Sediment toxicity in River Kolbäcksån

– toxicity tests with *Chironomus riparius* and *Gammarus pulex*

Master´s thesis, 20 credits.

by

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2003 Rapport 2003:5

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ISSN 1403-977X

Abstract

During a long time the river Kolbäcksån has been influenced by mining activities in the catchment area and consequently the lakes in the area have been affected by high metal discharges. The most contaminated lake in river Kolbäcksån is L. Saxen, which is situated in the upper part of the river system. The lake is heavily polluted with Cu, Zn, Cd, Pb and Cr, which is due to the old mine on Saxberget.

Toxicity tests on profundal and littoral sediments from different lakes in the river system were made to investigate if the metal concentrations in the sediments had any effect on benthic fauna. A profundal sediment toxicity test with the endpoints survival, emergence rate and development rate was performed on the midge *C. riparius*. *C. riparius* is an invertebrate and the larvae are frequently used in toxicity tests. It has been shown that tests with *C. riparius* are good estimates of pollutant toxicity in natural sediments. A littoral sediment toxicity test with the endpoint survival was performed on *Gammarus pulex*. *G. pulex* is an amphipod that is only occasionally found in the benthic fauna in river Kolbäcksån. The sediments were taken from five lakes in river Kolbäcksån; L. Saxen, L. Norra Barken, L. Stora Aspen, L. Östersjön and L. Freden. Sediments from L. Erken were used as a control.

In the profundal experiment with *C. riparius* there were variations in the total emergence, and in L. Saxen the smallest number of midges emerged. In L. Östersjön and L. Freden the metal concentrations are similar, but the emergence rate of the midges parted considerably from each other. This implicates that the variations in the total emergence might not have been due to the high metal concentration in the sediments, per se, but might have been caused by other factors like pH, ammonia concentration and/or oxygen level. In L. Saxen the development rate of the midges was low. A low development rate can be due to sublethal stress; the more stress the lower development rate for the midges. In addition, mouthpart deformities can be a result of sublethal toxicity, which means that the organism is affected of the pollutant in some other way then by death. However, this was not investigated in this experiment. Even if the pH in some of the test vessels at some times were very low, it did not seem to affect the larvae negatively, *C. riparius* larvae are known to tolerate very low pH. In the controls, where the pH was around seven all the time, the midges did not emerge to a larger extent than in the other vessels.

In the littoral experiment with *Gammarus pulex*, there was a large variation in survival for the animals exposed to the different sediments. The survival was the highest in vessels with L. Stora Aspen sediments, whereas all specimens died in the vessels exposed to L. Saxen sediments. This is probably due to the high metal concentration in L. Saxen sediments. In two of the control vessels the survival was very low, which coincided with a high occurrence of leeches. The mean weight for the Gammarids after the experiment was higher than the mean weight of those animals that was sacrificed at the start of the experiment. The mean weight was the highest in L. Östersjön and the lowest in L. Stora Aspen. This indicates that the animals fed and grew during the test. L. Östersjön sediments consisted mainly of detritus and could probably be used by the Gammarids as complementary food source. In L. Stora Aspen however the sediment consisted of clayey sand and probably did not provide the animals with any additional food.

In general, the Chironomids managed better in the sediments than the Gammarids, which is probably due to that they are not as sensitive to pollutants as Gammarids. Sediments are very complex and the exposure to the animals includes many different factors, which makes it hard to give a simple answer to the outcome of a test, but it gives a clue on the potential effect on the animals.

Sammanfattning

Under en lång tid har Kolbäcksån blivit påverkad av gruvaktiviteten i området och därmed har sjöarna under lång tid varit påverkade av omfattande metallutsläpp. Den mest förorenade sjön i Kolbäcksån är Saxen som ligger i den övre delen av vattensystemet. Sjön är väldigt förorenad av Cu, Zn, Cd, Pb och Cr på grund av den gamla gruvan på Saxberget.

Toxicitetstest med profundal- och litoralsediment från olika sjöar utmed Kolbäcksån utfördes för att se om metallkoncentrationen i sedimenten hade någon effekt på bottenfaunan. Ett toxicitetstest med profundal-sediment med försöksvariablerna överlevnad, utvecklingshastighet och kläckningsfrekvens utfördes med fjädermygglarven *Chironomus riparius*. *C. riparius* är en invertebrat och larven har använts mycket i toxicitetstest. Det har visat sig att sådana test ger en bra uppskattning av föroreningars giftighet i naturliga sediment. Ett toxicitetstest med litoralsediment med försöksvariabeln överlevnad utfördes med sötvattensmärlan *Gammarus pulex*. *G. pulex* är en amphipod som bara har hittats enstaka gånger i Kolbäcksån. Sediment hämtades från fem sjöar i Kolbäcksån, Saxen, Norra Barken, Stora Aspen, Östersjön och Freden. Sediment från sjön Erken användes som kontroll.

