Hydrobiologia **235/236**: 597–603, 1992.
B. T. Hart & P. G. Sly (eds), Sediment/Water Interactions.
© 1992 *Kluwer Academic Publishers. Printed in Belgium.*

Bacterial enzyme activity and metal speciation in urban river sediments

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

Key words: Stormwater, enzyme activity, heavy metals, speciation, sediments

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.

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 both long-term and short-term ecosystem damage on urban rivers (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 the receiving water. 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 water, as well as the sensitivity of the receiving water community to such pollutant changes (Morrison *et al.*, 1989).

Studies of metal speciation in urban runoff show that both dissolved and suspended forms are discharged throughout the storm profile (Morrison *et al.*, 1989). The dissolved concentrations of toxic metals, which can be analysed by the double acidification electrochemical method of Morrison *et al.* (1990), can exceed established water quality criteria (Morrison *et al.*, 1987) and can therefore pose a direct threat to the receiving water. Metal species in the suspended solid phase, analysed by sequential chemical extraction (Morrison *et al.*, 1984), can accumulate in the sediments of the receiving system and exert long-term ecological damage.

The connection between pollutant speciation in storm water and ecotoxicological effect in the receiving waters is poorly understood. 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 (a) to assess the bacterial enzyme status in urban river sediments, (b) to identify the metal speciation, and (c) to determine the ecotoxicological effect of metals discharged from urban areas to rivers and streams in Göteborg.

Experimental

Urban river sites and sampling

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

Table 1. Urban rivers sampled

Urban river	No. of sample sites	Chemical oxygen demand in sediments (mg/g)
A Delsjöbäcken	5	10–40
B Kvibergsbäcken	4	0.4–2
C Kvillebäcken	5	69–253
D Mölndalsån	7	5–165
E Balltorpsbäcken	4	95–248

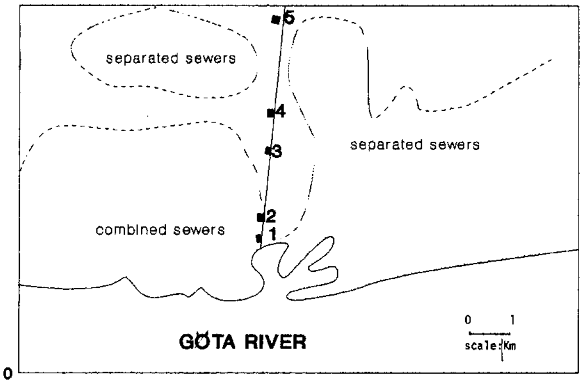


Fig. 1. Sampling sites in Kvillebäcken.

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). On the other hand, Kvillebäcken, Mölndalsån and Balltorpsbäcken were fairly stagnant and showed evidence of a build-up of organic matter in the sediments from urban discharges.

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

Enzyme activity

Wet sediment (0.5 g) was added to 50 ml of ultrapure 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 after this treatment was measured by weighing aliquots of the suspended material which had been dried at 105 °C.

The dehydrogenase activity in the sediment was determined using the method of Zimmerman *et al.* (1978). One ml of suspended solution was placed into a test tube. Four ml of 0.1 M phosphate buffer at pH 7.4 (50 ml 0.1 M KH_2PO_4 + 39.1 ml 0.1 M 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. The 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 formazan produced, and the formazan measured spectrophotometrically at 480 nm after further centrifugation.

The activity of glucosidase, galactosidase and phosphatase was measured by the method of Sayler *et al.* (1979) and Morrison *et al.* (1977). In this assay, the substrates p-nitrophenyl-galactoside, p-nitrophenyl-glucoside and p-nitrophenyl-phosphate were cleaved by the bacterial enzymes galactosidase, glucosidase and phosphatase, respectively to leave p-nitrophenol. p-Nitrophenol gives a yellow solution and can be measured in a spectrophotometer at 418 nm.

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 1 ml concentrated $HClO_4$ to 0.5 g dried sediment 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.

To obtain the EDTA-extractable fraction, 0.5 g of dried sediment was shaken with 10 ml of 0.01 M EDTA (pH 8.0) for 5 hours, the mixture centrifuged and the supernatant separated and made up to volume (25 ml) with ultra-pure water after centrifugation. The NH_4OAc-extractable fraction was obtained by shaking 0.5 g dried sediment with 10 mL of 0.1 M NH_4OAc at pH 7.0 for 1 hour, separating the supernatent by centrifugation and making up to 25 ml with ultrapure water.

