Enzyme activity variations along Kvillebäcken	15
Figure 6.	EDTA-Cu in Kvillebäcken	15
Figure 7.	Variation of DHA and Cu concentration downstream from a CSO discharge	16

Glossary of abbreviations

CSO	Combined sewer overflow
SSR	Separate stormwater runoff
COD	Chemical oxygen demand
DHA	Dehydrogenase activity
APA	Alkaline phosphatase activity
GLA	Glucosidase activity
GAA	Galactosidase activity
MIC	Microtox
INT	2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenoltetrazolium chloride
EDTA	Ethylenediaminetetraacetic acid
ETS	Electron transport system
NP	p-nitrophenol
SD	Standard deviation

Publications appended

1. Morrison, G. M. and Chen, W., 1991. Electroanalysis of metal speciation and its relevance to ecotoxicology. Analytical proceedings, 28: 70-71.

2. Chen, W. and G.M. Morrison, 1991. Bacterial enzyme activity and metal speciation in urban river sediments. Hydrobiologia, (in press).

3. Chen, W. and G.M. Morrison, 1991. Enzyme activity in urban rivers. New technologies in urban drainage, Maksimovic, C.,(ed.), Elsevier Science Publishers Ltd, England: 267-272.

4. Chen, W. and G.M. Morrison, 1991. Inhibition of bacterial enzyme activity and luminescence by urban water-course sediments (draft manuscript).

Acknowledgements

During my stay in Sweden, I have received so much assistance that it is impossible to mention all of the people who helped me with my work and living. They all have my gratitude.

Dr. Greg Morrison, my supervisor, in particular has taken hours of his time to detail the background and aims of this project leading up to the establishment of methods and application of new aspects in this project. It is due to his instruction and advice that this work could be undertaken efficiently. He provided thoughtful and valuable comments on this manuscript many times. I wish to thank him greatly not only for his constant encouragement, stimulating discussion and technical instruction in the past one and half years, but also for his friendship.

Professor Torsten Hedberg, who supported this work all the time, provided helpful and critical remarks on the preliminary draft of this paper. I would like very much to thank him.

Lars Ove Sörman should be most thanked for helping me to take all the samples and for breaking the ice in the field.

I wish to thank Evy Axen and Gabriella Kaffehr for skillful instrumental analysis and laboratory assistance, Bela Kaffehr and Åsa Edell for providing me with relevant references and Inger Hessel for helping to sort out many of the formalities.

Annika Carlson, who not only shares the office with me, but also is willing to share her personal knowledge, is thanked for very interesting and stimulating discussions.

Malin Torell, Teresia Wengström and Gittan Horkeby are also to be thanked for help and friendship.

Dr. Humberto Gonzalez is also to be thanked for correcting this manuscript.

Junjie Yao, my friend, should receive many thanks for encouragement and support.

I am indebted to Mr. Lennart Brandin and Ms. Anne-Marie Stamou for great help for my stay in Sweden, especially in the early months of my coming, which I will never forget.

This study was supported financially by Elof Hansson Research Foundation.

1. Introduction

Water quality in urban rivers is increasingly being degraded by receiving combined sewer overflow, storm runoff and industrial discharge. This results in high chemical oxygen demand (COD), toxic metals, hydrocarbons, nutrients, and pesticides (Hunter *et al.*, 1979; Wilber *et al.*, 1977; Hoffman *et al.*, 1982; Cole *et al.*, 1984). These pollutants may exert both long-term and short-term ecosystem damage on urban rivers (Moriaty, 1988; Hall, *et al.*, 1988). The extent of these effects depends on the individual pollutant concentration in the urban runoff relative to the flow or circulation of the receiving water, as well as on the pollutant speciation and the sensitivity of the recipient ecosystem (Morrison *et al.*, 1989).

In the early 1970's, researchers at the Department of Sanitary Engineering, Chalmers University of Technology, initiated a study on pollutants in storm runoff. The concentrations of nitrogen, phosphorus, lead, zinc, copper, and organic pollutants in some urban areas in Göteborg were measured (Malmqvist 1983).

Table 1. Concentrations of pollutants in urban runoff mean values and comparison with freshwater toxicity criteria for intermittent discharge

Parameter	pH	COD (mg/l)	Zn (µg/l)	Pb (µg/l)	Cu (µg/l)	N_{total} (mg/l)	P_{total} (mg/l)
Urban runoff	6.8	114	436	232	254	2.5	0.28
Freshwater toxic criteria[a]			380	150	20		

a. (U.S. EPA, 1983)

Table 1 summarises the results of the study in Göteborg and it is obvious that the mean pollutant concentrations associated with wet-weather urban discharges can be substantially in excess of water quality criteria. Other studies have shown that total Cd, Cu, and Pb concentrations in undiluted storm runoff regularly exceed U.S. EPA acute criteria for about 75 %, 70 %, and 20 % respectively of the storm duration and exceed chronic criteria for some 80 %, 85 % and 95 % of the time (Morrison *et al.*, 1987).

The ecological effect of separated storm runoff (SSR), combined sewer overflow (CSO) and industrial discharge on the receiving waters is poorly understood. Evidence has been provided that the freshwater shrimp, *Gammarus pulex*, accumulates metals when transferred to urban rivers (Davis, *et al.*, 197; Bascombe, *et al.*, 1990).

Recently, *in situ* chronic toxicity tests have been developed using benthic invertebrates (Hellawell, 1982), and bacterial enzymes (Bitton, 1983). These tests measure the activity or the amount of a metabolic product of living organisms. It is microbial assay which is generally used or recommended for use in the assessment of water quality (Burton, 1988; Williamson *et al.*, 1981). The oxido-reductase and hydrolase enzyme classes are useful indicators of environmental activity because they are components of major metabolic processes in all life forms. Enzymes are sensitive indicators of the present

microbial environment as microbial metabolism reacts quickly to environmental conditions (Wieser *et al.*, 1976). The advantages in using microbes for studying the ecological effect of pollutants are summarised below:

1. Changes in enzyme activity can give information on the level of pollution in the urban river.

2. The technique is extremely sensitive and requires only a small amount of sediment. This may be important when the sediment is limited.

3. The method is straightforward and inexpensive compared with fish toxicity test.

Tests using several different plant and animal species for toxicity testing have been suggested by U.S. EPA because various species often reveal different levels of sensitivity when exposed to the same toxicant at similar concentrations (Barnhart *et al.*, 1983; Dutka, *et al.*, 1989). The tests used in this paper were dehydrogenase activity (DHA) assay, alkaline phosphatase activity (APA) assay, glucosidase activity (GLA) assay, galactosidase activity (GAA) assay and Microtox (MIC) test. The latter uses the luminescent bacterium, *Photobacterium phosphoreum*.

In this work, experiments have been carried on the sediments. Sediment was chosen as a suitable medium for the following reasons:

1. Pollutants are concentrated in sediment (Fuller, *et al.*, 1990).

2. Sediment has a long-term effect with interactions between sediment/water due to changes in pH, ionic strength, redox and bacterial activity (Keramida, *et al.*, 1989).

3. It has been shown that organisms which are in contact with the sediment accumulate pollutants (Davis, *et al.*, 1987).

4. Sediment is relatively easy to collect and store, and it provides a time-integrated sample.

Since the extremely complex chemical nature of the urban river limits the usefulness of toxicity as a detector for the identification of individual components, a primary fractionation using a chemical or physical extraction followed by a biological toxicity test is necessary (Burkhard, *et al.*, 1983). This pre-fractionation has been effective in the identification of toxic metal species (Samant, *et al.*, 1990). However, components causing toxicity vary widely. In addition, when multiple toxicants are present, synergistic and antagonistic effects and the proportion of the overall toxicity due to each toxicant often vary significantly. A concept of eliminating toxicity by sample contaminant fractionation has been applied successfully previously (Dutka, 1991).

2. Objectives

The objectives of this study were:

* to select a proper enzyme assay as an indicator of the effect of urban runoff. The enzyme activity of the selected assays should be easy for analysis, highly sensitive, highly reproducible and represent the enzyme status in the sediment.

* to investigate the effect of CSO, SSR and industrial discharge on receiving water sediment.

* to identify the contribution of inhibiting fractions of various toxicants in urban river sediments.

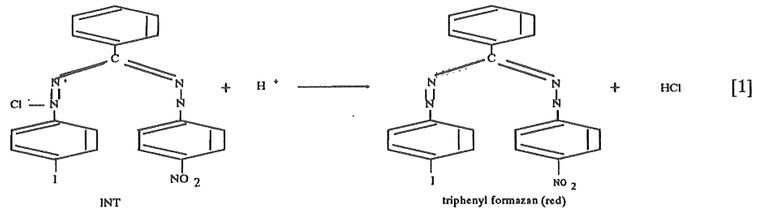
3. Theoretical aspects of the chosen methods and their applicability

Two methods were chosen to identify the toxic and ecological effects of pollutant fractions in urban river sediments. Dehydrogenase activity of bacteria living in the sediment should be directly affected by sediment-bound pollutants, therefore a measurement of dehydrogenase activity is a sensitive indicator of the ecological effects of pollutants. The second method was the Microtox toxicity test which is a laboratory-based method and, although not as ecologically relevant as dehydrogenase activity, has a good precision in analysis.

Dehydrogenase enzymes are the major representatives of the class of oxido-reductase enzymes. They catalyze the oxidation of substrates by transfer of electrons through the electron transport system (ETS) which consists of a complex chain of intermediates (flavoprotein, cytochrome, etc) which transport electrons from food to O_2, the final electron acceptor. A specific dye such as INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenoltetrazolium chloride) can be used as an indicator of ETS activity. The dye acts as an artificial hydrogen acceptor and changes colour in the reduced state. Thus, activity may be measured easily with the aid of a spectrophotometer (Bitton, 1983).

The INT reaction for dehydrogenase is in equation [1]

$$(\text{cyt.b-coenz.Q})_{red.} \longrightarrow (\text{cyt.b-coenz.Q})_{ox.}$$



INT is reduced to formazan, an insoluble red precipitate, according to the above reaction. Following incubation, the produced formazan is dissolved in an organic solvent and the red color is measured at 480 nm. Dehydrogenase is expressed as a rate, μg formazan per gram biological material per incubation time.

Dehydrogenase activity was suggested to assess the impact of toxicants, particularly in sediment (Broberg, 1983). This comparatively new method has been used in sedimentological studies by Christianson and Packard (1977), Broberg (1980) and Jones (1982), and in detecting toxicant effects in soil and sludge (Bitton, 1983). Lanhard (1968) observed that this method is more sensitive to metals than to organics. Trevors (1984) compared this method with O_2 uptake by using three sediment samples with and without amended substrate. A good correlation was found between the inhibition of dehydrogenase and values of O_2 consumption. Similar results were obtained by Anderson (1988) in an evaluation of DHA for assessing heavy metal inhibition of activated sludge.

From the investigations it would appear that DHA assay may be a sensitive and reliable means for estimating the ecological effects of toxicants, especially metals, in environmental samples.

Microtox (Microbics) is an instrument which uses the light emitting bacterium, *Photobacterium phosphoreum*. The results are expressed as an EC_{50} value which is the toxicant concentration that causes a 50 % reduction in light emission of the bacteria. The principle of this reaction is illustrated in equation [2]:

$$FMNH_2 \xrightarrow{\text{luciferases(E)}} \text{E-}FMNH_2 \xrightarrow{\text{Oxygen}} \text{E-}FMNH \cdot \xrightarrow{\text{RCHO---->RCOOH}} \text{Light} + FMN \qquad [2]$$

The method is rapid, and sensitive. McFeter (1983) compared the Microtox EC_{50} of 35 test chemicals to fish LD_{50} obtained from a traditional fish test. A good correlation between Microtox and fish assay data was obtained. Dutka (1983) observed that Microtox was the most sensitive among three bioassays studied. A similar observation was also found by Giesy (1988) in comparing Microtox bioassay with *Daphnia magna* assay and *Chironomus tentans* assay using river sediments.

4. Experimental Methodology

The method for enzyme activity was developed for use in urban river sediments.

4.1 Study sites and sampling

Samples were collected from three urban rivers in the Göteborg region, Sweden (Fig.1). All these rivers ultimately discharge into the Göta älv which drains the Göteborg urban area. Sampling sites are described in Table 2.

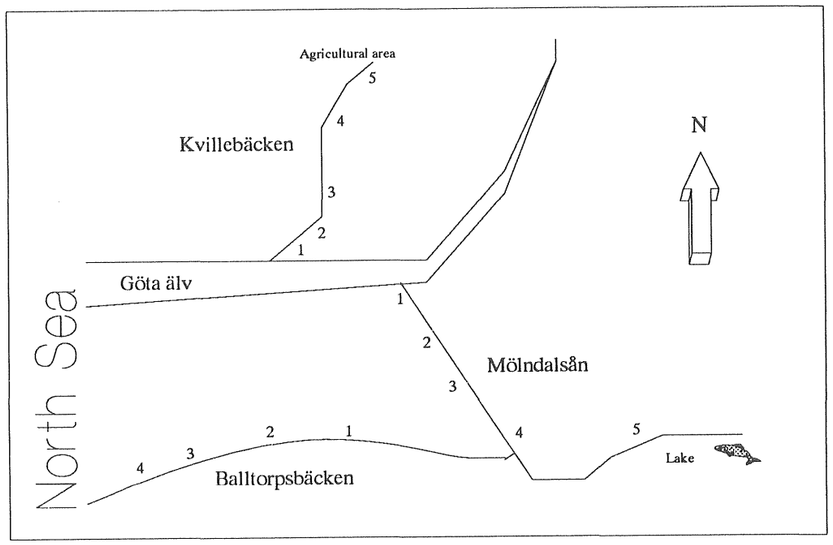


Figure 1. Location of urban rivers and sampling sites

Sediments were collected from the top of the river bed using a grab sampler. Ten samples were taken at each sampling site for enzyme analysis, metal speciation and COD.

Surface water was collected at each site as a buffer solution for sediment incubation.

Table 2. Description of urban rivers and quality

Urban river	No. of sampling sites	Contamination sources	pH	COD mgO_2/g	Cu µg/g
Kvillebäcken	5	Combined sewer overflow, Separate stormwater runoff, agricultural runoff	4.3-5.6	70-250	80-150
Mölndalsån	5	Combined sewer overflow,	4.6-5.3	5-165	130-440
Balltorpsbäcken	4	Separate stormwater runoff, Industrial discharges	5.7-6.3	95-250	30-540

4.2 Sediment processing

Each sediment was split into three portions prior to chemical analysis and toxicity tests.

One portion of sediment was dried (105°C) before chemical analysis for total metals, and 0.01 M EDTA (ethylenediaminetetraacetic acid) extractable metal fractions.

The second portion of the sediment (0.1 g dried at 65°C) was extracted with 10 ml of each one of the following extractants: 0.01 M EDTA; ethanol; pentane/benzene (1:1); and pentane. Each extract was then centrifuged at 4000 rpm for 20 minutes. The supernatant was decanted from the sediment. After further washing with Milli-Q water or ethanol, the sediment was dried at 65°C over-night. Microtox test was carried out on the dry extracted sediment.

The third portion of the sediment (0.5 g) was added to 50 ml of Milli-Q water in a beaker (100 ml), then mixed with a magnetic stirrer. One ml of this suspended solution and 10 ml surface river water, amended with nutrients were placed into the test tube for the enzyme tests and COD analysis. One ml of the suspended solution was extracted with 10 ml of 10^{-4} M EDTA; 0.5 % ethanol; and sparged with air for 20 minutes. Following extraction, 10 ml of surface river water amended with nutrients was added to sediment after filtering the aliquot through a 0.45 μm filter. Dehydrogenase activity was carried out after the suspended solution had been incubated in a water bath at 25°C for 48 hours. The concentration of sediment suspended in water was measured by weighing aliquots of the suspended material which had been dried at 105°C.