I profundalförsöket med *C. riparius* var det en stor variation i den totala utvecklingen av myggor och vid exponeringarna med sediment från Saxen utvecklades det minst myggor. Östersjön och Freden har liknande metallkoncentrationer i sedimenten, men myggutvecklingen skiljde sig väsentligt åt. Det kan tyda på att utvecklingen av myggorna inte bara beror på metallhalterna utan att den också kan bero på andra faktorer som pH, ammoniumkoncentrationen och/eller syrgashalten. För Saxen var kläckningsfrekvensen låg. En låg kläckningsfrekvens kan bero på subletal stress, vilket innebär att ju mer stressade organismerna är desto lägre kläckningsfrekvens. Mundelsdeformationer kan vara en subletal toxicitetseffekt, med det menas att organismen blir påverkad på något annat sätt av föroreningen än genom att dö, men mundelarna undersöktes inte i det här experimentet. Även om pH ibland var lågt i några av försöksburkarna verkade det inte påverka larverna negativt, *C. riparius* är känd för att kunna klara av väldigt låga pH. I kontrollerna där pH låg runt sju hela tiden utvecklades inte fler myggor än i någon annan försöksburk.

I litoralförsöket med *G. pulex* var det stora variationer i överlevnaden hos djuren. Överlevnaden var högst vid exponeringar med Stora Aspen sediment, medan alla gammarider som utsattes för sediment från Saxen dog, vilket förmodligen beror på de höga metallkoncentrationerna i Saxens sediment. I två av kontrollburkarna var överlevnaden väldigt låg, vilket sammanföll med att det också fanns väldigt många iglar i dessa burkar. Medelvikten för gammariderna efter försöket var högre än för de djur som togs ut innan försöket. Det tyder på att djuren åt och växte under försöket. Medelvikten var högst i Östersjön och lägst i Stora Aspen. Östersjöns sediment bestod mest av detritus och kunde förmodligen ge gammariderna tillskott i födan, medan Stora Aspen sedimentet bestod av lerig sand och förmodligen inte gav någon extra föda.

Generellt sett klarade sig chironomiderna sig bättre än gammariderna i sedimenten, vilket förmodligen beror på att de inte är lika känsliga för föroreningar som gammariderna. Sediment är väldigt komplexa och det är många faktorer som inverkar när djuren exponeras för dem. Detta gör det svårt att ge enkla svar på resultaten av dessa försök, men de kan ge en ledtråd på hur sedimenten kan påverka djuren.

Introduction

Metals are naturally present in fresh waters at low concentrations. In the sediment the metal content is higher due to natural enrichment caused by the high particle affinities of metals. Anthropogenic discharge of metals to waters has generally increased the concentration of metals in many lakes. The increased metal exposure to organisms can be a danger. Even small doses can result in biological disturbance and affect phyto- and zooplankton (SEPA, 2000). Heavy metals have a density of more than 5 g/cm^3 (Johansson, 1997). The toxicity of heavy metals can generally be listed by decreasing toxicity order: Hg, Cd, Cr, Zn, Ni, Pb, Cr, Co (Abel, 1989). Many of the heavy metals are essential to organism in small doses e.g. Co, Cu, Fe, Mn, Zn, Cr, and V (Klaassen *et al*, 1986).

Metals in the water phase can settle to the sediment through adsorption to particles or precipitation (Claesson, 2000). Adsorption means that the metals bind to the surface of a particle in the sediment or in the water phase. Metals can easier bind to fine materials like clays because it has larger surface/volume ratio. Additionally, clay rich sediments are often covered with iron- and manganese oxides. These oxide coatings have a high particle affinity so other metals can easily bind to the surface under neutral pH conditions. Redox conditions and pH affects the mobility of the metals in the sediment. When the redox potential is low, e.g. due to high decomposition, Fe and Mn are reduced and the metals that are bound to their surface are released. Precipitation of metals occurs when dissolved metals aggregate, e.g. due to changed pH/redox conditions, precipitate, and settle to the bottoms. Dissolved metal ions are generally easy for organism uptake (Johansson, 1997). However, metals bound to small organic molecules can be even easier to take up, e.g. methyl mercury and alkyl lead, because they are lipophilic (Klaassen *et al*, 1986). For organisms that live in the sediment there are two ways to take up metals, by the food through the digestive system or directly from the sediment through the skin.