Metal concentration was determined using either flame Atomic Absorption Spectrophotometry (AAS – Perkin-Elmer Model 2380) or furnace AAS (Perkin Elmer Model 603A with HGA 76B furnace).

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 to the concentration of oxidisable organic material (estimated by COD analysis) to compare urban rivers with different sediment organic concentrations. However, all bacterial communities sampled were stimulated by the addition of the glucose substrate. The addition of substrate was also found to give more reproducible results.

Similar trends were observed for the activity of all enzymes (dehydrogenase, glucosidase, galactosidase and phosphatase) measured in the urban rivers (Table 2). Activity was high in the free flowing Kvibergsbäcken, where there was no evidence of the presence of CSO or SWO sediments. The total metal concentrations are also low in this river (Table 3). In contrast the enzyme activity was found to be inhibited in the more stagnant urban rivers (Kvillebäcken, Mölndalsån and Balltorpsbäcken). These rivers also had higher metal concentrations due to the accumulation of metal-contaminated sediments from urban discharges.

Metal speciation in the sediments from these urban rivers was measured using two separate chemical extractants. 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 frac-

Table 2. Range of enzyme activity normalised to COD in sediments taken from the five urban rivers

River	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

Table 3. Range of metal concentrations ($\mu g\ g^{-1}$) in urban river sediments

Metals	River				
	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

tion, 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 highest in the rivers with the highest total metal concentrations (Table 3). Suspended solids in urban discharges have been shown to contain high concentrations of metals, often with a considerable percentage in extractable forms (Morrison *et al.*, 1984). In one urban river (Kvillebäcken), the percentage of metal in an EDTA extractable form was found to increase 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 the metal fraction extracted with ammonium acetate.

Variations along the urban rivers

Kvillebäcken

Kvillebäcken is a small river draining the northwest urban area of Göteborg. The river originates in agricultural land (site 5) and then flows through separately sewered areas (sites 3, 4) and then 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 flows of 3 times the river baseflow. Site 5 is unaffected by urban runoff and had low levels of extractable and total metals in the sediments (Fig. 2a). The enzyme activity was high at this site (Fig. 2b) 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. En-

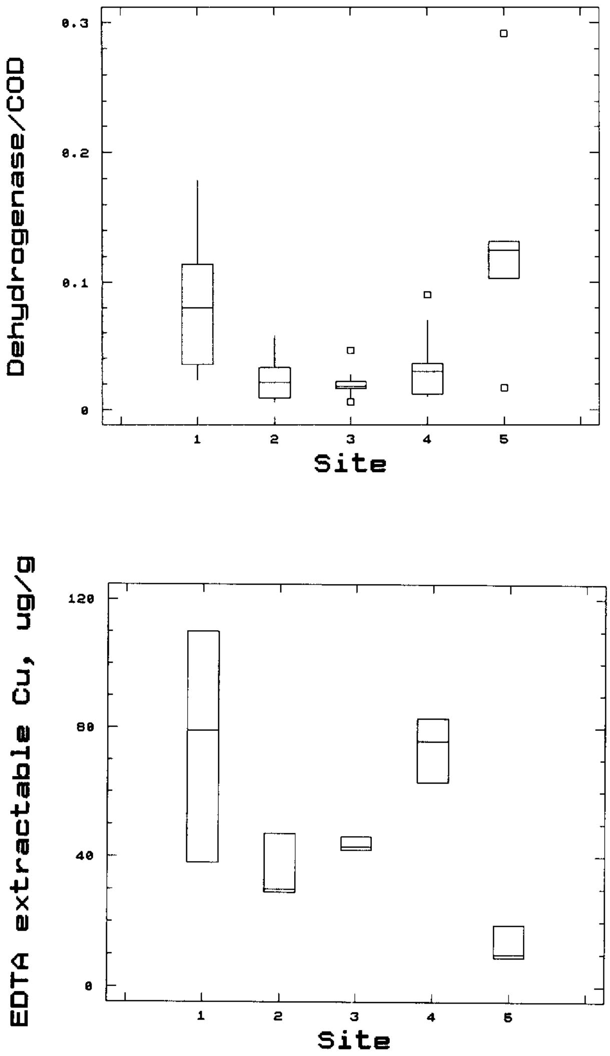


Fig. 2. (a) EDTA-extractable copper in Kvillebäcken sediments;(b) Dehydrogenase activity normalised to COD in Kvillebäcken sediments. The Box and Whisker plot gives the median as the central line while the central box covers the upper and lower quartiles. The whiskers extend to those extreme points which are within 1.5 times the interquartile range.