4.3 Microbial activity assays

Dehydrogenase activity assay (Zimmerman *et al.* 1978) was based on the reduction of INT (Sigma) to INT-formazan by active microbial electron transport systems. One ml of substrate, 0.2 % INT, was added to a test tube containing sediment and buffer. After vortex, the sample was incubated in a dark water bath at 25°C. One ml of 37% formalin was added after one hour to stop the reaction. The suspended solution was centrifuged at 4000 rpm for 10 minutes. After carefully decanting the supernatant, 10 ml of ethanol was added. The extraction was carried out under vortex for 5 minutes. The red extract was clarified by centrifugation at 4000 rpm for 10 minutes. The absorbance of the extract was read in a spectrophotometer at 480 nm.

Alkaline phosphatase activity (APA) was determined by the method of Sayler *et al* (1979). In this assay, the substrate p-nitrophenyl-phosphate (Sigma) was cleaved by bacterial alkaline phosphatase to leave p-nitrophenol (NP), which is a yellow coloured compound and is measured with a spectrophotometer at 418 nm. One ml of substrate, p-nitrophenyl-phosphate (1 mg/ml) in 0.2 M $NaHCO_3$ buffer (pH 7.6) was added to a test tube containing sediment and river water amended with nutrients. Sample was mixed in a vortex mixer, and was then put in a dark water bath at 25°C for a one hour incubation. 1.0 ml of 1 M NaOH was added to the sample to stop the reaction and to develop the colour at the end of incubation. The suspended sample was centrifuged at 4000 rpm for 10 minutes. After centrifugation, the absorbance of supernatant was measured in a spectrophotometer at 418 nm. Absorbance reading was converted to μg of p-nitrophenol per gram weight sediment by comparison to a p-nitrophenol standard curve. A blank was prepared by the same process but without sediment in the test tube.

Glucosidase and galactosidase activities (GLA and GAA) were assayed using the method of Morrison *et al* (1977). Test substrates were p-nitrophenyl-ß-D-glucoside and p-nitrophenyl-ß-D-galactose (Sigma). NP formation was measured at 418 nm. The performance of these assays was similar to that of APA assay.

Microtox is an instrument which uses the light emitting bacterium, *Photobacterium phosphoreum*. The results are expressed by EC_{50} which is the toxicant concentration that causes a 50 % reduction in light emission. Microtox (Model 500) was performed on dried sediment. Freeze-dried *photobacterium phosphoreum* bacterium was reconstituted for use in the test. All tests were carried out at 15°C. Data were analysed according to the manufacturers operating manual (Microbics).

4.4 Metal analysis

Total and EDTA extractable Cu, Zn, Pb, and Cd in the sediments were measured.

Total sediment bound metals were released by adding 10 ml concentrated HNO_3 and one ml concentrated $HClO_4$ to dried sediment (0.5 g) and boiling the sediment for 2 hours in a covered beaker. After digestion, the sediment was removed by centrifugation and the supernatant was diluted to 25 ml with ultra-pure water and retained for analysis.

EDTA-extractable metals were extracted by shaking with 10 ml of 0.01M EDTA (pH 8.0) for 5 h. The supernatant was made up to volume (25 ml) with ultra-pure water after centrifugation.

Metal concentration was determined using a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer with flame and Perkin-Elmer 603A Atomic Absorption Spectrophotometer with HGA 76B furnace techniques.

4.5 COD and pH

COD was determined by the standard reflux method (American Public Health Association, 1985) using dichromate/sulphuric acid oxidation and spectrophotometric determination of residual Cr^{3+}.

Sediment pH was measured by a combined pH electrode in a 10 ml aqueous extract of 1 gram of sediment.

4.6 Data analysis

All data was processed using a data analysis program, Statgraphics on a Personal Computer. Standard Deviation (±SD) was obtained from the results of ten samples at each site.

5. Results and discussion

The following discussion summarises the results obtained from the analysis of urban river sediments. Further details are presented in the appended articles.

5.1 COD and metal results in urban rivers

Tables 3-5 summarise the results obtained from the metal analysis and COD of sediment for all sites in the three urban river sediments (Fig.1).

Table 3. Chemical analysis results from Kvillebäcken

Site No.	COD (mg/g)	Cu(µg/g) Total	Cu(µg/g) EDTA	Zn(µg/g) Total	Zn(µg/g) EDTA	Pb(µg/g) Total	Pb(µg/g) EDTA	Cd(µg/g) Total	Cd(µg/g) EDTA
1	250	98	76	830	460	190	100	0.30	0.28
2	98	79	35	390	170	100	74	0.05	0.02
3	120	120	44	610	310	160	99	0.08	0.02
4	140	150	74	450	200	110	67	1.3	0.42
5	69	41	13	200	56	50	10	0.01	<0.01

Table 4. Chemical analysis results from Mölndalsån

Site No.	COD (mg/g)	Cu(µg/g) Total	Cu(µg/g) EDTA	Zn(µg/g) Total	Zn(µg/g) EDTA	Pb(µg/g) Total	Pb(µg/g) EDTA	Cd(µg/g) Total	Cd(µg/g) EDTA
1	44	130	5	86	<0.01	28	18	0.01	<0.01
2	130	140	61	320	160	110	9	2.1	0.57
3	160	440	82	990	200	310	180	4.3	0.53
4	25	52	2	51	<0.01	23	<0.01	<0.01	<0.01
5	5	36	5	64	<0.01	31	10	<0.01	<0.01

Table 5. Chemical analysis results from Balltorpsbäcken

Site No.	COD (mg/g)	Cu(µg/g) Total	Cu(µg/g) EDTA	Zn(µg/g) Total	Zn(µg/g) EDTA	Pb(µg/g) Total	Pb(µg/g) EDTA	Cd(µg/g) Total	Cd(µg/g) EDTA
1	160	150	70	680	160	66	31	1.2	0.25
2	170	100	26	1100	390	150	130	3.9	0.52
3	230	540	260	1500	590	300	96	1.4	0.68
4	110	91	45	180	71	400	50	0.03	<0.01

Highly elevated COD values were observed in the sites which were affected by urban runoff, especially by CSO. This is because fine settled sediments, high in pollutants and organic material are typical for stormwater and combined sewer overflow. The COD was low in the lake area (site 5 in Mölndalsån), but decreased COD values were also observed in the industrial and agricultural areas (site 5 in Kvillebäcken and site 4 in Mölndalsån).

Metal concentrations at site 5 (in both Mölndalsån and Kvillebäcken) were significantly lower than in the rest of the sites which were affected by urban runoff. Stormwater is a significant source of metals and enters the urban rivers at a large number of wet weather discharge points. Metal concentrations in Balltorpsbäcken and at site 2 and 3 in Mölndalsån were significantly higher than at the rest of sites. It could be anticipated that the contribution of metals in urban rivers was not only from urban runoff but also from industrial discharges.

5.2 Variations in toxicity

Tables 6-8 summarise the results obtained from the microbial activity assays, and standard deviations for ten of each sample assay.

Table 6. Bioassay results from Kvillebäcken

Site	DHA[a] (±SD)	APA[b] (±SD)	GLA[b] (±SD)	GAA[b] (±SD)	MIC[c]
1	7.6 (5.5)	15.00 (7.7)	36.00 (12)	23.00 (28)	0.023
2	2.5 (1.9)	9.9 (2.7)	9.6 (2.7)	9.6 (2.7)	0.025
3	1.6 (0.69)	15 (3.4)	46 (3.8)	15 (3.8)	0.0073
4	4.9 (3.9)	22 (7.7)	54 (2.4)	15 (2.4)	0.013
5	8.4 (0.88)	17 (3.1)	16 (2.4)	15 (2.4)	0.0017

Table 7. Bioassay results from Mölndalsån

Site	DHA[a] (±SD)	APA[b] (±SD)	GLA[b] (±SD)	GAA[b] (±SD)	MIC[c]
1	0.67 (0.45)	15 (5.2)	47 (20)	81 (40)	0.22
2	0.37 (0.11)	21 (3.8)	23 (0.00)	24 (6.9)	0.036
3	0.66 (0.11)	18 (2.8)	33 (3.9)	32 (5.4)	0.019
4	0.20 (1.6)	23 (4.9)	34 (6.1)	34 (9.0)	3
5	0.50 (0.74)	19 (2.4)	55 (9.2)	51 (18)	0.2

Table 8. Bioassay results from Balltorpsbäcken

Site	DHA[a] (±SD)	APA[b] (±SD)	GLA[b] (±SD)	GAA[b] (±SD)	MIC[c]
1	0.40 (0.0)	8.8 (0.38)	13 (1.1)	11 (0.69)	0.059
2	0.92 (0.33)	10 (2.5)	22 (11)	17 (5.4)	0.016
3	2.40 (0.75)	18 (2.6)	27 (5.5)	25 (4.6)	0.019
4	1.30 (0.33)	17 (4.5)	36 (8.8)	30 (3.9)	0.7

a. DHA bioassay is expressed as produced formazan (mg) per gram sediment dry weight per assay incubation time.
b. APA, GLA and DAA bioassays are expressed as produced nitrophenol (μg) per gram sediment dry weight per assay incubation time.
c. MIC stands for Microtox EC_{50} expressed as sediment (gram) dry weight per liter solution

The standard deviation for dehydrogenase activity of one sample was found to be very good (Table 9). However, in site variations for sediment samples were much greater (Tables 6-8). Therefore the enzyme analysis gave reliable and consistent results, but there were more difficulties in obtaining a representative sample.

In all enzyme assays, DHA was much higher than the others, followed by GLA, GAA and APA. GAA was only higher than GLA at site 1 in Mölndalsån. Microtox responses observed were mixed.

Table 9. The standard deviation (SD %) for dehydrogenase activity

Site	1	2	3	4	5
Kvillebäcken	1.38	10.70	0.16	4.19	4.78
Mölndalsån	14.29	3.48	4.43	9.37	7.6
Balltorpsbäcken	1.99	3.21	2.23	0.51	

Enzyme activities were normalised to the concentration of oxidisable organic material (estimated by COD analysis) to compare urban rivers with different sediment organic concentration. It can be used as an index to indicate the level of enzyme activity. The higher the enzyme activity index value, the lower the inhibition of activity by substances suggested. Figures 2-4 show variations of dehydrogenase activity/COD in the urban river sediments.

In general, dehydrogenase activity was higher in Kvillebäcken which receives mostly CSO, SSR and agricultural runoff, while Mölndalsån and Balltorpsbäcken receive not only CSO and SSR but also industrial discharges. It may therefore be generally proposed that some additional inhibition of dehydrogenase activity occurs in Mölndalsån and Balltorpsbäcken due to industrial discharge. At most sampling sites in the three rivers luminescence was inhibited and this suggests that pollutants from urban runoff have a significant effect.

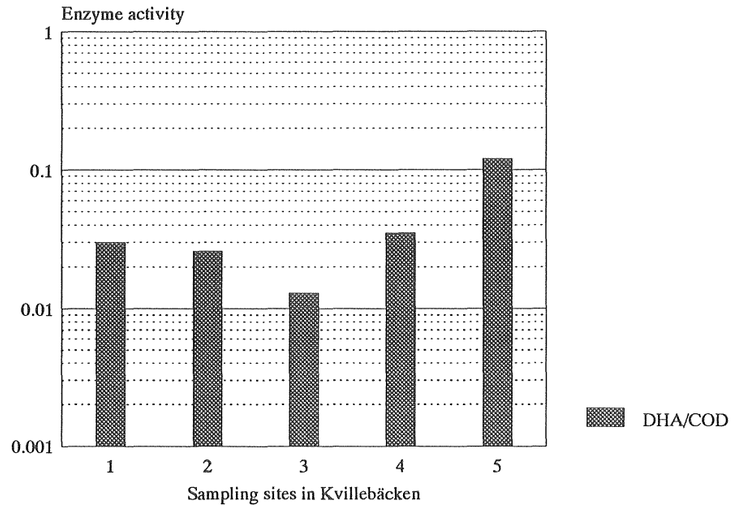


Figure 2. Enzyme activity in Kvillebäcken

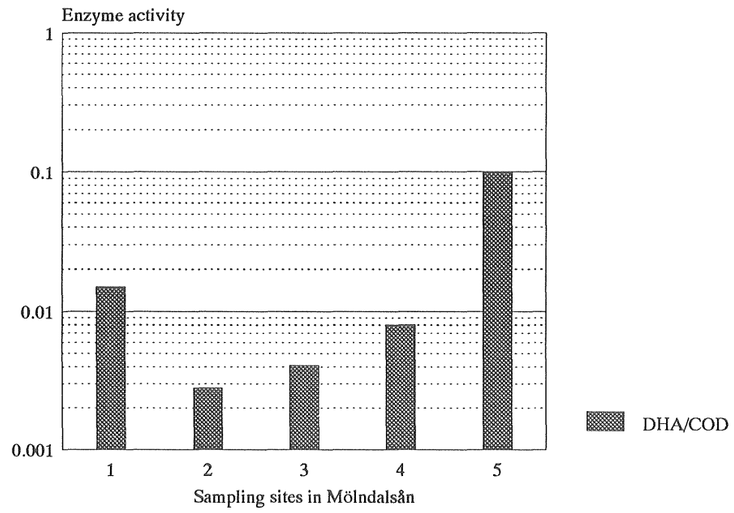


Figure 3. Enzyme activity in Mölndalsån

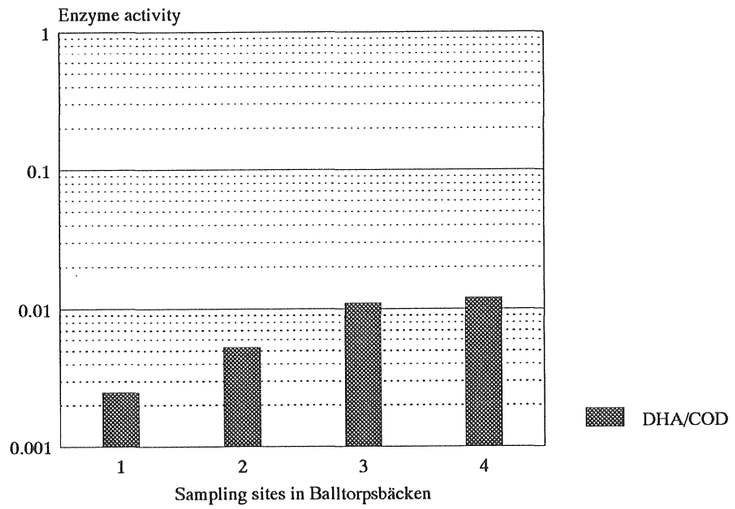


Figure 4. Enzyme activity in Balltorpsbäcken

DHA/COD was high in the agricultural areas. Increased activity was also observed at two CSO discharges in Kvillebäcken (site 1, 2) because domestic CSO contains a lot of nutrients which stimulate the enzyme activity. Enzyme activity was low at sites 3 and 4 in Kvillebäcken due to the effect of stormwater runoff. In a lake (site 5 in Mölndalsån), enzyme activity was high but decreased on entering the industrial area along Mölndalsån. An exception is site 1 where enzyme activity was again high. The enzyme activity was high at this site although metal concentrations were still relatively high. The toxicity of metals could be reduced by low EDTA extractable metals (Table 4) because enzyme inhibitory metals were strongly bound to the sediment and unavailable to the bacteria. Low enzyme activity was also observed in the area with low metals concentrations (site 4 in Mölndalsån). The toxicants in this site causing inhibition to enzyme activity may not be metals but organic compounds. Low enzyme activity was obtained in Balltorpsbäcken sites in which high metal concentrations were also found.