The river Kolbäcksån drainage area is 3120 km^2, which includes approximately 9 % lake surface (County Administration Board, 1998). The river stretches from Lake Bysjön about 50 km north of Ludvika to L. Freden south of Strömsholm where it reaches L. Mälaren. The tributary is L. Mälaren second largest inflow. The main stream is 180 km long and runs through the municipalities Ludvika, Smedjebacken, Fagersta, Surahammar and Hallsta-hammar with small inflows from Norberg and Lindesberg. During a long time the river has been influenced by mining activities in the catchment area (Lasu, 2001) and consequently the lakes in the area have been affected by high metal discharges for a long time. The discharges have been reduced since the beginning of 1970's mainly due to better refining methods and the shutting down of industries (Sonesten and Goedkoop, 2002). In spite of the reduced dis-charges the watercourses are still polluted by leakage from the old mines and slag deposits as well as leakage from the contaminated sediments (Lasu, 2001). The most contaminated lake in river Kolbäcksån is L. Saxen, which is situated in the upper part of the river system. The lake is heavily polluted with Cu, Zn, Cd, Pb and Cr, which is due to the old mine on Sax-berget. Zn is a comparatively mobile metal, which implicates that the metal affects the whole river system downstream. On the contrary metals like Cd, Cu and Pb are rather immobile within the lake and therefore those metals do not affect the downstream lakes to the same extent as i.e. Zn. In L. Stora Aspen there are high levels of both Cr and Ni that is due to local discharge mainly from Fagersta Stainless AB. The highest content of Co is found in L. Stora Aspen, which originates from discharge from Seco Tools AB. Other important point dis-charges in river Kolbäcksån are the Gårlången- and Mölntorp sewage-treatment plants (Sonesten and Goedkoop, 2002).

The aim of the study was to investigate how the metal concentration in the sediment affected the test animals *Chironomus riparius* and *Gammarus pulex*. For *C. riparius* a profundal sediment toxicity test with the endpoints survival, emergence rate and development rate was performed. The midge *C. riparius* is an invertebrate that has been used frequently in toxicity tests. It has been shown that tests with *C. riparius* are good estimates of pollutant toxicity in natural sediments (Wästlund, 1999).

For *Gammarus pulex* a littoral sediment toxicity test with the endpoint survival was performed. *G. pulex* is an amphipod that is only occasionally found in the benthic fauna in river Kolbäcksån.

The sediments were taken from five lakes in river Kolbäcksån; L. Saxen, L. Norra Barken, L. Stora Aspen, L. Östersjön and L. Freden. The reason I selected these five lakes was to get a gradient over the whole system. To get an extreme lake I included L. Saxen due to the outstanding high metal concentrations in the sediment (Sonesten and Goedkoop, 2002). All lakes are well documented since they are subject of an annual recipient control program performed by the Department of Environmental Assessment, Swedish University of Agricultural Sciences in Uppsala. As a reference lake, L. Erken, north of Norrtälje was chosen.

Materials and methods

Site Description

L. Saxen, which is situated in the upper part of the system, is a moderately eutrophic lake with quite low total phosphorus levels (table 1). The nutrient levels increases in the lakes further down in the river system due to increased load from sewage-treatment plants and more arable land. The water in L. Saxen has high or very high content of several metals and the lake is the most metal contaminated lake in the river system (table 3). The high metal concentrations originate from the residues of the old mine, Långfallsgruvan, on Saxberget, which closed down in 1988. A lot of the metals in the water also come from internal loading of the old sediments that are heavily contaminated (Sonesten and Goedkoop, 2002).

Table 1. Maximum depth, lake area, total phosphorus and total nitrogen levels and sampling locations in the investigated lakes.

Lake	Maximum depth (m)	Area (km^2)	Tot P (µg/l)*	Tot N (µg/l)*	Sampling coordinates, according to the Swedish National Grid
Saxen		0,8	8	440	1454394 E, 6671881 N
Norra Barken	37	20	17	450	1501566 E, 6651072 N
Stora Aspen	25	6	18	600	1522045 E, 6620019 N
Östersjön	7	1,3	24	550	1522045 E, 6620019 N
Freden	15		27**	930**	1526077 E, 6598874 N
Erken (control)	21	24	27***	660***	1657857 E, 6639292 N

*Mean value of surface sample, 2001 (Sonesten and Goedkoop, 2002)

**Mean value of surface sample, 1999 (Sonesten *et al*, 2000)

*** Yearly mean value for P and N, data from 1933-1998 (Weyhenmeyer, 1999)

L. Norra Barken is the second largest lake in the river system and it is a deep and moderately eutrophic lake with a clear temperature stratification during summer time (table 1). L. Stora Aspen is moderately eutrophic and has a clear temperature stratification in the summer time (County Administration Board, 1998). The high nitrogen level (table 1) originates from industries, mainly Fagersta Stainless AB, and from households in Fagersta and Västanfors (Sonesten and Goedkoop, 2002). High levels of Cr and Ni are also discharged from the industries in Fagersta. High discharges of these metals are also taken place from mechanical industries in Virsbo, Surahammar and Hallstahammar all situated further down in the system (Lasu, 2001). Lake Östersjön is a small lake with a large flow-through, which makes the water residence time short (County Administration Board, 1998). The lake is one of the most eutrophic lakes in Kolbäcksån and has a fairly high level of total phosphorus (table 1) (Sonesten and Goedkoop, 2002). L. Freden is the bay where river Kolbäcksån emerges to Lake Mälaren. L. Freden is a shallow eutrophic lake with a high flow-trough, which prevents seasonal temperature stratification (County Administration Board, 1998).