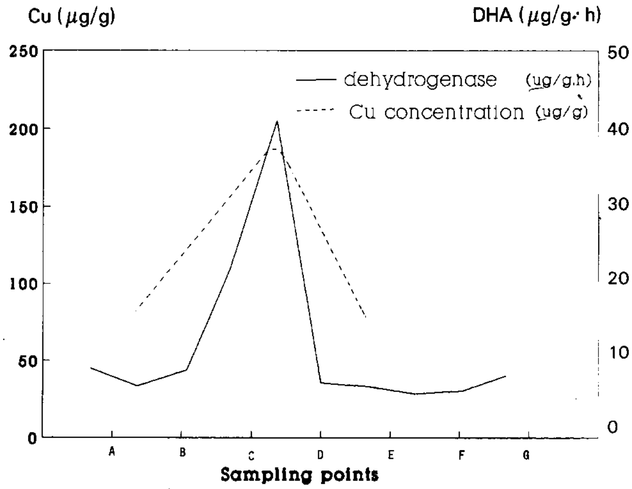


Fig. 3. Variations in the enzyme activity and copper concentration in sediment taken at 10 metre intervals downstream of site 1 at Kvillebäcken.

zyme activities reflect the influence of stormwater runoff with inhibition noted at both sites (Fig. 2b). Ten enzyme measurements were made at each site and the range of measured values are shown in Fig. 2b. Metal concentrations were also considerably higher than at site 5; the EDTA extractable fraction represents $42 \pm 6\%$, $61 \pm 1\%$, $47 \pm 3\%$ and $75 \pm 1\%$ for Cu, Pb, Zn and Cd respectively. Ammonium acetate extractable metal concentrations were also high being $18 \pm 4\%$, $6 \pm 2\%$, and $75.5 \pm 0.5\%$ for Pb, Zn, and Cd respectively; the Cu values were below the detection limit.

The two sites receiving combined sewer overflow (site 1 and 2) generally showed elevated metal concentrations and reduced enzyme activity similar to the two sites influenced by the stormwater runoff. This is not surprising since 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. Copper concentrations and dehydrogenase activity are shown in Fig. 3. The enzyme activity (and COD) was highest at a point 30–40 m downstream of this input, indicating deposition of sewage on the streambed. Further evidence for the presence of sewage overflow was the elevated COD and biochemical oxygen demand values also found at 30–40 m downstream (unpublished data). The elevated metal concentrations may be due to metal chelation by the high organic matter concentrations in the stormwater. Morrison *et al.* (1984) reported that stormwater contains high concentrations of free and weakly complexed metal species, and it is possible that these could be removed by sewage solids and subsequently deposited in the river.

Urban rivers receiving sewage overflows are characterised by sediments containing high enzyme activity and high metal concentrations. However, analysis of enzyme/COD ratios shows that enzyme activity is inhibited.

Mölndalsån

Mölndalsån river (Fig. 4) begins in a lake (Stensjön) which receives no urban discharges (site 7). It then proceeds through an industrial area (sites 4, 5 and 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 flows 3 times the baseflow.

Enzyme activities along the river reflect the industrial and stormwater discharge (Fig. 5a). In the lake sediment (site 7) the enzyme activity was high, but decreased on entering the industrial area. Metal concentrations were low through the industrial area. It may be that toxic organic compounds inhibit the enzyme activity in this region.

The stormwater-affected sites (1–4) are characterised by a relatively low enzyme activity and high total metal concentrations (e.g. Cu in Fig. 5b). The exception is at site 1, where the enzyme activity increased although the total Cu concentration was still relatively high. However, it is significant that the EDTA-extractable Cu concentration was very low at this site, suggesting that the enzyme inhibiting 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, Mölnsdalsån is typical of urban rivers with mixed industrial and sewer discharges.