5.3 Selection of toxicity test

By applying test of enzyme activity which was in the sediment it is possible to confirm the actual results representing reality in the urban river. It was found from the analysis of enzyme activity, that dehydrogenase activity assay is an assay which is less disturbed by background colour. In addition, the dehydrogenase assay can provide comparative estimates of microbial activity. Because of its ease of analysis, high sensitivity, and high reproducibility, dehydrogenase activity therefore was selected as representative of enzyme activities in this study.

Microtox test was chosen as an indicator of toxicity in the sediment although the marine bacterium, *Photobacterium phosphoreum* used in Microtox test is not directly relevant to the study ecosystem, and it does not necessarily behave in the same way as those bacteria living in the urban river. The pattern of Microtox toxicity is highly dependent on the type of river for assay (Ankley, *et al.*, 1989) and individual chemicals (Hermens, *et al.*, 1985). However, the advantage of using Microtox test to detect the toxicity is that marine bacteria may be very sensitive to the contaminants which do not necessarily lead to toxicity to organisms living in sediment.

5.4 Variations in toxicity along the urban rivers

5.4.1 Kvillebäcken

Kvillebäcken is a small urban river draining the northwest urban area of Göteborg. Figure 1 shows that the river originally drains agricultural land (site 5) followed by separately sewered areas (sites 3,4) and combined sewered areas (sites 1,2) before finally flowing through a culvert into Göteborg's main river, the Göta älv. Sites 1 and 2 are located at points of combined sewer overflow.

DHA/COD was high and the other enzyme activities were slightly increased at site 5 with the exception of decreased GLA/COD and Microtox. Increased DHA/COD in the areas of agricultural runoff would be expected (Burton, 1987). Low MIC was observed in this site, which implies that MIC is very sensitive to the toxicity of agricultural runoff.

At sites 3 and 4, separate stormwater drainage (i.e. only urban surface runoff with no contribution from sewage) affects the river sediments. Enzyme activities reflect the influence of a high concentration of total and extractable metals from stormwater runoff at both sites (Table 3, and Fig. 2). High concentration of EDTA extractable Cu observed in these sites was anticipated causing inhibition of enzyme activity in sediment (Fig. 5 and 6). The Box and Whisker plot gives the median as the central line while the central box covers the upper and lower quartiles. The whisker extend to those extreme points which are within 1.5 times the interquartile range. Ten enzyme measurements were made at each site and SD is shown in Table 6.

The two sites receiving combined sewer overflow (site 1 and 2) generally showed high total metal concentrations, and elevated enzyme activity as well. This is not surprising as the sewage water can stimulate the enzyme activity in sediment. Evidence for sewage overflow deposited in the urban river sediment was found at site 1. A profile of the streambed was made by sampling at 10 metre intervals downstream from the sewer overflow. Metal concentrations and enzyme activities are shown in Figure 7.

The results show that enzyme activity, and COD, are high at 40-50 m downstream which indicates sewage deposition on the streambed. Further evidence for the presence of sewage overflow was the elevated COD (412 mg/g) downstream. The elevated metal concentrations may be due to metal chelation by the highly organic material in the carrying stormwater. Stormwater contains high concentrations of free and weakly

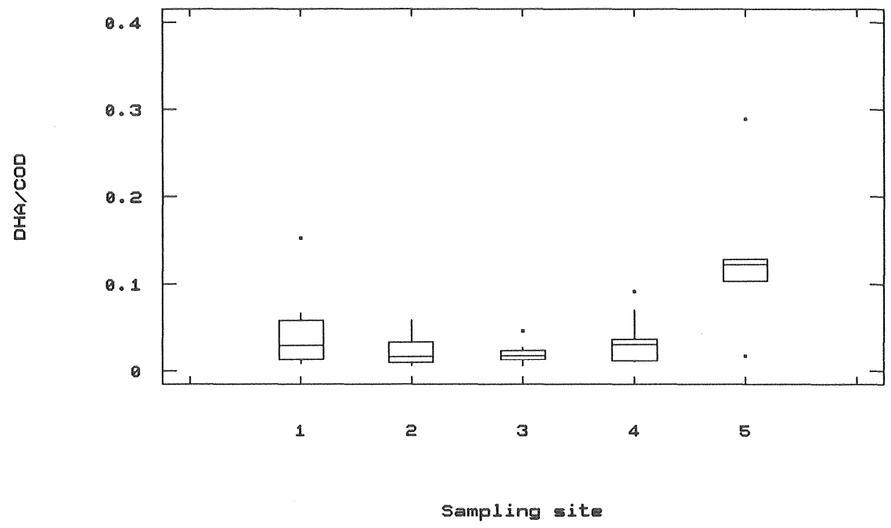


Figure 5. Enzyme activity variations along Kvillebäcken

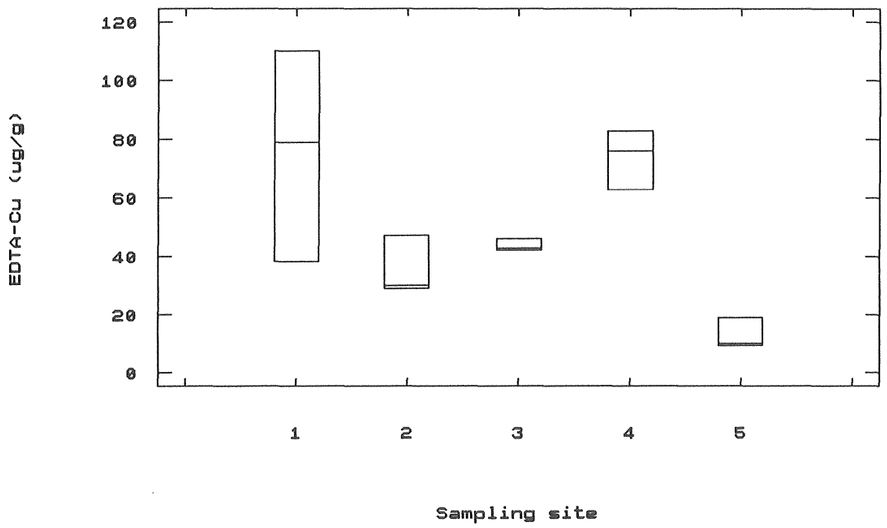


Figure 6. EDTA-Cu concentration in Kvillebäcken

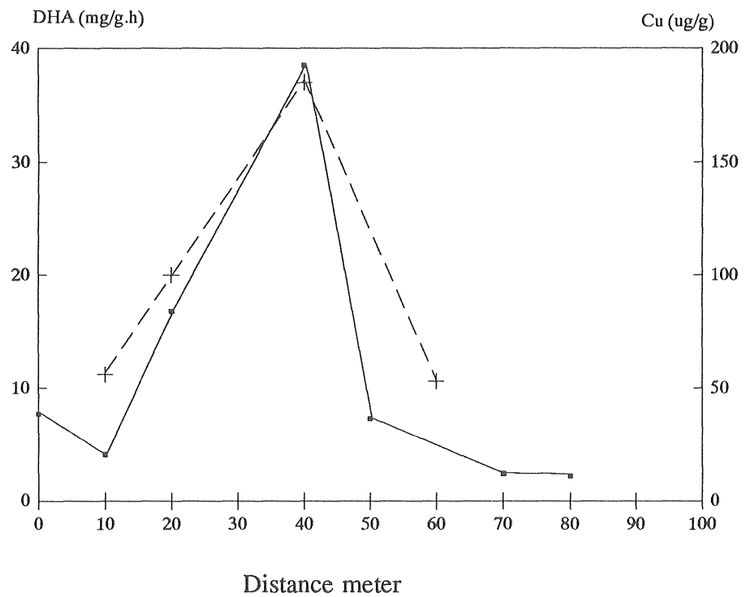


Figure 7. Variation of DHA and Cu concentration downstream from a CSO discharge.
_____ DHA (mg/g.h) - - - Cu (µg/g)

complexed metal species (Morrison *et al.*, 1984), which could readily be removed by sewage solids. Sewage overflow in urban rivers is therefore characterised by high enzyme activity and metal concentrations in the sediment. However, analysis of enzyme/COD shows that enzyme activity is inhibited.

5.4.2 Mölndalsån

Mölndalsån (Fig. 1) begins in a lake (Stensjön) which has a sampling site. It then proceeds through an industrial area (sites 2,3,4) which includes a paper producer (site 2), and automobile repair companies (site 3 and 4). The major inputs from stormwater are on entering the urban area (sites 1-4) and include a number of combined sewer overflows.

Enzyme activities along the river reflect industrial and stormwater discharge (Figure 3). In the lake sediment (site 5) the enzyme activity is high, but decreases on entering the industrial area. Metal levels are at site 4 but enzyme activity was still low compared to site 5, and it may be anticipated that it is toxic organic compounds which are inhibiting the enzyme activity of the bacterial biomass.

The stormwater affected sites (1-4) are characterised by a relatively low enzyme activity and high total metal concentrations as shown in Figure 3 and Table 4. The exception is at site 1, where the enzyme activity increases, although total Cu is still relatively high. This may be explained by consideration that EDTA-extractable Cu is very low at site 1 and therefore enzyme inhibitory metals are strongly bound to the sediment and unavailable to the bacteria.

The presence of industrial activity in Mölnsdalsån complicates the interpretation of the effects of urban stormwater discharges. However, Mölndalsån is typical of urban rivers with mixed industrial and sewer discharges.

5.4.3 Balltorpsbäcken

Balltorpsbäcken is a small slow-flowing river which receives SSR and industrial discharges. Normally, it only receives SSR and some small industrial discharge. However, during periods of heavy rain, deposited pollutants backflow from Mölndalsån to Balltorpsbäcken and relocate themselves in the sediment (Fig.1).

Enzyme activities along the river reflect industrial and stormwater discharge. In relatively less polluted sediment (site 4) the activities were high, but decreased along the distribution of contaminant influences (Fig.4). The concentrations of heavy metals were high in this river (Table 5). The results suggest that sites 1-4 are characterised by redistribution of pollutants transferred from Mölndalsån to Balltorpsbäcken during heavy rainfall.

Higher Microtox value was observed at site 4. This may be due to the influence of seawater on the urban river sediments.

5.5 Inhibition of microorganism response by sediment-bound pollutants

The presence of the toxic fraction by separation of extracts was suggested by decreased inhibition on microbial activity (Table 10). It has been shown that pollutants in urban river sediments inhibit enzyme activity and luminescence. However, it was not known which pollutants are causing sediment toxicity. Therefore, to separate the pollutants from the sediment by chemical extractions was decided and in this way metals or organics could be identified as causing toxicity (Lanza, *et al.*, 1988).

In the case of enzyme activity it was only possible to use chemical extractants which are non-toxic to the sediment-dwelling bacteria. EDTA and ethanol have been tested and caused no change in enzyme activity at the chosen concentration, but nonpolar solvents, such as pentane or benzene could not be used.

For Microtox test the sediment is only exposed to the organisms after extraction and therefore it was possible to use more nonpolar solvents, such as pentane for hydrocarbons and pentane/benzene for aromatic hydrocarbons.

EDTA is a reagent which removes metals. Inhibition of sediment was decreased by 23 %, 14 % and 13 % in Mölndalsån, Balltorpsbäcken and Kvillebäcken sediments respectively after EDTA extraction (Table 10). Ethanol may extract lipid soluble contaminants which may come from CSO and some industrial discharge. The inhibition of contribution by these two fractions was low. Polyaromatic hydrocarbon (PAH) and chlorinated hydrocarbon may be present in Mölndalsån and Balltorpsbäcken sediments because a significant increase percent of microbial activity was obtained after fractionation of the sediment by pentane and benzene. Measurements of 17 PAHs give total concentrations of 3-19 µg/l in urban runoff (Marsalek, 1990).

Table 10. Effect of chemical extraction on Microtox test in the sediments

	% average increase of Microtox EC_{50} after extraction with			
River	0.01 M EDTA	Ethanol	Pentane/Benzene (1:1)	Pentane
Kvillebäcken	13	18	12	0
Mölndalsån	23	17	335	474
Balltorpsbäcken	14	5	196	269

The fractionation experiment was also carried out using the DHA assay. Results are shown in Table 11.

Table 11. Effect of physico-chemical extraction on DHA in the sediments

	% average increase in DHA after extraction with		
River	10^{-4}M EDTA	0.5% Ethanol	Air 20 min.
Kvillebäcken	0	0	2.6
Mölndalsån	14.7	14.2	0
Balltorpsbäcken	4.2	11.2	24

The effect of the extractable fraction on DHA in Mölndalsån and Balltorpsbäcken sediments were very similar with that of Microtox test. The metal fraction contributes to DHA inhibition as 14.7 % and 4.2 %, and the lipid fraction 14.2 % and 11.2 % in Mölndalsån and Balltorpsbäcken respectively. No effect was observed for the EDTA and ethanol extractable fraction in Kvillebäcken. This may be due to either incomplete extraction with low concentration of extractants or insufficient sensitivity of DHA assay to the contaminants in the sediments removed by extraction. However, studies have shown that DHA assay is more sensitive to metals than organics (Lenhard, 1968).

Aeration was designed to determine whether toxicity can be attributed to volatile or oxidisable compounds in the sediments. The results show that inhibition of the volatile or oxidisable fraction may exist in Balltorpsbäcken sediment.

6. Conclusions

The conclusions can be drawn as follows:

* By using a natural organism more complex answers are obtained but they are ecologically more realistic since natural interactions between organisms and the abiotic environment are maintained. Dehydrogenase activity can represent the enzyme activities in sediment because the values of it were much higher than others, the results of it were more reliable, and activity pattern of it in the urban river sediments were correlated with others. The Microtox test showed a varied sensitivity pattern. However, it can be employed as an indicator for unexpected contaminants in the assessment of urban river. Dehydrogenase activity assay presented here may contribute to developing ecotoxicologically based quality standards for urban river management under wet-weather conditions.

* Based on the data obtained, three urban river profiles were made according to the level of biological activity and distribution of COD and metal concentrations. Mölndalsån is the greatest contaminated river with low enzyme activity, high concentration of COD and metals. Balltorpsbäcken is influenced greatly by toxicants transferred from Mölndalsån during heavy rain and recoveres gradually along the river. Kvillebäcken was characterised by low DHA and high extractable metal concentrations due to the discharges of CSO and SSR. It is very obvious that not only some industrial discharges affect urban rivers in Göteborg region, but also CSO and SSR are causing degradation of receiving water quality.

* Inhibition proportion on microorganism can be identified successfully by using chemical extraction of the sediment prior to a toxicity test. Toxicity of metals, lipid fraction and polyaromatic hydrocarbons in the sediment were revealed in Mölndalsån by increased Microtox EC_{50} after chemical extraction. Significant inhibition from polyaromatic hydrocarbons was also observed with Microtox in Balltorpsbäcken. In Kvillebäcken, only metals inhibition was obtained. Inhibition of metals was also obtained by increased DHA assay after chemical extractions throughout the three investigated urban rivers.