L. Erken is a moderately eutrophic lake, which is stratified during summer time. It is situated north of Stockholm close to the Swedish east coast approximately 20 km north of Norrtälje (59˚25’ N, 18˚15’ E) (Weyhenmeyer, 1999). L. Erken was used as a control because it is not so much affected by anthropogenic activities so the effect on the benthic fauna is supposed to be small (Forsberg and Pettersson, 1998)

Profundal sediment toxicity test

Sediment collection

Sediment from five different lakes in the river Kolbäcksån were investigated, L. Saxen, L. Norra Barken, L. Stora Aspen, L. Östersjön and L. Freden. Sediment from L. Erken was used as a reference. Twelve profundal sediment cores were collected in each lake (except for Lake Östersjön where only ten cores were collected) with a core sampler, (∅ 67 mm) on 5-6 September 2002. Only the top centimeter of the sediment was used in each core. On the 19th of September nine sediment cores were collected in Lake Erken. The sediment samples were kept cool during transport (~10˚C) and stored 41 days and 27 days, respectively, (4˚C) before use.

Test organism

Chironomus riparius larvae were cultured at room temperature (~20˚C) with a photoperiod of 16 hours light and 8 hours dark at the department of Environmental Assessment, Swedish University of Agricultural Sciences in Uppsala. The larvae were held in a small aerated aquarium which was placed in a cage to allow adult midges to swarm. The larvae were fed daily with pulverized TetraPhyll®. When egg ropes were produced they were put in petri dishes to let the larvae hatch. The toxicity test for the midges followed the proposed OECD guideline 218 (2001) except that natural sediments were used in spite of artificial.

Experimental setup

Approximately 80g sediment was put in each glass test vessel (one liter), which corresponds to a sediment depth of approximately 15 mm. Before adding the sediment to the vessels it was sieved through a 0.5 mm sieve. For each lake three replicates were used. Sediment from the different lakes was put in a freezer (-20˚C) for later analyses.

Lake water (approximately 330 cm^3), sampled at the same time as the sediments, and thereafter kept cool (4˚C), was added carefully to the vessels to prevent the surface to be disturbed. First a gas-washing bottle was used, but this was found to be hard due to clogging caused by particles in the lake water. Instead the water was pored gently on to a plastic disc (∅ 10 cm). To get the same amount of water in every vessel it was weighted during the addition.

Oxygen concentration and pH were measured at several occasions during the experiment. In some of the test vessels pH were quite low, below 5.8. To raise the pH in these vessels a buffer, $NaHCO_3$ (M=1.2 µmol/l), was added before the animals were added. In some cases the water had to be buffered at two more occasions during the experiment (appendix 1). At the experiment start, twenty newly hatched *Chironomus riparius* larvae were added to each vessel. The larvae were mixed from four different egg ropes. The aeration was turned off for a few hours after the larvae additions to let them settle in the sediment. The Chironomids were fed five days a week with 10 mg pulverized TetraPhyll per vessel the first two weeks, then 20 mg, five days a week. The test vessels were kept in a climate room at 20˚C with a 16:8 hour light:dark regime. The experiment lasted for 28 days. The vessels were gently aerated to keep a high redox potential and to provide the animals with a high oxygen supply. After the first adult midge had emerged the vessels were controlled every day for new midges.

After the termination of the experiment, the sediments were checked for remaining larvae that had not emerged and sediment samples for chemical analyses were put in a freezer (-20˚C). The sediment from the test vessels, together with previously frozen sediments from the start of the experiment, was later freeze-dried. The sediments from the test vessels were analyzed for TOC and total nitrogen (Carlo Erba NCS- analyzer 1500). The start sediment was analyzed for TOC, total nitrogen and the metals (ICP-MS); Al, Fe, Mn, Cu, Zn, Pb, Cd, Cr, Co, Ni, As and V. Water from the test vessels was analyzed for ammonia (Phenol method according to Berthelot, at 660 nm).

Littoral sediment toxicity test

Sediment collection

Littoral sediment (table 2) was collected on 5-6 September 2002. The sediment was scraped of the bottom at 1-2 m depth with the upper part of a plastic bottle attached to a 2 m long handle. On the 19th of September reference sediment were collected in Lake Erken.

Test organism

Gammarus pulex were collected on the 7th of October 2002 in Enstabäcken near Tierp church in Uppland (coordinates according to the Swedish National Grid 159180 E, 668749 N). The animals were put in small pots with a leaf for protection and food. The pots were gently aerated and the animals were acclimatized for twenty-four hours to the temperature in the climate room (20˚C). Thirty animals were put in a freezer (-20˚C), and later freeze-dried. The mean dry weight of these animals was compared to the dry weight of the animals at the end of the experiment to measure if the animals gained or lost weight during the experiment.