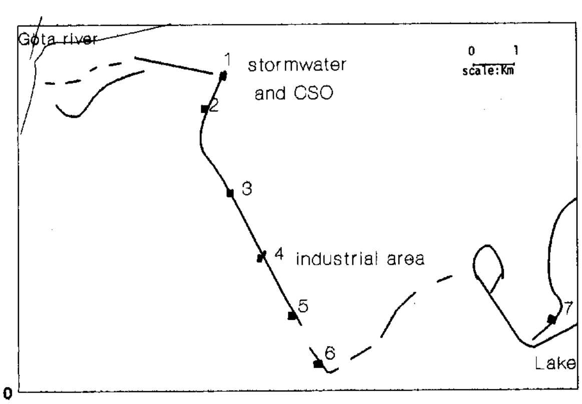
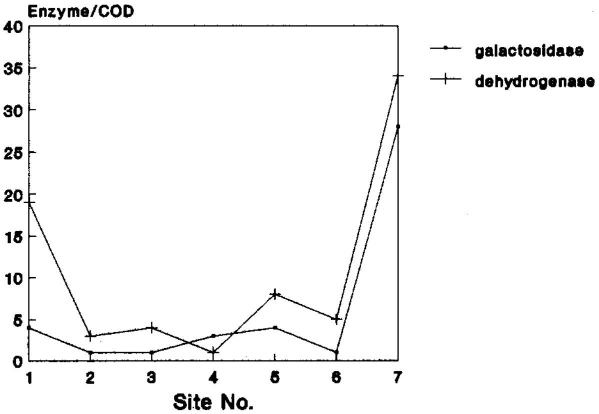


Fig. 4. Sampling sites in Mölndalsån.

Conclusions

Urban rivers in Göteborg affected by stormwater are characterised by low bacterial enzyme activ-



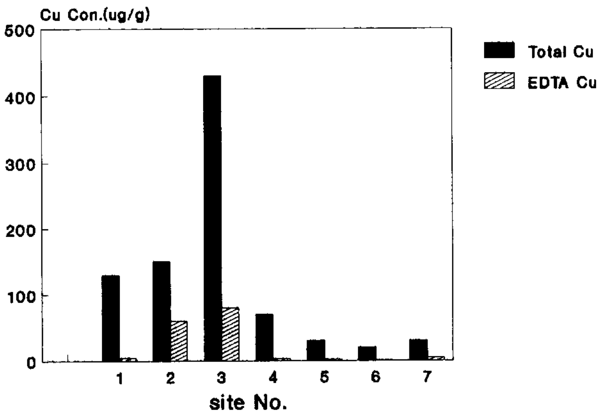


Fig. 5. Enzyme activity normalised to (a) COD (b) and copper speciation in Mölndalsån sediments.

ities and high extractable metal concentrations. Sewage, deposited 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.

Acknowledgements

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

References

Berkas, R. W., 1980. Effects of urban runoff and wastewater effluent on rivers. U.S. Geological Survey, Water-Resources Investigations, Washington D.C.: 80–27.

Chung, Y-C. & J. B. Neethling, 1989. Microbial activity measurements for anaerobic sludge digestion. J. Wat. Pollut. Cont. Fed. 61: 343–349.

Davis, J. B. & J. J. George, 1987. Benthic invertebrates as indicators of urban and motorway discharges. Sci. Tot. Envir. 59: 291–302.

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.

Moriarty, F., 1988. Ecotoxicology: The Study of Pollution in Ecosystems. Academic Press, London.

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., G. E. Batley & T. M. Florence, 1989. Metal speciation and toxicity. Chem. Brit. 25: 791–796.

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, 1989. Sources and storm loading variations of metal species in a gullypot catchment. Sci. Total Envir. 80: 267–278.

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 (ed.), 3rd International Conference On Urban Storm Drainage, Vol. 3. Chalmers University of Technology, Göteborg, Sweden: 989–1000.

Morrison, S. J., J. D. King, R. J. Bobbie, R. E. Bechtold & D. C. White, 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. Envir. Sci. Technol. 14: 314–318.

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.

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.