7. Recommendations and future work

There is a shortage of satisfactory ecotoxicological methods developed to investigate the effects of urban runoff on urban rivers. The methods developed in this study only considered the bacterial community. For further work, wild species which are supposed to give the most sensitivity to variation of toxicants and physical changes in the sediment are suggested for use in the assessment. Other ecological levels also need to be considering such as plankton and fish.

This study suggests that urban runoff has an ecological effect in receiving waters. Therefore, there is required an investigation of methods for wet-weather management, for example, the effectiveness of retention ponds or reed bed.

References

American Public Health Association, 1985. Standard methods for the examination of water and wastewater. New York.

Anderson, K., B. Koopman & Bitton, G.,1988. Evaluation of INT-dehydrogenase assay for heavy metal inhibition of activated sludge. Wat. Res. 22: 349-353.

Ankley, G. T., Hoke, R.A., Giesy, J.P., & Winger, P.Y., 1989. Evaluation of the toxicity of marine sediments and dredge spoils with the microtox bioassay. Chemosphere. 18: 2069-2075.

Barnhart, C. L. H. & Vestal, J.R., 1983. Effects of environmental toxicants on metabolic activity of natural microbial communities. Applied and Environmental Microbiology. 46: 970-977.

Bascombe, A., 1988. Urban pollution. Middlesex Polytechnic Research & Consultancy. Middlesex, 129 pp.

Bascombe, A.D., Ellis, J.B., & Revitt, D.M., 1990. The development of ecotoxicological criteria in urban catchments. Water Sci. and Tech. 22:173-180.

Bitton, G., 1983. Bacterial and biochemical tests for assessing chemical toxicity in the aquatic environmental: A review. CRC Critical Reviews on Environmental Control. 13:51-67.

Broberg, A., 1983. Effects of heavy metals on electron transport system activity (ETSA) in freshwater sediments. Environmental Biogeochemistry., 35: 403-418.

Broberg, A., 1980. Measurement of electron-transport-system activity in freshwater sediment. Andersen, F. Ö., *et al.*, (eds). Odense :172-193

Burkhard, L. P. & G. T. Ankley, 1989. Identifying toxicants: NETAC's toxicity based approach. Environ. Sci. Technol., 23: 1438-1443.

Burton Jr, G. A. & Lanza, G.R., 1987. Aquatic microbial activity and macrofaunal profiles of an Oklahoma stream. Wat. Res., 10: 1173-1182.

Burton, G. A., 1988. Stream impact assessments using sediment microbial activity tests. Chemical and biological Characterization of Sludges, Sediments, Dredge Spoils, and Drilling Muds. ASTM STP 970, J. J. Lichtenberg (ed.), American Society for Testing and Materials, Philadelphia,: 300-310.

Christianson, J.P., & Packard, T.T., 1977. Sediment metabolism. Deep-sea Res., 24: 331-343.

Cole, R.H., Frederick, R.E., & Rolan, R.G., 1984. Preliminary findings of the priority pollutant monitoring project of the nationwide urban runoff program.JWPCF, 56: 898-908.

Davis, J. B. & George, J.J., 1987. Benthic invertebrates as indicators of urban and motorway discharges. The Science of the Total Environment, 59: 291-302.

Dutka, B. J., Nyholm, N., & J. Petersen, 1983. Comparison of several microbiological toxicity screening tests. Water Res., 17: 1363-1368.

Dutka, B. J., Kwan, K.K., Jurkovic, S.S., & D. Liu, 1991. River evaluation using ecotoxicological and microbiological procedures. Envir. Monitoring and Assessment, 16: 287-313.

Ellis, J. B., 1989. The quality of urban stormwater: a state of the art review. Middlesex Polytechnic. London, UK.

Fuller, C. C., Davis, J.A., Cain, D.J., & Lamothe, P.J., 1990. Distribution and transport of sediment-bound metal contaminants in the Rio Grande De Tarcoles, Costa Rica (Central America). Wat. Res., 7: 805-812.

Giesy, J. P., Graney, R.L., & Newsted, J.L., 1988. Comparison of three sediment bioassay methods using Detroit river sediments. Envir. Toxicology and Chemistry, 7: 483-498.

Hall, K. & Anderson, B.C., 1988. The toxicity and chemical composition of urban stormwater runoff. Can. J. Civ. Eng., 15: 98-106.

Hammons, A. S. (editor), 1981. Ecotoxicological test systems. Proceedings of a series of workshops. EPA-560/6-81-004, 183 pp.

Hermens, J., Busser, F., Leeuwangh, P., & Musch, A., 1985. Quantitative structure-activity relationships and mixture toxicity of organic chmemicals in Photobacterum phosphoreum: the microtox test. Ecotoxicology and Environmental Safety, 9: 17-25.

Hoffman, E.J., Latimer, J.S., & Quinn, J.G., 1982. Petroleum hydrocarbons in urban runoff from a commercial land use area. JWPCF, 54: 1517-1525.

Hunter, J.V., Sabtino, T., & McKenzie, M.J., 1979. Contribution of urban runoff to hydrocarbon pollution. JWPCF, 51: 2129-2138.

Keramida, V., T. Renner & B. Neilson, 1989. Study of toxic compounds in river bottoms at metropolitan areas. 43rd Purdue Insdustrial Waste Conference Proceedings, Lewis Publishers, Inc., Chelsea, Michigan 48118,: 7-17.

Lanza, G. R., Burton, G.A.Jr., & Dougherty, J.M., 1988. Microbial enzyme activities: potential use for monitoring decomposition processes. Functional Testing of Aquatic Biota for Estimation Hazards of Chemicals, ASTM STP 988, J. Cairns, Jr. & J. R. Pratter,(eds.), American Society for Testing and Materials, Philadelphia,: 41-54.Lenhard, G., Nourse, L.D., & Schwartz, H.M., 1964. The measurement of dehydrogenase activity of activated sludge. Baars, J.K. (ed.), Pergamon Press, Oxford, 2: 105-119.

Loellen, L., Chuang, J., Nishioka, M., & Petersen, B., 1990. Bioassay-directed fractionation of the organic extract of srm 1649 urban air particulate matter. Intern. J. Envir. Anal. Chem., 39: 245-256.

Malmqvist, P.A., 1983. Urban stormwater pollutant sources. Department of sanitary Engineering, Chalmers University of Technology, ISBN 91-7032-106-X.

Marsalek, J., 1990. Evaluation for pollutant loads from urban nonpoint sources. J. Wat. Sci. Tech., 22: 22-30.

McFeters, G. A., Bond, P.J., Olson, S.B., & Tchan, Y.T., 1983. A comparison of microbial bioassays for the detection of aquatic toxicants. Water Res., 17: 1757-1762.

Microbics Microtox Co., Manual of How to Run Microtox M500.

Moriaty, F., 1988. Ecotoxicology, Academic Press, Harcourt Pub.

Morrison, S. J., King, J.D., & Bobbie, R.J., 1977. Evidence for microfloral succession on Allochthonous Plant Litter in Apalachicola Bay, Florida, USA. Marine Biology, 41: 229-240.

Morrison, G.M.P., Revitt, D.M., & Ellis, J.B., 1984. The physico-chemical speciation of zinc, cadmium, lead and copper in urban stormwater. In proc. 3rd Int. Conf. Urban Storm Drainage, Göteborg, Sweden 1984, 3: 989-1000

Morrison, G.M.P., Revitt, D.M., & Ellis, J.B., 1987. Heavy metal exceedance of water quality standards during storm events. Gujer, W. and Krejei, V.(eds): Topics in Urban Stormwater Quality, Planning and Management, Ecole Polytechnique Federal, Lausanne, : 91-96.

Morrison, G., Batley, G.E., & Florence, T.M., 1989. Metal speciation and toxicity. Chemistry in Britain, 25: 791-796.

Samant, H. S., Doe, K.G., & Vaidya, O.C., 1990. An integrated chemical and biological study of the bioavailability of metals in sediments from two contaminated harbours in new brunswick, Canada. The Science of the Total Environment, 96: 253-268.

Sayler, G. S., Puziss, M., & Silver, M., 1979. Alkaline phosphatase assay for freshwater sediments: application to perturbed sediment systems. Applied and Environmental Microbiology, 38: 922-927.

Trevors, J. T., 1984. The measurement of electron transport system (ETS) activity in freshwater sediment. Wat. Res., 5: 581-584.

U.S. EPA, 1983. Toxicity guidelines, Final report to the National Urban Runoff Programme, Water Planning Division, Washington D.C.

Wieser, W. & Zech, M., 1976. Dehydrogenases as tools in the study of marine sediments. Mar. Biol., 36: 113-122.

Wilber, W.G., & Hunyrt, J.V., 1977. Aquatic transport of heavy metals in the urban environment. Water Resources Bulletin, 13: 721-734.

Williamson, K.J. & Johnson, D.G., 1981. A bacterial bioassay for assessment of wastewater quality, Wat. Res., 15: 383-386.

Zimmerman, R., Iturriaga, R., & Becker-Berck, J., 1978. Simulations determination of the total number of aquatic bacteria and the number thereof involved in respiration. Appl. Environ. Microbiol., 36: 926-935.

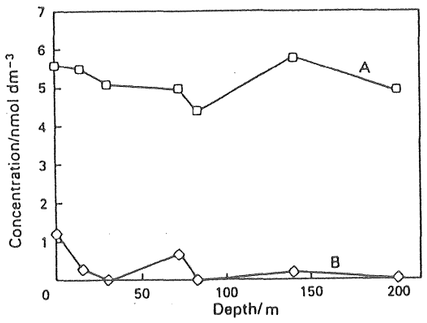


Fig. 4 Profile of labile Cr^{VI} and Cr^{III} concentrations in sea-water from the north-west Mediterranean (April 1990; 20 nautical miles off Marseille, France). A, Cr^{VI}; and B, Cr^{III}

20 nautical miles off Marseille (France). The quantification of Cr^{III} was not very accurate because our procedures had not been fully optimized. Nevertheless, the fairly constant Cr^{VI} profile confirms the vertical mixing of the water column in this area at this time of the year. The mean concentration of labile Cr^{VI} in open sea-water from the Mediterranean was found to be about 5 nmol dm^{-3}.

References

1 Murray, J. W., Spell, B., and Paul, B., in *Trace Metals in Sea Water*, eds. Wong, C. S., Boyle, E., Bruland, K. W., Burton, J. D., and Goldberg, E. D., Plenum Press, New York and London, 1983, pp. 643–669.
2 Cranston, R. E., and Murray, J. W., *Anal. Chim. Acta*, 1978, **99**, 275.
3 Golimowski, J., Valenta, P., and Nurnberg, H. W., *Fresenius Z. Anal. Chem.*, 1985, **322**, 315.
4 Crossmun, S. T., and Mueller, T. R., *Anal. Chim. Acta*, 1975, **75**, 199.

Electroanalysis of Metal Speciation and Its Relevance to Ecotoxicology

Gregory M. Morrison and Chen Wei
Department of Sanitary Engineering, Chalmers University of Technology, S-412 96 Göteborg, Sweden

Metal Speciation Analysis

Undoubtedly metal speciation analysis is an essential step in the study of the significance of priority metal pollutants during discharge, mobilization or transformation in natural water.[1] It is known that certain forms of metals, for example ionic copper or methylmercury, are more toxic than complementary forms of the same metals, for example copper bound to fulvic acid and inorganic mercury.

Electroanalysis, usually involving anodic stripping voltammetry (ASV), is frequently used because of its intrinsic capability in separating ionic metal species from electroinactive forms and its estimate of the potential metal detoxification capacity of the water sample, *viz.*, complexation capacity.[2]

Consequently, attempts have been made to compare metal speciation and toxicity in water samples and to relate further the results to in-stream ecology. These ideas are illustrated for an urban discharge in Fig. 1.

Metal Speciation and Toxicity

Laboratory comparisons of the interactions between model ligands, such as fulvic acid and NTA (nitrilotriacetic acid), and metals, have shown consistencies in the metal detected by bioassay and ASV (Table 1). It should be stressed that lability depends on the experimental conditions at the mercury electrode.[3]

The flexibility of these speciation measurements was enhanced by the introduction of mercury electrodes coated with Nafion (perfluorosulphonate) and cellulose acetate. These allow the separation of metal complexes which are negatively charged and have low molecular size, respectively.[4]

The empirical comparisons of bioassay with electrochemical analysis might be satisfactory in laboratory studies with a controlled sample matrix, but difficulties arise in water samples where surfactants significantly affect the transport of ionic metal to the mercury electrode. By a combination of medium exchange and controlled acid additions it has been possible to estimate that the interference of surfactants amounts to a 60–100% decrease in metal lability in water samples.[5] Clearly, as surfactants in natural or polluted waters are the dominant factor in the decrease of ASV lability and at the same time do not decrease metal toxicity to aquatic test organisms, then ASV will seriously underestimate the toxic fraction.

This difficulty can be overcome by the use of the acidification procedure outlined in Table 2. The measurement required for toxicity studies (*i.e.*, the effect of complexing agents on deposition) is [100 − (2) + (1)] where the values are in per cent. of the total metal. Lability values in natural waters

Table 1. Speciation and toxicity of copper to algae

	Copper toxicity or lability (%)				
	Bioassay		ASV		
	Nitzschia closterium	*Chlorella pyrenoidosa*	HMDE*	MFE†	NMFE‡
Fulvic acid	8	60	21	8	36
Fe–humic colloid	60	109	36	4	78
NTA	20	ND§	67	ND§	3

* HMDE = Hanging mercury drop electrode.
† MFE = Mercury film electrode.
‡ NMFE = Non-mercury film electrode.
§ ND = Not determined.

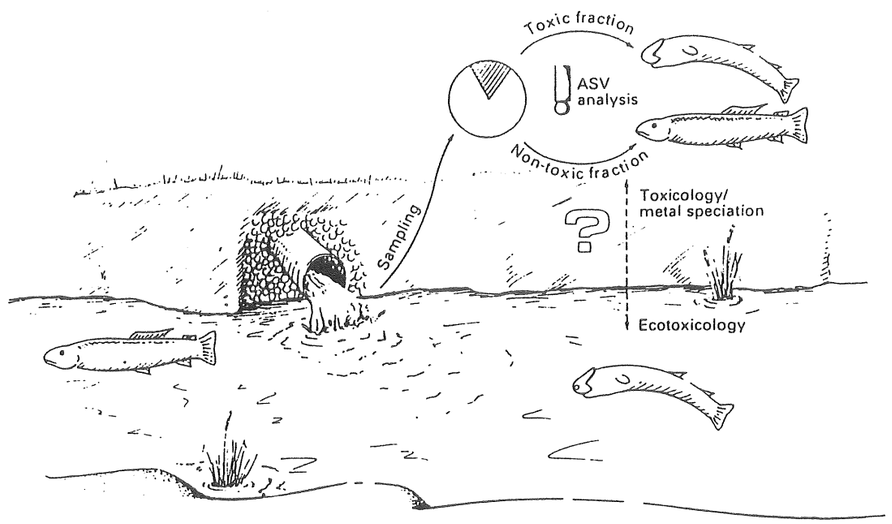


Fig. 1 Relationship between metal speciation, toxicology and ecotoxicology

Table 2. Effects of complexing agents and surfactants on the deposition and stripping steps of ASV

	Complexing agents		Surfactants	
ASV technique	Deposition	Stripping	Deposition	Stripping
(1) Deposition, pH 7.0 Stripping, pH 1.9	Yes	No	Yes	Yes
(2) Deposition, pH 7.0 Stripping, pH 1.9	No	No	Yes	Yes

increase significantly owing to the absence of surfactant effects. That this is a correct value is confirmed by medium exchange during stripping, which also gives an increase in lability owing to surfactant removal.[5]

Metal Speciation and Ecotoxicology

It might be the case that metal speciation procedures, which have been verified under controlled laboratory conditions and in parallel with toxicity studies, will require further verification in order to determine their ecological significance. Fig. 1 illustrates the latter relationship and identifies the problem of deciding whether toxic metal species are likely to cause ecotoxicological effects.