Table 2. Ocular determination of sediment type for the different lakes in the littoral sediment toxicity test.

Lake	Sediment type
Saxen	Gravels with small pebbles
Norra Barken	Soft sediment with sand
Stora Aspen	Clayey sand with some small twigs.
Östersjön	Detritus, small twigs, leaves, shells from gastropods. Soft material with some gravel.
Freden	Gravel, largest pebble ∅ 2 cm.
Erken (control)	Gravels with small pebbles and shells from gastropods.

Experimental setup

The littoral sediment was put in approximately one to two centimeter layers in the test vessels. It was not sieved because it consisted of such different materials, some of the sediments consisted mostly of gravel whereas other sediments consisted mainly of very fine material (table 2). Lake water (approximately 200-300 cm^3), collected at the same time as the sediments and thereafter kept cool (4˚C) was added very carefully to not disturbance the sediment surface (see description for the profundal sediment). However, the surface of the littoral sediment was not as sensitive to disturbances as the profundal sediments.

Twenty *Gammarus pulex* were added to each test vessel. The vessels were put in a climate room (20˚C) with gentle aeration and a 16:8 hour, light:dark regime. The Gammarids were fed five days a week with 10 mg pulverized TetraPhyll per vessel the first two weeks, then 20 mg, five days a week. The experiment lasted for 21 days and oxygen concentration and pH were measured at several occasions during the experiment.

After the termination of the experiment the sediments were checked for surviving Gammarids and other animals. The surviving Gammarids were put in the freezer and later freeze-dried and weighed.

Statistical analyses

Data was analyzed using ANOVA with a significance level of 5 %. A post hoc test, Fischer's PLSD, was used for a pair-wise comparison (Statview). The emergence rate for the midges was arcsin-transformed prior to statistical analyses (OECD, 2001).

Results

Profundal sediment toxicity test

There were differences found in the total emergence of *C. riparius* exposed to profundal sediments from the different lakes (fig 1). The control (L. Erken) had on average enough emerged midges to be a valid control according to the OECD guideline. In L. Saxen the smallest number of midges emerged with a significant difference to L. Norra Barken (p=0.010), L. Stora Aspen (P=0.025) and L. Östersjön (p=0.026), (fig 2).

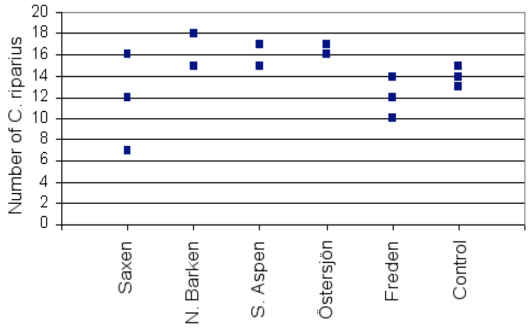


Figure 1.Total number of emerged C. riparius *for all replicates of the lakes in the profundal sediment toxicity test. Lake Erken sediments were used as control.*

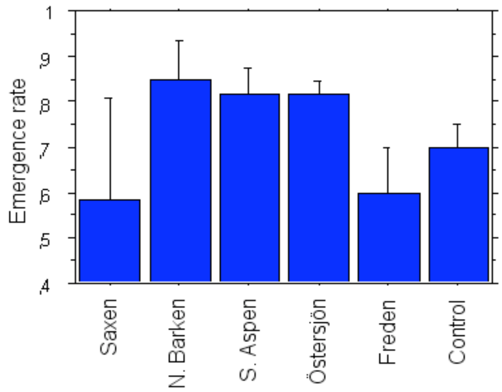


Figure 2. Mean emergence rate (± standard deviation) for C. riparius *for the different lakes in the profundal sediment toxicity test. Lake Erken sediments were used as control.*

In L. Freden also a small number of midges emerged with a significant difference to L. Norra Barken (p=0.012), L. Stora Aspen (p=0.032) and L. Östersjön (p=0.033), (fig 2). There were very large variations in larvae emergence between the different test vessels with L. Saxen sediments. In one of these vessels only seven midges emerged out of the 20 from start, which made the average number for the whole lake very low (fig 2). The midges exposed to L. Freden sediments had a high development rate (fig 3), but the emergence rate was low (fig 2). This high development rate depends on that the few midges that emerged did so during the first few days. L. Saxen had a low development rate; the midges emerged during the whole experiment typically with one midge per day (fig 3). There was only a significant difference in development rate between L. Saxen and L. Freden (p=0.009).