It does appear that complexation capacity and toxic metal species analysis might profitably be combined with ecotoxicological methodology.

Bioaccumulation of metals in transferred or *in situ* populations and community diversity indices are notoriously difficult to relate to metal speciation measurements.[6] However, metal speciation analysis appears to be relevant to the enzyme activity of the *in situ* sedimentary microbial community.[7] The tolerance of the periphyton community[8] and metallothionein accumulation and avoidance behaviour in fish populations might also prove promising. These developments provide a challenge for analytical chemists to develop metal speciation methods that have ecotoxicological relevance.

References

1 Morrison, G. M. P., Batley, G. E., and Florence, T. M., *Chem. Br.*, 1989, 791.
2 Florence, T. M., *Analyst*, 1986, **111**, 489.
3 Morrison, G. M. P., and Florence, T. M., *Anal. Chim. Acta*, 1988, **209**, 97.
4 Morrison, G. M. P., and Florence, T. M., *Electroanalysis*, 1989, **1**, 485.
5 Morrison, G. M. P., Florence, T. M., and Stauber, J. L., *Electroanalysis*, 1990, **2**, 9.
6 Luoma, S. N., *Sci. Total Environ.*, 1983, **28**, 1.
7 Morrison, G. M. P., and Chen, W., *Hydrobiologia*, in the press.
8 Blanck, H., and Wängberg, S., *Can. J. Fish. Aquat. Sci.*, 1988, **45**, 1816.

Bacterial enzyme activity and metal speciation in urban river sediments

Chen Wei and Greg Morrison

Department of Sanitary Engineering,
Chalmers University of Technology,
S-412 96 Göteborg, Sweden

Abstract

The effects of stormwater and combined sewer overflows on receiving waters were investigated using measurements of bacterial enzyme activity and metal speciation in the sediments of five urban rivers.

Free flowing urban rivers had high enzyme activity and low metal concentration in sediments, indicating a lack of contribution by stormwater sediments. More stagnant urban rivers, which tended to trap sewer-discharged sediments, were characterised by inhibited enzyme activity and high ammonium acetate- and EDTA-extractable metal concentrations.

Profiles along two urban rivers showed a direct inhibition of enzyme activity at sites of stormwater and industrial discharge. Deposited sewage, from combined sewer overflows, was indicated by highly elevated enzyme activity and metal concentrations.

The results of this study demonstrate that the ecologically relevant enzyme activity measurement

may be a useful complement to metal speciation analysis when investigating the effects of stormwater discharges on urban rivers.

Keywords: stormwater; enzyme activity; heavy metals; speciation; sediment

INTRODUCTION

Urban runoff discharges metals, polyaromatic hydrocarbons and oxygen demanding substances at separate stormwater sewer outfalls (SWO) and combined sewer overflows (CSO) into the receiving waters (Berkas, 1980). These pollutants may exert, on the urban river, both long-term and short-term ecosystem damage (Moriarty, 1988). The extent of such damage is to some extent dependent on the individual pollutant concentration in the urban runoff relative to the flow or circulation of receiving waters. However, a full assessment of the effect cannot be made without consideration of the speciation of pollutants in the discharge and their transformations in the receiving waters, as well as the sensitivity of the receiving water community to such pollutant changes (Morrison et al., 1989).

Metal speciation studies of urban runoff show that both dissolved and suspended solid species are discharged throughout the storm profile (Morrison et al.,1989). Toxic dissolved species concentrations, which can be analysed by the double acidification electrochemical method (Morrison et al.,1990), exceed established water quality criteria recommendations (Morrison et al., 1987) and can therefore provide a direct threat to receiving waters. Metal species in the suspended solid phase, analysed by sequential extractions (Morrison et al., 1984), can accumulate in the receiving water sediments and exert long-term ecological damage.

The connection between pollutant speciation and ecotoxicological effect in the receiving waters

for stormwater is still an uncertain area. Evidence has been provided that the freshwater shrimp, Gammarus pulex, accumulates metals when transferred to urban rivers (David et al., 1989). However, the in-stream environment of urban rivers can change frequently as a result of storm events. For this reason it may be more relevant to concentrate on organisms which easily adapt to the changing environment of the urban river and can be measured in situ between storm events. The bacterial community is ideal in this respect and the status of this community can be assessed by measurements of enzyme activity.

Bacterial enzyme activity has been widely used in monitoring active sewage sludge (Chung et al., 1989) and has recently been applied to environmental monitoring (Dutka 1986). Dehydrogenase (i.e. enzymes which oxidise organic compounds leading to a loss of protons) and phosphatase activity are commonly measured and have been reported to be inhibited by metal pollution (Wood et al., 1987). In addition these assays are straightforward, sensitive and inexpensive to carry out.

The objectives of the present research are (1) to assess the bacterial enzyme status in urban river sediments, (2) to identify the metal speciation, and (3) to determine the ecotoxicological effect of metals discharged from urban areas to rivers and streams in Göteborg.

EXPERIMENTAL

1. Urban river sites and sampling.

Samples were collected from five urban rivers in the Göteborg region, Sweden (Table 1). All urban rivers ultimately discharge into the Göta älv (a river), although Kvibergsbäcken discharges first into the Säveån. Sightings of migratory salmon are common in Mölnsdalsån and Kvillebäcken, while Delsjöbäcken has a visible resident population of trout.(Table 1.)
All urban rivers receive CSO at 3x the baseflow (except Kvibergsbäcken where it is 5x) and also considerable quantities of SWO.

Delsjöbäcken and Kvibergsbäcken are free flowing, and the sampled sediments showed little evidence of organic material, as indicated by analysis of COD (chemical oxygen demand) (Table 1). Kvillebäcken, Mölndalsån and Balltorpsbäcken, on the other hand, were fairly stagnant and showed evidence of organic compounds from urban discharges.

Sediments were collected by a grab sampler from the top surface of the river bed. Ten samples were taken at each sampling site for enzyme analysis, of which 3 samples were analysed for metal speciation and COD.

2. Enzyme activity

Wet sediment (0.5 g) was diluted in 50 ml of ultra-pure water (double distilled water passed through a Milli-Q system) in a beaker (100 ml) and sonicated for 1 minute (in an Artex model 150 sonic dismembrator, the power was at 30% relative output). The concentration of sediment suspended in the water was measured by weighing aliquots of the sediment slurry, which had been dried at 105°C.

The dehydrogenase activity in the sediment was determined using the method of Zimmerman et al. One ml of suspended solution was placed into a test tube. Four ml of 0.1M phosphate buffer at pH 7.4 (50 ml 0.1M KH_2PO_4 + 39.1 ml 0.1M NaOH) and 1 ml of 0.2% INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride) were added to the test tube followed by 0.1 ml of 0.2% (w/v) glucose as stimulatory substrate. After vortex mixing, the tubes were incubated in the dark at 20°C. The reaction was stopped after 1 hour by adding 1.0 ml of 37% formalin. Supernatant was decanted from the INT-treated mixture after centrifugation at 4000 rpm for 20 minutes. Five ml of 99.5% ethanol was added to the tube, to extract the formed formazan. Formazan was measured, and standardised in ethanol, spectrophotometrically at 480 nm after further centrifugation.

Activity of glucosidase, galactosidase and phosphatase was measured by the method of Sayler (Saylar et al., 1979), and Morrison (Morrison et al., 1977). In this assay, the substrates p-nitrophenyl-galactoside, p-nitrophenyl-glucoside, and p-nitrophenyl-phosphate were cleaved, to leave p-nitrophenol, by the bacterial enzymes galactosidase, glucosidase and phosphatase,

respectively. p-Nitrophenol gives a yellow solution and can be measured in a spectrophotometer at 418 nm.

3. Metal speciation

Sediment was dried (105°C) before analysis for total metals and for ammonium acetate and EDTA (ethylenediaminetetraacetic acid) extractable metal fractions.

Total sediment bound metals were released by the addition of 10 ml concentrated HNO_3 and one ml concentrated $HClO_4$ to dried sediment (0.5 g) and boiling for 2 hours in a covered beaker. After digestion, the sediment was removed by centrifugation and the supernatant was diluted to 25 ml with ultra-pure water.

Sediment species were extracted by shaking with 10 ml of 0.01M EDTA (pH 8.0) and 10 ml of 1M NH_4OAc (ammonium acetate) at pH 7.0 for 5 and 1 h respectively. The supernatant was made up to volume (25 ml) with ultra-pure water after centrifugation.

Metal concentration was determined using a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer with flame and Perkin-Elmer 603A Atomic Absorption Spectrophotometer with HGA 76B furnace techniques.

RESULTS AND DISCUSSION

Enzyme activities and metal speciation

Enzyme activity and metal speciation results for the urban rivers studied are shown in Tables 2 and 3, respectively. Enzyme activities were normalised with respect to the oxidisable organic material (estimated by COD analysis) in order to compare urban rivers with different organic concentrations. However, all sampled bacterial communities were stimulated by the addition of glucose substrate. The addition of substrate was also found to give more reproducible results.

All enzyme activity measurements (dehydrogenase, glucosidase, galactosidase and phosphatase) gave similar trends in urban rivers (Table 2). Enzyme activity was high in the free flowing Kvibergsbäcken where no evidence for the presence of CSO or SWO sediments was found. This is reflected in total metal concentrations (Table 3) which are also low in this river. Enzyme activity was found to be inhibited in the more stagnant urban rivers (Kvillebäcken, Mölndalsån and Balltorpsbäcken) and the high metal concentrations found reflect the accumulation of sediments from urban discharges.

Metal speciation in the urban river sediments was measured using two extractants separately. The ammonium acetate selects metals adsorbed to the sediment surface, while the EDTA extracts chelated as well as adsorbed metal (Rendell et al., 1980). Neither extractant has been shown to

extract the "toxic" metal fraction, although EDTA is known to effectively mask copper toxicity in algal bioassay (Morrison et al., 1989). EDTA also does not suffer from the readsorption problems found with other sediment extractants (Morrison et al., 1987).

The availability of Zn, Cu,and Pb in urban river sediments, as measured by ammonium acetate and EDTA extractions, was found to be higher in the urban rivers with the highest metal concentrations (Table 3). Suspended solids in urban discharges have been shown to contain high concentrations of metals, with a considerable percentage in extractable forms (Morrison et al., 1984). In one urban river (Kvillebäcken) it was found that the percentage metal in an EDTA extractable form increased from a background site unaffected by urban discharge (Cu,32%; Zn, 21%; Pb,15%; Cd,1%) to a site receiving separate stormwater discharge (Cu,43%; Zn,21%; Pb,67%; Cd,14%). Similar trends were found for ammonium acetate extracted metal.

Variations along urban river profiles

Kvillebäcken

Kvillebäcken is a small urban river draining the northwest urban area of Göteborg. Figure 1A shows that the river originally drains agricultural land (site 5) followed by separately sewered areas (sites 3,4) and combined sewered areas (sites 1,2) before finally flowing through a culvert into Göteborg's main river, the Göta älv.

Sites 1 and 2 are located at points of combined sewer overflow which discharge at 3x river baseflow. Site 5 is unaffected by urban runoff and has low levels of sediment bound metal both in terms of the extractable fractions (Figure 1C) and total metal. The enzyme activity is high at this site (Figure 1B) and comparable to the values found in Kvibergsbäcken where stormwater sediments were apparently absent.

At sites 3 and 4 separate stormwater drainage (i.e. only urban surface runoff with no contribution from sewage) affects the river sediments. Enzyme activities reflect the influence of stormwater runoff with inhibition at both sites (Figure 1B). Ten enzyme measurements were made at each site and the range of measured values are shown in Figure 1B. Metal concentrations increase considerably, compared to site 5, and the EDTA extractable fraction represents 42$\pm$6 %, 61$\pm$1 %, 47$\pm$3 %, and 2$\pm$1 %, for Cu, Pb, Zn, and Cd respectively. Ammonium acetate extractable metal is also high with 18$\pm$4 %, 6$\pm$2 %, and 75.5$\pm$0.5 % for Pb, Zn, and Cd respectively, although the Cu values were too low for detection.

The two sites receiving combined sewer overflow (site 1 and 2) generally showed metal concentrations and enzyme activity reflecting stormwater runoff. This is not surprising as the sewage released at these sites is considerably diluted with stormwater (it is estimated that > 80% of the discharge is stormwater even under extreme rainfall conditions). However, evidence for sewage overflow was found at site 1. A profile of the streambed was made by sampling at 10 metre intervals downstream from the sewer overflow. Metal concentrations and enzyme activities are shown in Figure 3. The results show that enzyme activity, and COD, are high at sampling

point 1C which indicates sewage deposition on the streambed. Further evidence for the presence of sewage overflow was the elevated COD and biochemical oxygen demand values also found at point 1C. The elevated metal concentrations may be due to metal chelation by the highly organic material in the carrying stormwater. Stormwater contains high concentrations of free and weakly complexed metal species (Morrison et al., 1984). which could readily be removed by sewage solids. Sewage overflow in urban rivers is therefore characterised by high enzyme activity and metal concentrations in the sediment. However, analysis of enzyme/COD shows that enzyme activity is inhibited.

Mölndalsån

Mölndalsån river (Figure 3A) begins in a lake (Stensjön) which receives no urban discharges (site 7). It then proceeds through an industrial area (sites 4,5,6) which includes a paper producer and automobile repair companies. The major inputs from stormwater are on entering the urban area (sites 1-4) and include a number of combined sewer overflows which release at 3x baseflow. Enzyme activities along the river reflect the industrial and stormwater discharge (Figure 3B). In the lake sediment (site 7) the enzyme activity is high, but decreases on entering the industrial area. Metal levels are low through the industrial area and it may be anticipated that it is toxic organic compounds which are inhibiting the enzyme activity of the bacterial biomass. The stormwater affected sites (1-4) are characterised by a relatively low enzyme activity and high total metal concentrations, Cu is given as an example and is shown in figures 3C. The exception is at site 1, where the enzyme activity increases, although total Cu is still relatively

high. This may be explained by consideration that EDTA extractable Cu is very low at site 1 and therefore enzyme inhibitory metals are strongly bound to the sediment and unavailable to the bacteria.

The presence of industrial activity in Mölnsdalsån complicates the interpretation of the effects of urban stormwater discharges. However, Mölndalsån is typical of urban rivers with mixed industrial and sewer discharges.

CONCLUSIONS

1) In Göteborg, urban rivers affected by stormwater are characterised by low bacterial enzyme activities and high extractable metal concentrations.

2) Deposited sewage, from combined sewer overflow, is indicated by highly elevated enzyme activities and metal concentrations.

Further studies are being directed towards separating the individual effects of metals and organics on enzyme activity in urban rivers and comparing the results with other toxicity tests.

ACKNOWLEDGEMENT

Bernt Persson, Water And Sewage Supply Works of Göteborg, is thanked for help with the project.

REFERENCES

BERKAS,R.WAYNE, 1980. Effects of urban runoff and wastwater effluent on rivers. U.S. Geological Survey, Water-Resources Investigations. Washington D.C.: 80-27.