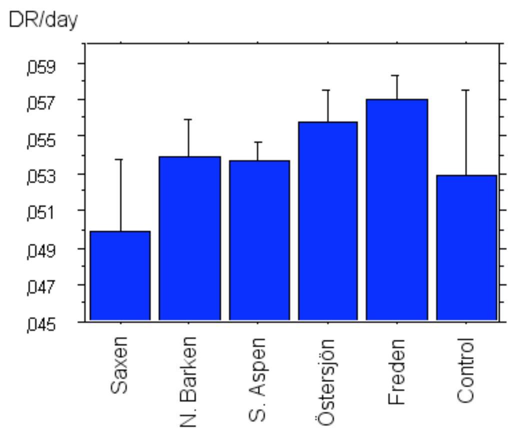


Figure 3. Mean development rate (± standard deviation) per day of emerged midges exposed to profundal sediments from different lakes. Lake Erken sediments were used as control.

The pH did not seem to have any direct effect on the midge development (fig 4). In the controls were the pH was stable and quite high during the experiment (appendix 1), the midges do not emerge more successfully or faster than compared to the other lakes (figs 2 and 3). In L. Stora Aspen and L. Östersjön where the emergence rate was comparably high the water was buffered twice during the experiment.

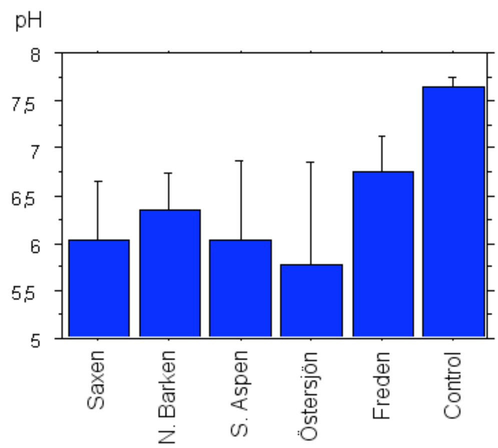


Figure 4. Mean pH (± standard deviation) in the water phase in the experiment with profundal sediments from the different lakes. Water from the Lake Erken test vessels were used as control.

The measured oxygen concentration was between 4.4 and 8.4 mg O_2/l in the test vessels during the whole experiment (appendix 2), which is above the minimum concentration of 2-3 mg O_2/l for the test to be valid (OECD, 2001). The oxygen concentration was measured approximately 3 cm below the water surface in the test vessels.

The ammonia concentration showed large variation between different lakes (fig 5) and a quite large variation between the different replicates (appendix 3). However, it did not seem to have any negative effects on the midges. In L. Stora Aspen were the ammonia concentration was the highest the midges emerged successfully and in L. Östersjön were the concentration was the lowest a high number also emerged (fig 2).

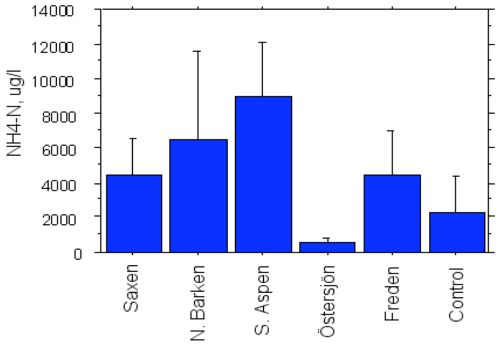


Figure 5. Mean NH_4-N concentration (± standard deviation) in the water phase in the experiment with profundal sediments from the different lakes. Water from the Lake Erken test vessels were used as control.

There were large variations in the C/N ratio between the different profundal sediments at the end of the experiment (fig 6). L. Saxen had a high C/N ratio, which was significantly different to L. Norra Barken (p=0.009), L. Stora Aspen (p=0.032), L. Östersjön (p=<0.001) and to the control (p=0.007). Also L. Freden had a high C/N ratio, which was significantly different to L. Östersjön (p=0.004) and to the control (p=0.045).

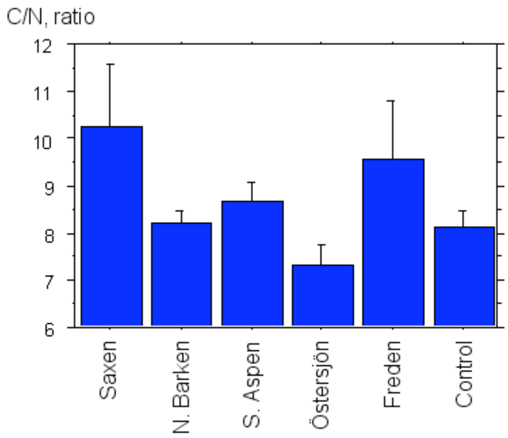


Figure 6. Mean C/N ratio (± standard deviation) for the profundal sediments at the end of the experiment from the different lakes. Lake Erken sediments were used as control.

L. Saxen has by far the highest metal concentrations in the profundal sediment (tables 3 and 4). The concentration of Cu, Zn, Pb and Cr are in the highest "environmental quality class" according to SEPA (2000). In L. Stora Aspen the concentrations of Cr and Ni are in the highest "environmental quality class" when compared to the local background levels from L. Bysjön (table 4).

Table 3. Metal content in the top layer of the sediment (0-1 cm). The colors show environmental quality class according to SEPA (2000).Red indicates a high divergence to natural background levels and blue a small divergence. The order between those levels is orange, yellow and green.

Lake	Cu	Zn	Pb	Cd	Cr	Co*	Ni	As	V*
				mg/kg d.w.					
Freden	44.1	467	48.9	0.986	95.3	18.6	81.0	5.47	61.2
Östersjön	41.3	556	59.9	0.766	100	17.3	84.1	5.41	56.3
S. Aspen	76.8	1088	239	2.29	403	35.5	286	8.47	51.6
N. Barken	62.3	1085	160	2.89	133	8.39	17.6	5.75	50.4
Saxen	1977	11418	7469	23.4	1770	8.72	7.24	7.07	30.7
Control	37.7	154	41.6	0.770	46.3	15.0	41.5	4.09	59.7

** SEPA does not give any quality class for Co and V.*

Table 4. Divergence ratio in metal content compared to deep sediments (19-20 cm) from lake Bysjön 1991 considered to be local background levels (Sonesten and Goedkoop, 2002). The color shows environmental quality class according to SEPA (2000). Red indicates a high divergence to the background levels and blue a small divergence.The order between those levels is orange, yellow and green.

Lake	Cu	Zn	Pb	Cd	Cr	Co*	Ni	As	V*
			Measured content/background levels						
Freden	2.75	3.46	0.815	1.23	4.14	1.24	7.57	1.30	3.06
Östersjön	2.58	4.12	0.998	0.958	4.35	1.16	7.86	1.29	2.82
S. Aspen	4.80	8.06	3.99	2.87	17.5	2.37	26.7	2.02	2.58
N. Barken	3.89	8.04	2.67	3.61	5.79	0.559	1.64	1.37	2.52
Saxen	123	84.6	124	29.2	77.0	0.581	0.677	1.68	1.53
Control	2.35	1.14	0.693	0.962	2.01	0.999	3.88	0.975	2.98

** SEPA does not give any quality class for Co and V.*

Littoral sediment toxicity test

There were very large variations in the survival between the *G. pulex* exposed to littoral sediments from the different lakes (fig 7). The survival was the highest in L. Stora Aspen vessels whereas all specimens died in the vessels exposed to L. Saxen sediments. In the vessels with sediment from L. Östersjön there was a large variation, in one of the vessels all Gammarids died, but in the other two 50% or more of the animals survived. In the control there was a high survival in one of the test vessels, but in the other two only five Gammarids survived (fig 7) which coincided with high occurrence of leeches (table 5). L. Freden had a low survival, but it was only significantly different to L. Stora Aspen (p=0.013), (fig 8).

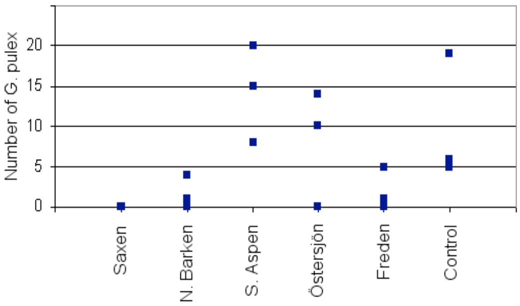


Figure 7. Total number of surviving G. pulex *for all replicates after 21 days for all the lakes in the littoral sediment toxicity test. Lake Erken sediments were used as control.*

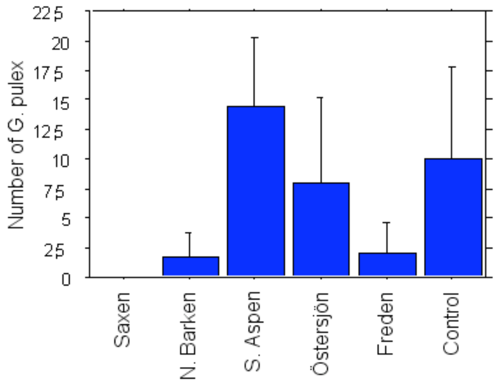


Figure 8. Mean survival (± standard deviation) of G. pulex *after 21 days in the littoral sediments from the different lakes. Lake Erken sediments were used as control.*

There were a large variety of animals other than the Gammarids in the sediments at the end of the experiment (table 5).

Table 5. Other animals found in the test vessels for the different lakes at the termination of the littoral sediments experiment.

Lake	Animals found in the test vessels
Saxen	Oligochaetes
Norra Barken	No animals found
Stora Aspen	Ephemeroptera (mayfly), *Ephemera vulgata* *Caenis horaria*
Östersjön	*Asellus aquaticus* Oligochaetes *Chironomus sp.*
Freden	*Erpobdella octoculata* (leech) *Chironomus sp.* Oligochaetes
Erken (control)	*Erpobdella octoculata* (leech)

The pH was fairly stable during the experiment (fig 9). The low mean pH for L. Östersjön was caused by a low pH in one of the vessels at the beginning of the experiment.