MORIARTY,F., 1988. Ecotoxicology, The study of pollution in ecosystems. Academic press. Harcourt Brace Jovanovich, London.

MORRISON,G.M.P., G.E.BATLEY & T.M.FLORENCE, 1989. Metal speciation and toxicity. Chem.Br. 25: 791-796.

MORRISON,G.M.P., D.M.REVITT & J.B.ELLIS, 1989. Sources and storm loading variations of metal species in a gullypot catchment. Sci. Total Environ. 80: 267-278.

MORRISON,G.M.P., T.M.FLORENCE & J.L.STAUBER, 1990. The effects of complexing agents and surfactants on the deposition and stripping processes in differential pulse anodic stripping voltammetry of metals at the hanging mercury drop electrode. Electroanalysis 2: 9-14

MORRISON,G.M.P., D.M.REVITT & J.B.ELLIS, 1987. Heavy metal exceedance of water quality standards during storm events. In W.Gujer & V.Krejci (eds.). Proceedings of Fourth International Conference On Urban Storm Drainage, Ecole Polytechnizue Federale,Lausanne: 91-94.

MORRISON,G.M.P., D.M.REVITT, J.B.ELLIS, G.SVENSSON, & P.BALMER, 1984. The physico-chemical speciation of zinc, cadmium, lead and copper in urban stormwater. In P.Balmer et al.(eds.). Proceedings of the 3rd International Conference On Urban Storm Drainage, Vol.3, Chalmers University of Tecchnology, Göteborg, Sweden: 989-1000.

DAVIS,J.B. & J.J.GEORGE, 1987. Benthic invertebrates as indicators of urban and motorway discharges. Sci. Total Environ. 59:291-302.

CHUNG,Y-C. & J.B.NEETHLING, 1989. Microbial activity measurements for anaerobic sludge digestion. J. Water Pollut. Control Fed. 61: 343-349.

DUTKA,B.J., K.JONES, K.K.KWAN, H.NAILEY, & R.MCINNIS, 1986. Use of Microbial and Toxicant Screening Tests for Priority Site Selection of DEgraded Areas in Water Bodies. Wat. Res. 22: 503-510.

WOOD,J.M., 1984, Microbiological strategies in resistance to metal ion toxicity. In H. Sigel(ed.), Metal Ions in Biological Systems, Vol.18, Marcel Dekker, N.Y.: 333-351.

ZIMMERMAN,R., R.ITURRIGA, & J.BECKER-BERCK, 1978. Simultaneous determination of the total number of aquatic bacteria and the number therefore involved in respiration. Appl. Envir. Microbiol 36: 926-935.

SAYLAR,G.S., M.PUZISS, & M.SILVER, 1979. Alkaline phosphatase assay for freshwater sediments: Application to perturbed sediment systems. Appl. Envir. Microbiol 38: 922-927

MORRISON,S.J., et al, 1977. Evidence for microfloral succesion on allochthonous plant litter in apalachicola bay, U.S.A.. Mar. Biol. 41: 229-240

RENDELL,P.S., G.E.BATLEY, & A.J.CAMERON, 1980. Adsorption as a control of metal concentrations in sediment extracts. Environ. Sci. Technol. 14: 314-318.

Table 1. Urban rivers sampled

Urban river	No.of Sample sites	Chemical oxygen demand mg/g
Delsjöbäcken	5	10-40
Kvibergsbäcken	4	0.4-2
Kvillebäcken	5	69-253
Mölndalsån	7	5-165
Balltorpsbäcken	4	95-248

Table 2. Enzyme activity/COD in urban river sediments

Site *	Dehydrogenase /COD	Glucosidase /COD	Galactosidase /COD	Phosphatase /COD
A	0.02-0.11	0.18-0.68	0.12-0.47	0.14-0.50
B	0.50-3.10	34-122	14-56	4-48
C	0.02-0.13	0.20-0.60	0.20-0.30	0.14-0.30
D	0.002-0.02	0.20-1.60	0.20-1.80	0.14-0.80
E	0.002-0.014	0.08-0.38	0.06-0.32	0.06-0.50

* A. Delsjöbäcken; B. Kvibergsbäcken; C. Kvillebäcken; D. Mölndalsån; E. Balltorpsbäcken

Table 3. Metal speciation in urban river sediments
(metal concentration range gg $^{-1}$)

	Site				
metals	A	B	C	D	E
$Cu(HNO_3)$	10-68	8-22	79-153	133-436	26-543
(EDTA)	4-13.5	1.0-7	13-76	0.1-82	45-104
(NH_4OAC)	<0.1	<0.1	<0.1	<0.1-69	<0.1-11
$Zn(HNO_3)$	21-43	19-37	199-829	47-991	183-1501
(EDTA)	50-113	18-35	56-457	<0.1-204	71-594
(NH_4OAC)	9-21	3-8	1-73	<0.1-25	<0.1-131
$Pb(HNO_3)$	29-78	16-29	50-188	9-312	31-400
(EDTA)	7.2-44	6-16	103-10	<0.1-180	50-130
(NH_4OAC)	<0.1-6.7	<0.1-7	4-182	<0.1-9	<0.1-17

Figure legends:

Figure 1A: sampling sites at Kvillebäcken

Figure 1B: Dehydrogenase activity / COD in Kvillebäcken sediments. The Box and Whisker plot gives the median as the central line while the central box covers the upper and lower quartiles. The whiskers extend to those extreme points which are within 1.5 times the interquartile range

Figure 1C: EDTA-extractable copper in Kvillebäcken sediments

Figure 2: Enzyme activity and copper concentrations variations in sediment at sampling points taken at 10 metre internals downstream of site 1 at Kvillebäcken

Figure 3A: Sampling sites in Mölndalsån

Figure 3B: Enzyme activity /COD in Mölndalsån

Figure 3C: Copper speciation in Mölndalsån sediments

Fig 1

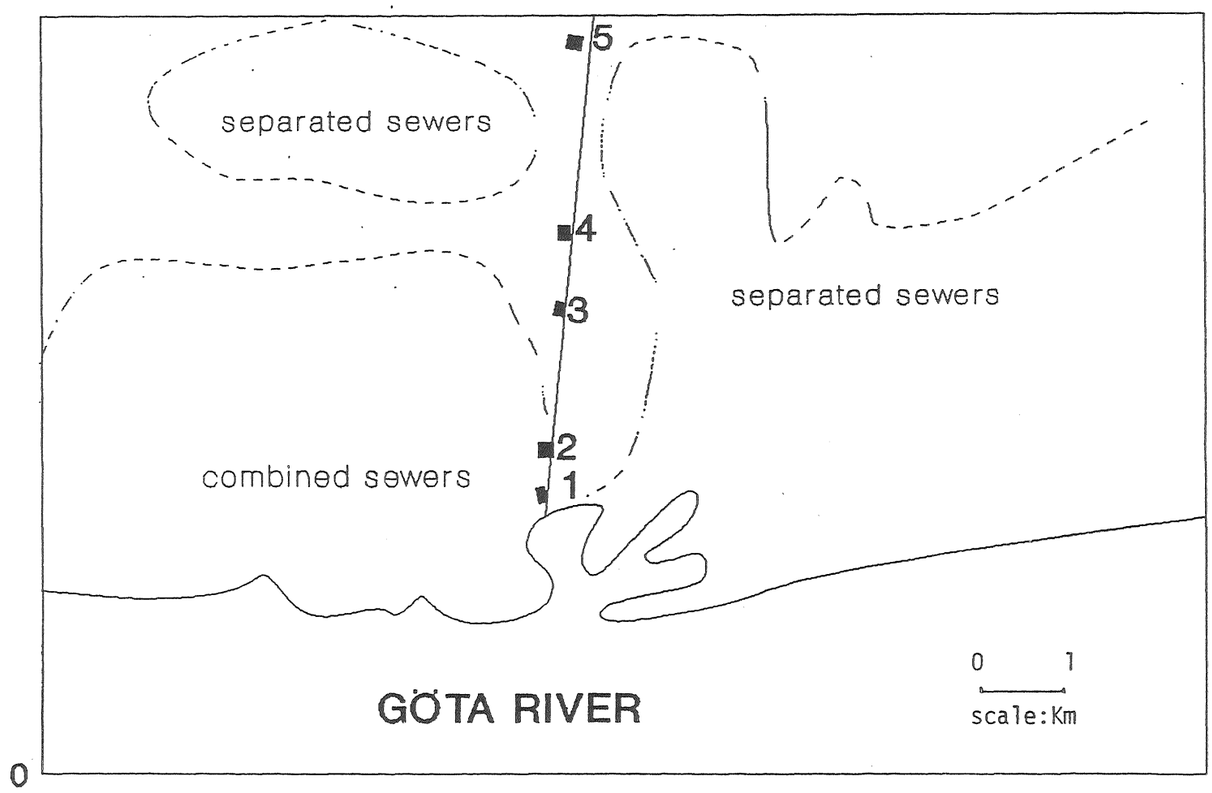
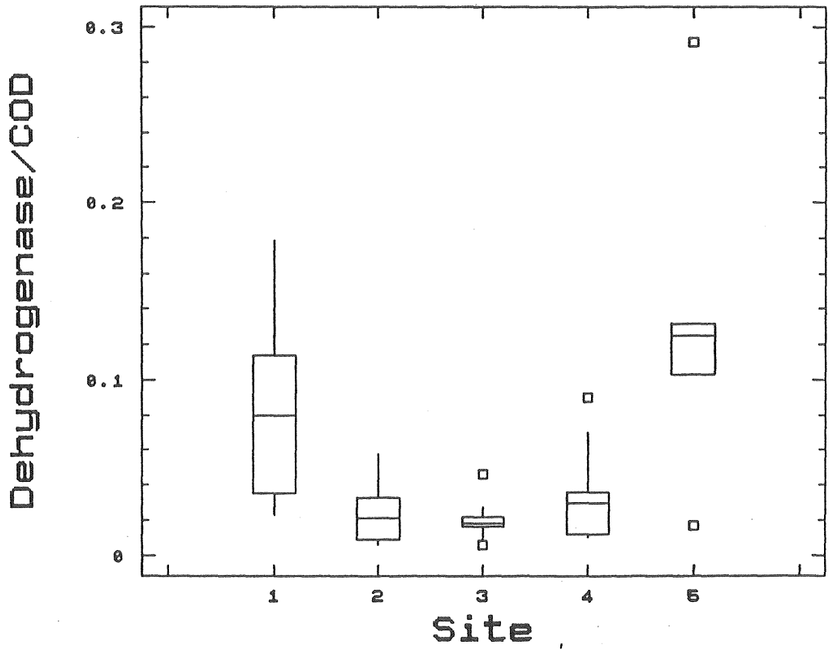

5
separated sewers
4
separated sewers
3
2
combined sewers
1
0
1
scale:Km
GÖTA RIVER
0

Fig 1B



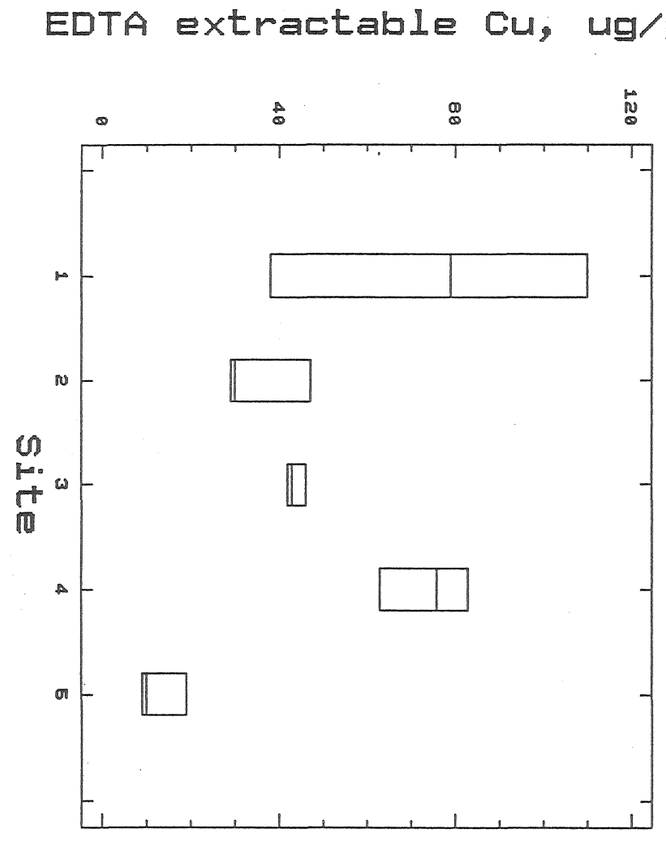


Fig 2.5

Fig B2

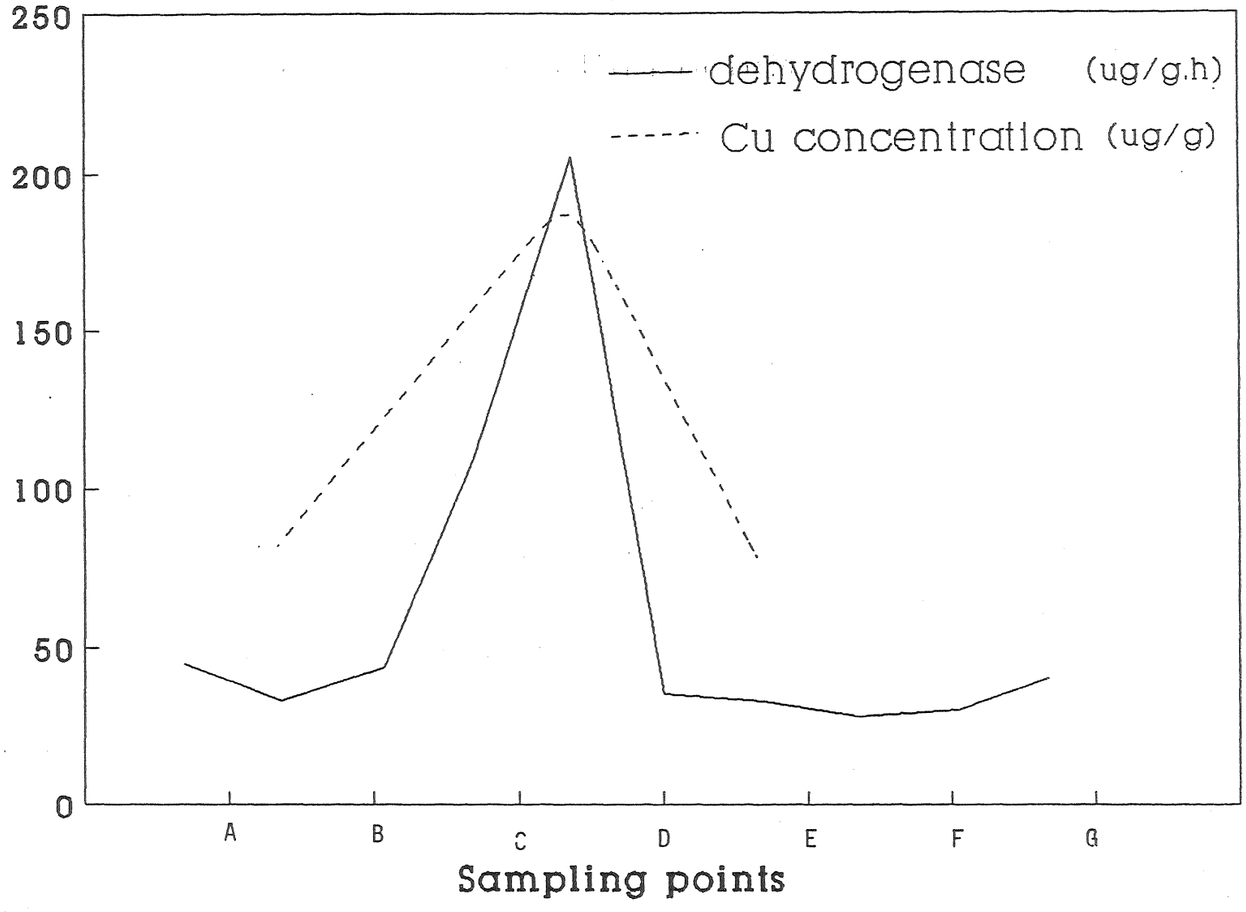

250
200
150
100
50
0
dehydrogenase (ug/g.h)
Cu concentration (ug/g)
A
B
C
D
E
F
G
Sampling points

Fig 3A

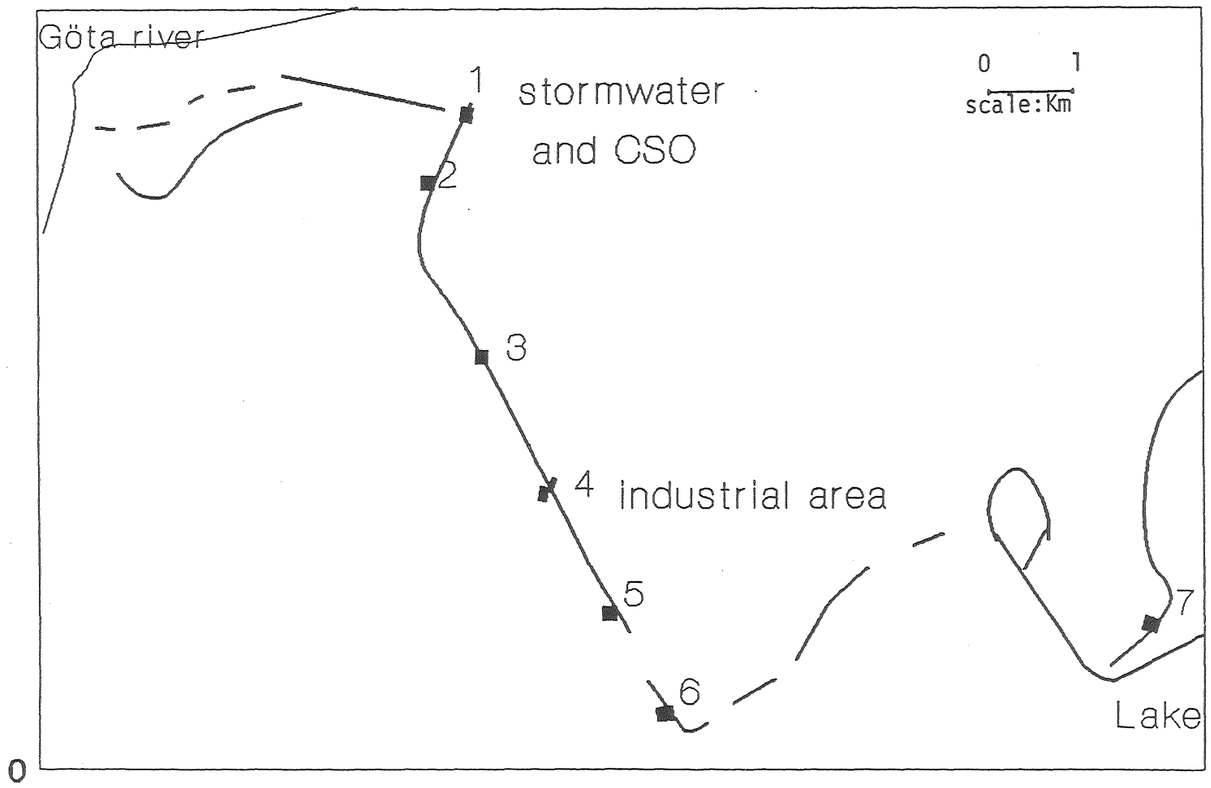

Göta river
1
stormwater
and CSO
2
3
4
industrial area
5
6
7
Lake
0
1
scale:Km
0

Fig 36

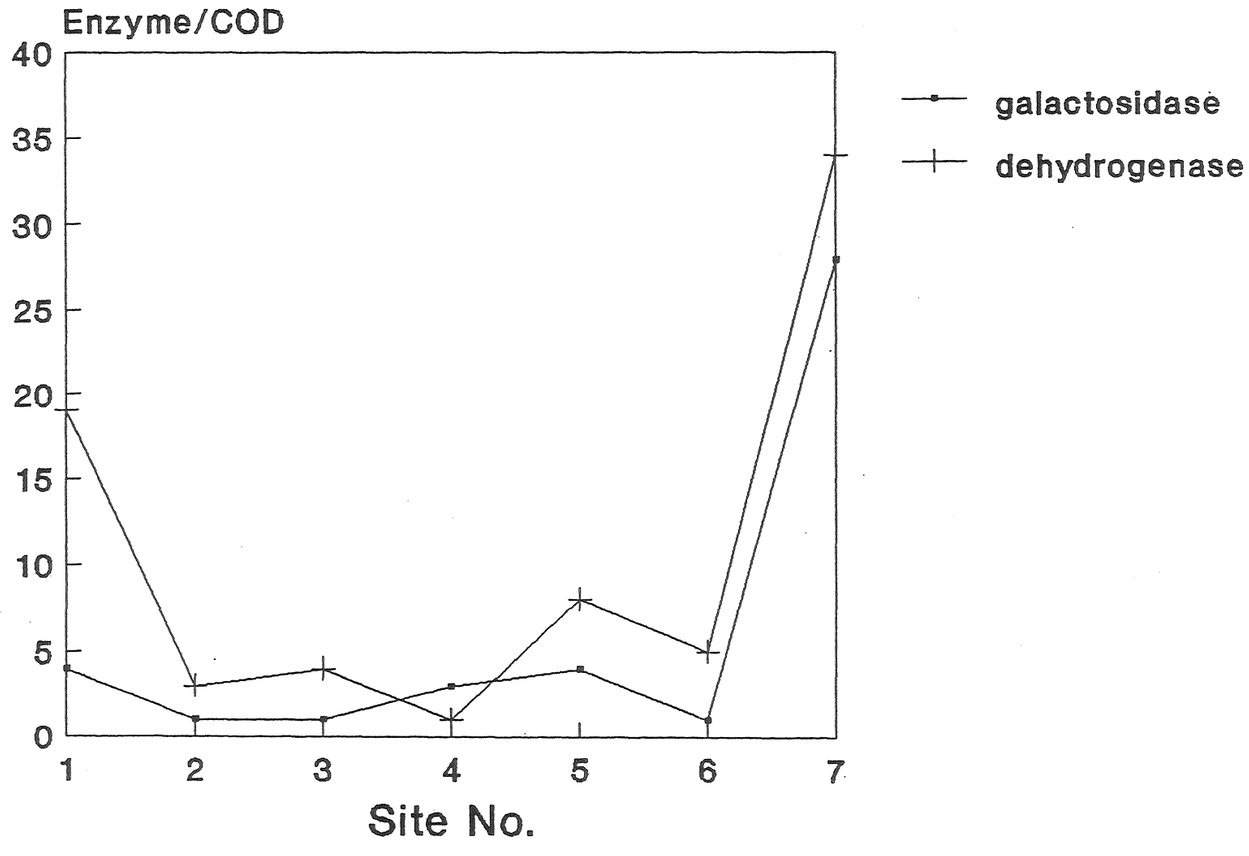
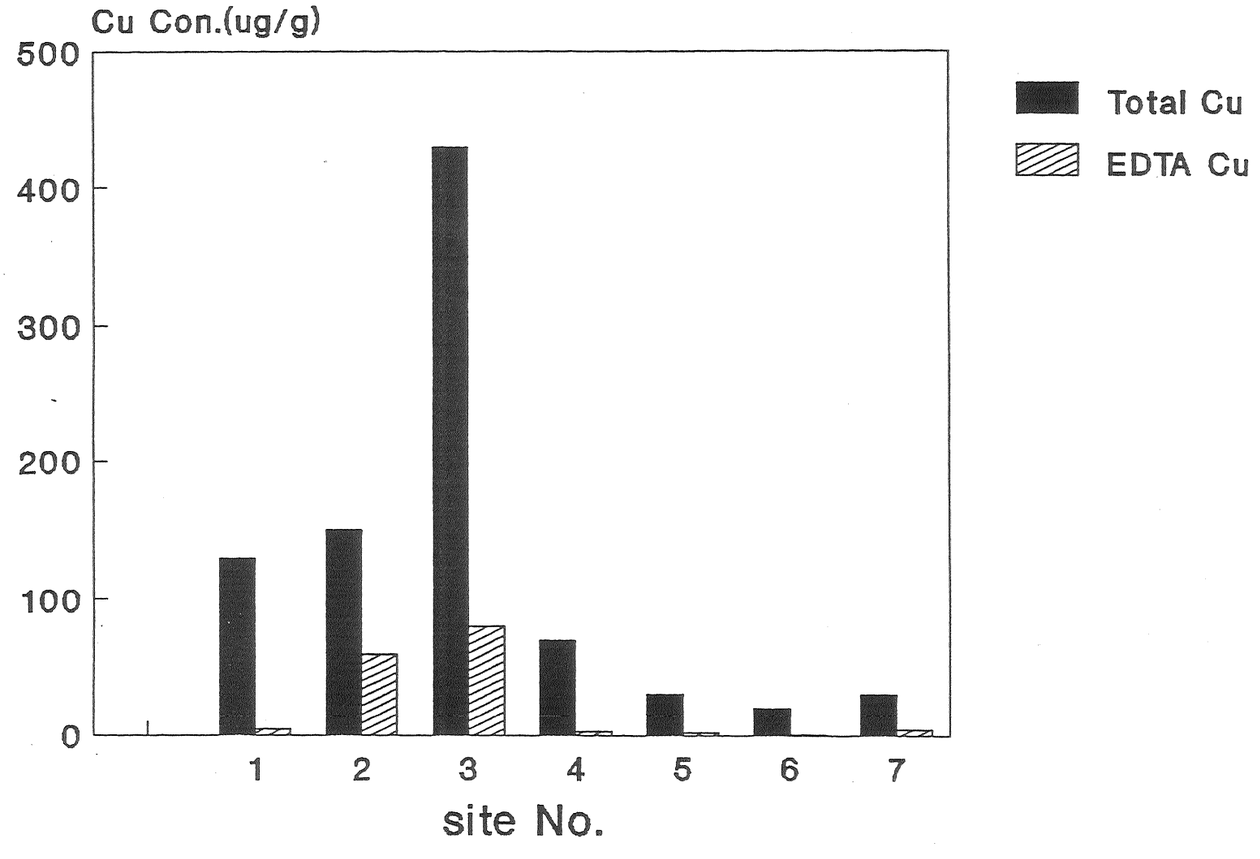

Enzyme/COD
40
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
Site No.
galactosidase
dehydrogenase

Fig3C


Cu Con.(ug/g)
500
400
300
200
100
0
1
2
3
4
5
6
7
site No.
Total Cu
EDTA Cu

ENZYME ACTIVITY IN URBAN RIVERS

C. Wei and G. Morrison

Department of Sanitary Engineering, Chalmers University of Technology, Göteborg, SWEDEN

Abstract

Inhibition of the enzyme activity of bacteria in urban river sediments was investigated. Urban rivers which accumulate stormwater sediments had inhibited enzyme activity and increased concentrations of metals. The enzyme method was further developed to allow an assessment of which physically/-chemically separated fractions provide inhibitory pollutants. Urban river sediment was selectively extracted with EDTA and ethanol and the remaining pollutants, bacteria and sediment incubated for 48h. The ethanol-soluble fraction was found to account for up to 67% of inhibition.

Introduction

Stormwater and combined sewer overflow from urban areas release metals, polyaromatic hydrocarbons and oxygen-demanding substances, without treatment, to receiving waters (1). These pollutants can, in urban rivers, lead to both chronic and acute ecological effects (2). The extent of these effects depends on firstly, the concentration of each individual pollutant relative to flow and water volume in the recipient and secondly, on pollutant speciation and sensitivity of the recipient ecosystem (3).

Attempts have been made to establish a relationship between pollutant speciation and ecological effect in urban rivers. Concentrations of toxic dissolved metal species in stormwater have been shown to exceed recommended water quality criteria (4). The freshwater crustacean, *Gammarus pulex*, accumulates metals when transferred to urban rivers (5).

The urban river environment as an ecological habitat can be considerably altered as a result of stormwater discharge. It is therefore most relevant to study organisms which can readily adapt to the urban river environment and which can be studied *in situ*. Status of the bacterial community is ideal in this respect and can be assessed through analysis of enzyme activity.

Bacterial enzyme activity has been widely used in monitoring active sewage sludge (6) and has been recently applied to environmental monitoring (7,8). Dehydrogenase (i.e. enzymes which oxidise organic compounds leading to a loss of protons) and phosphatase activity are commonly measured and have been reported to be inhibited by metal pollution. In addition these assays are relatively straightforward, sensitive and inexpensive to carry out.

The objective of ongoing research is to determine the ecological effects of pollutants discharged from urban areas to rivers and streams in Göteborg.

Experimental

Urban river sites and sampling

Sediment samples were collected from five urban rivers in the Göteborg region, Sweden (Table 1).

Table 1 Urban rivers sampled

Urban river	No of sampling sites	Chemical oxygen demand mg/g
Kvibergsbäcken	4	0.4-2
Delsjöbäcken	5	10-40
Kvillebäcken	5	69-253
Mölndalsån	7	5-165
Balltorpsbäcken	4	95-248

All urban rivers receive combined sewer overflow and separate stormwater discharge. Delsjöbäcken and Kvibergsbäcken are free flowing and the sampled sediments showed little evidence of organic material, as indicated by analysis of chemical oxygen demand (Table 1). Kvillebäcken, Mölndalsån and Balltorps

bäcken, on the other hand, were fairly stagnant and showed evidence of accumulated sediment from stormwater discharge.

Sediments were collected by a grab sampler from the top surface of the river bed. 10 samples were taken at each sampling site for enzyme and metal analysis.

Enzyme activity

Wet sediment (0.5g) was diluted in 50 ml of ultra-pure water (double distilled water passed through a Milli-Q system) in a beaker (100 ml) and sonicated for 1 minute (in an Artex sonic dismembrator, the power was at 30% relative output). The concentration of sediment suspended in solution was measured by weighing aliquots of the sediment solution, which had been dried at 105 C.

The dehydrogenase activity in the suspended solution (1 ml) was measured using the method of Zimmerman (10).

The effects of individual fractions of organic pollutant on enzyme activity was measured after extraction of fresh sediment with 0.5% ethanol or by purging with air for 20 minutes. The effects of metals on enzyme activity was measured by the addition of 0.01M EDTA. After extraction, or addition, the sediments were incubated with added nutrient and substrate (0.5% glucose) for 48h at 20 C. Enzyme activity was measured as described above.

Metal speciation

Sediment was dried (105 C) before analysis for total metal, ammonium acetate and EDTA extractable fractions.

Total sediment bound metals were released by the addition of concentrated HNO_3 (10 ml) and concentrated $HClO_4$ (1 ml) to dried sediment (0.5g) and boiling for 2h in a covered beaker. After digestion, the sediment was removed by centrifugation and the supernatant diluted to 25 ml with ultra-pure water.

Sediment species were extracted by shaking with 0.01M EDTA (10 ml) at pH 8.0 and 1M ammonium acetate (10 ml) for 5h and 1h respectively.

Metal concentration was determined using a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer with flame and Perkin-Elmer 603A Atomic Absorption Spectrophotometer with HGA 76B furnace techniques.

Results and discussion

During the early work on enzyme activity we concentrated on comparisons of enzyme activity with total and speciated metal concentrations in the urban river

sediments.

Enzyme activity was high in the free flowing rivers (Kvibergsbäcken and Delsjöbäcken) where no evidence for the presence of sewer discharge sediments was found (Table 2). This is reflected in total metal concentrations which are also low in these rivers. Enzyme activity was found to be inhibited in the more stagnant urban rivers (Kvillebäcken, Mölndalsån and Balltorpsbäcken) and the high metal concentrations found reflect the accumulation of sediments from urban discharges.

Table 2 Enzyme activity and copper in urban river sediments in Göteborg

Urban river	Dehydrogenase/COD	Copper µg/g
Kvibergsbäcken	0.5-3.1	8-22
Delsjöbäcken	0.02-0.11	10-68
Kvillebäcken	0.02-0.13	79-153
Mölndalsån	0.002.0.02	133-436
Balltorpsbäcken	0.002-0.014	26-543

The availability of Zn, Cu and Pb in urban river sediments, as measured by ammonium acetate and EDTA extractions, was found to be higher in the urban rivers with the highest metal concentrations. In one urban river (Kvillebäcken) it was found that the percentage metal in an EDTA extractable form increased from a background site unaffected by urban discharge (Cu,32%; Zn,21%; Pb,15%; Cd,1%) to a site receiving separate stormwater discharge (Cu,43%; Zn,21%; Pb,67%; Cd,14%). Similar trends were found for ammonium acetate extracted metal.

The results presented above indicate inhibition of enzyme activity in urban rivers due to the presence of stormwater and combined sewer sediments. It was decided to improve the methodology to allow an assessment of which category of pollutants causes the inhibition.

The following extractions were investigated:

1. EDTA - to detoxify metals
2. Purging with air for 20 minutes - to remove volatile organic substances
3. Extraction with 0.5% ethanol - to remove ethanol-soluble organics

The results shown in Table 3 are from four samples taken from an urban river which receives considerable industrial discharge. Significant inhibition can be attributed to ethanol-soluble organics, with only a minor role played by metals. Although it is perhaps not surprising that toxic organic substances provide the greatest inhibition of enzyme activity in an industrial discharge recipient, it will be interesting to see if the same applies to stormwater discharge sites.

Table 3 Inhibition of enzyme activity after physical and chemical extractions

Sample	% inhibition of enzyme activity due to Metals	Ethanol-soluble organics	Volatile organics
1	11	25	11
2	12	57	(12)
3	(4)	67	4
4	52	47	29

References

1. Ellis, J.B., in Urban Drainage Systems, R.E. Featherstone and A. James, eds., Pitmans, London, 1982, 39-57.

2. Moriaty, F., in Ecotoxicology - the study of pollution in ecosystems, Academic Press, Harcourt Pub., 1988.

3. Morrison, G.M.P., Batley, G.E. and Florence, T.M., Metal speciation and toxicity, Chemistry in Britain, 1989, 791-796.

4. Morrison, G.M.P., Revitt, D.M. and Ellis, J.B., Heavy metal exceedance of water quality standards during storm events, Fourth international conference on urban storm drainage, 1987, 91-94.

5. Bascombe, A.D., Ellis, J.B., Revitt, D.M. and Shutes, R. B. E., The development of ecotoxicological criteria in urban catchments, 2nd Wageningen conference om urban storm water quality and effects upon receiving waters, 1989.

6. Chung, Y.C. and Neethling, J.B., Microbial activity measurements for anaerobic sludge digestion, Journal of the Water Pollution Control Federation, 1989, 61, 343-349.

7. Russell, L.L., Impact of priority pollutants on publicly owned treatment works processes: a literature review, Proc. 37th Indus Conf., 1982, 871-883.

8. Koopman, B., in Toxicity testing using microorganisms, Dukta, B.J. and Bitton, G., eds., 1986, 101-113.

Inhibition of bacterial enzyme activity and luminescence by urban watercourse sediments

Chen Wei and Gregory M. Morrison

Department of Sanitary Engineering, Chalmers University of Technology, S-412 96 Göteborg, Sweden

The wet weather discharge of stormwater and sewage into watercourses is a problem in urban areas. Control over point discharges has reduced pollution in many rivers, but uncontrolled separate stormwater and combined sewer overflow continue to be a problem [1]. Metals and polyaromatic hydrocarbons are found in high concentrations in urban runoff [2] and it has been demonstrated that these pollutants bioaccumulate [3] and increase toxicity [4,5]. Here we present a study which demonstrates that pollutants in the sediments of urban watercourses can have toxicological and ecological effects. The urban watercourse sediments were chemically fractionated using selective extractants to separate the effects of metal and organic contaminants. In many cases the enzyme activity of the sediment-dwelling bacteria is inhibited by metals. Variations in inhibition are attributed to differences in sediment complexation of, rather than bacterial community tolerance to, metals. Nonpolar organic compounds significantly increase the toxicity of urban watercourse sediments and it is proposed that polyaromatic hydrocarbons from stormwater are an important source of sediment toxicity.

Three urban watercourses which ultimately feed into the Göta river in Göteborg were studied. The watercourses are partly natural and partly man-made drainage channels and are characterised by a considerable increase in volume during storm events. Sediment pH was slightly acidic (Table 1) as the Göteborg area is dominated by a non-calcareous granite bedrock. Urban runoff provides organic material [6] which is evidenced by the high chemical oxygen demand (COD) values. Metal concentration was also high, indicating the input of stormwater solids. Kvillebäcken alone has over twenty separate stormwater outfalls and two major combined sewer overflow sites. Mölndalsån and Balltorpsbäcken additionally receive diverse industrial discharges and effluent from abandoned dumping sites.

Sediment toxicity was investigated by direct contact of dilutions of sediment sample with the marine bacterium *Photobacterium phosphorium* and the EC_{50} calculated from inhibition of bioluminescence (Table 1). The toxicity of individual fractions of sediment-bound pollutants was revealed by selective chemical extraction of the sediment prior to analysis. The method differs from previous work by exposing the bacteria to a sediment sample and not to a sediment sample extract [7]. The resulting decrease in toxicity indicates the extent of removal of the toxic pollutant.

Organic contaminants discharged in urban runoff include high concentrations of polyaromatic hydrocarbons (PAHs) [8]. Measurements of 17 PAHs give total concentrations of 3 - 19 g/l in urban runoff [9]. In our samples significant reduc-

tions in toxicity were obtained by extraction of the sediments with nonpolar solvents (Table 2). Pentane/benzene should remove the aromatic hydrocarbons [10] and it is evident that the toxicity test is sensitive to this group of compounds.

High loadings of cadmium, lead and copper are discharged in urban runoff and accumulate in urban watercourse sediments. Ethylenediaminetetraacetic acid (EDTA) was the extractant chosen to remove metal toxicity. EDTA has been shown to mask ionic metal toxicity [11], although lipid-soluble organometallic compounds such as methylmercury and alkyllead species would not be extracted.

Extraction of metals with EDTA gave a slight, but consistent decrease in toxicity (Table 2). That this decrease was not greater seemed surprising considering the high concentrations of heavy metals found in the sediment samples. However, comparison of the concentrations of EDTA-extractable copper in the sediments (Table 1), the reported EC_{50} for copper to *Photobacterium phosphorium*, 0.34 mg/l [12] and the EC_{50} for urban sediment (Table 1) indicates that the toxicity test is not necessarily sufficiently sensitive to metals at the concentrations found in urban watercourse sediments.

A weakness in the use of laboratory tests to separate toxic fractions is that the correlation with ecological effects may prove to be poor [13]. Enzyme activity has been shown to provide a possible *in situ* indication of the effects of pollutants on the status of the sediment-dwelling bacterial community [14,15].

The enzyme activity of bacteria in activated sewage sludge [16,17] and river sediments [18,19] is sensitive to elevated concentrations of heavy metals. In our study the dehydrogenase activity of the bacteria in urban sediments increased slightly after removal of metals with EDTA and controlled incubation (Table 3). However, occasionally considerable increases in enzyme activity were observed. Because urban sediments are disturbed during storm events a high variability was found even after repeat sampling at the same site.

Variations in enzyme inhibition by metals can also be accounted for by the complexation properties of the sediment (Table 4). Inhibition of enzyme activity occurred before the complexation capacity was reached and indicates that metals which are weakly complexed to the sediment surface are available for inhibition. Bacterial community tolerance has been reported previously [20], although our results suggest that sedimentary complexing characteristics are more important in determining metal availability.

Watercourses to direct wet-weather runoff are a common feature of urban areas. This study, combined with previous research, demonstrates the need for the management of toxic and ecologically inhibiting pollutants in separate storm-water and combined sewer overflow, which are discharged without treatment to urban watercourses.

References

1. Field, R. & Pitt, R.E. Wat.Sci.Tech., 22, 1-7 (1990).

2. Cole, R.H., Frederick, R.E., Healy, R.P. & Rolan, R.G. Journal WPCF, 56, 898-908 (1984).

3. Bascombe, A.D., Ellis, J.B., Revitt, D.M. & Shutes, R.B.E. Wat.Sci. Tech., 22, 173-179 (1990).

4. Medeiros, C, LeBlanc, R. & Coler, R.A. Environmental Toxicology and Chemistry, 2, 119-126 (1983).

5. Saeger, J. & Abrahams, R.G. Wat.Sci.Tech., 22, 163-171 (1990).

6. Hall, K.J. & Anderson, B.C. Can.J.Civ.Eng., 15, 98-106 (1988).

7. Dukta, B.J., Kwan, K.K., Rao, S.S., Jurkovic, A. & Liu, D. Environmental Monitoring and Assessment, 16, 287-313 (1991).

8. Valls, M., Bayona, J.M. & Albaiges, J. Intern.J.Environ.Anal.Chem., 39, 329-348 (1990).

9. Marsalek, J. Wat.Sci.Tech., 22, 22-30 (1990).

10. Lewtas, J., Chuang, J., Nishioka, M. & Petersen, B. Intern.J.Environ.Anal.-Chem., 39, 245-256 (1990).

11. Morrison, G.M.P. & Florence, T.M. Electroanalysis, 1, 107-112 (1989).

12. Ankley, G.T., Hoke, R.A., Giesy, J.P. & Winger, P.V. Chemosphere, 18, 2069-2075 (1989).

13. Cairns, J. & Mount, D.I. Environ.Sci.Technol., 24, 154-161 (1990).

14. Bitton, G. CRC Critical Reviews in Environmental Control, 13, 51-67 (1982).

15. Broberg, A. Environmental Biogeochemistry Ecol. Bull., 35, 403-418 (1983).

16. Anderson, K., Koopman, B. & Bitton, G. Wat. Res. 22, 349-353 (1988).

17. Chung, Y. & Neethling, J.B. Journal WPCF, 61, 343-349 (1989).

18. Sayler, G.S., Puziss, M. & Silver, M. Applied and Environmental Microbiology, 38, 922-927 (1979).

19. Burton, G.A. & Lanza, G.R. Wat. Res., 21, 1173-1182 (1987).

20. Pickup, R.W. JWSRT-Aqua, 38, 230-235 (1989).

21. Trevors, J.T. Water Res., 18, 581-584 (1984).

Table 1 Sediment quality characteristics. Surface sediment samples were collected on three occasions with 10 samples at each site and five sites in each watercourse. Sediment pH was measured by a combined pH electrode in a 10ml aqueous extract of 1g of sediment. COD was a standard reflux method using dichromate/sulphuric acid oxidation and spectrophotometric determination of residual Cr^{3+}. Copper was extracted with 0.01M EDTA or a boiling concentrated nitric/perchloric acid mixture (10:1). Analysis was by electrothermal atomic absorption spectrophotometry. Dehydrogenase activity [21] analysis was carried out on fresh wet sediment (1g) diluted in 50ml of ultrapure water. Suspended sediment was standardised by drying aliquots at 105 C. Dehydrogenase activity is expressed as analyte produced (formazan) per gram sediment dry weight per assay incubation time [19]. EC_{50} for sediment using *Photobacterium phosphorium* was a standard procedure using a Microbics Microtox M500.

	pH	COD mgO_2/g	Copper g/g	EDTA extractable copper g/g	Microtox EC_{50} (%wt:vol)	Dehydrogenase activity mg formazan /g sediment/h
Kvillebäcken	4.3-5.6	70-250	80-150	13-80	0.002-0.08	1.6-8.3
Mölndalsån	4.6-5.3	5-165	130-440	0.1-82	0.02-3,0	0.2-1.9
Balltorpsbäcken	5.7-6.3	95-250	30-540	45-257	0.04-0.7	0.4-2.4

Table 2 Effect of selective chemical extraction on *Photobacterium phosphorium* EC_{50} for urban sediments. Dried sediment (0.1g) was exhaustively extracted with aliquots of extractant (10ml). After further washing with ultrapure water or ethanol the sediment was dried. The Microtox test was carried out on the dry extracted sediment.

	% average increase of EC_{50} after extraction with			
	0.01M EDTA	Ethanol	Pentane/ Benzene (1:1)	Pentane
Kvillebäcken	13	18	12	0
Mölndalsån	23	17	335	474
Balltorpsbäcken	14	5	196	269

Table 3 Effect of physico-chemical extraction on dehydrogenase activity of fresh urban watercourse sediments. 1ml of a slurry of fresh sediment (20g) in ultrapure water (50 ml) was retained on a 0.45 m filter. After washing with the extractant (10 ml) or a 30 min air purge, followed by ultrapure water (3 x 10 ml) and filtration through a 0.45 m filter, the sediment was incubated for 48h in culture medium (1 ml) at 37 C. The culture medium was in 1l; 8.5g KH_2PO_4, 21.75g K_2HPO_4, 33.4g $Na_2HPO_4.7H_2O$, 1.7g NH_4Cl, 22.5g $MgSO_4.7H_2O$, 0.25g $FeCl_3.6H_2O$, 27.5g $CaCl_2$, 2g glucose. Dehydrogenase activity was measured before and after incubation.

	% average increase in dehydrogenase activity after extraction with		
	10^{-4}M EDTA	0.5% Ethanol	Air, 20 min
Kvillebäcken	0	0	2.6
Mölndalsån	14.7	14.2	0
Balltorpsbäcken	4.2	11.2	24

Table 4 Comparison of metal complexation capacity and the metal concentration causing 50% inhibition of dehydrogenase activity (IC_{50}) for five sediment samples from Kvillebäcken. Metal was added to sediment (1g) in ultrapure water (50ml) and equilibrated with stirring for 1h. Portions of sediment slurry were filtered and dissolved metal analysed by flame atomic absorption spectrophotometry. Complexation capacity was reached when further additions of metal gave a corresponding increase in dissolved metal.

Sampling site	Copper (mg/g)		Cadmium (mg/g)	
	Complexation capacity	IC_{50}	Complexation capacity	IC_{50}
1	3.9	1.1	8.8	2.1
2	3.5	0.2	3.7	2.8
3	3.1	0.2	3.0	1.5
4	3.7	0.5	2.9	0.5
5	4.9	1.1	4.6	2.8

Tryckt & Bunden
Vasastadens Bokbinderi
GÖTEBORG 1991