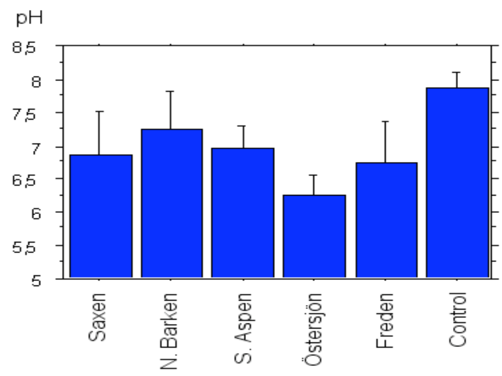


Figure 9. Mean pH (± standard deviation) in the water phase in the littoral experiment for the different lakes. Water from the Lake Erken test vessels were used as control.

In the beginning of the experiment there were some problems measuring the oxygen concentration due to a defect battery. This makes the second measurement uncertain (appendix 4). However, at the end of the experiment the battery was exchanged and the measurements are reliable.

The mean weight of the Gammarids was highest in L. Östersjön, significantly higher than L. Norra Barken (p=0.015), L. Stora Aspen (p=<0.0001), L. Freden (p=0.016) and the control (p=<0.0001), (fig 10). The sediment in L. Östersjön was very soft and consisted mostly of detritus with a few twigs (table 2). The mean weight for the Gammarids after the experiment was higher than the mean weight of those animals that was sacrificed at the start of the experiment (fig 10). In L. Stora Aspen, where the most animals survived (fig 8), the mean weight was the lowest (fig 10).

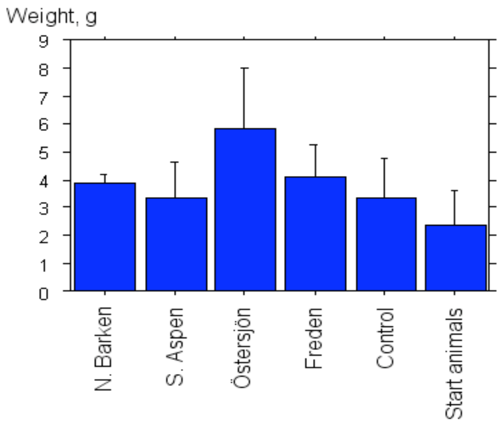


Figure 10. Mean weight (± standard deviation) of G. pulex *in the littoral sediments from different lakes at the start and at the end of the experiment. Lake Erken sediments were used as control.*

Discussion

The experiments on the midge *Chironomus riparius* and the amphipod *Gammarus pulex* were done to investigate if the metal concentrations in the sediments had any effect on the animals. Sediments are very complex and the exposure to the animals includes many factors, which makes it hard to give a simple answer to the outcome of a test, but it gives a clue on the potential effect on the animals. When the sediments are sampled some things changes in the sediments, e.g. redox potential, dissolved oxygen and particle distribution. These changes may influence the sediments toxicity (Burton and Scott, 1992). In L. Saxen the total metal concentration is the highest of all the lakes. This is probably the cause to that all Gammarids exposed to the littoral sediments from this lake died (fig 7), but the midge larvae did not seem to have been affected to the same extent by the profundal sediments.

No ammonia measurements were made on the experiment with littoral sediments. If the ammonia concentration was as high in these vessels as it was in the profundal experiment, the Gammarids might have been affected by this as well, since they are sensitive to high ammonia concentration (Berenzen *et al*, 2001). Probably, this was not such a big problem in the littoral sediments as they consist of less organic matter than the profundal sediments (more inorganic particles like gravels) and therefore the ammonia production ought to have been substantially lower in the littoral sediment test compared to the profundal. In general, the midges managed better in the sediments than the Gammarids, which is probably due to that they are not as sensitive to pollutants as Gammarids (Suedel *et al*, 1996). For all the lakes the mean total amount of emerged midges was more than half the total number of larvae at the beginning of the test. It is more difficult to interpret the results of the profundal experiment, as it is probably several factors in the sediments that affect the midges. Also, the sediment toxicity in the sediments can affect the midges in other ways than just by killing them, e.g. they can develop mouthpart deformities (Meregalli *et al*, 2000).

The control in this experiment was Lake Erken. An alternative would have been to use clean sand instead, which might have given larger differences between the control and the lakes. On the other hand, a more natural control sediment might reflect the reality in a better way. The control in the littoral experiment should have been checked for animals before adding the Gammarids since the leeches that were found at the termination of the test probably affected the outcome in a negative way.

Profundal sediment toxicity test

The mean emergence of the midges exposed to the highly contaminated L. Saxen sediments was the lowest (fig 2), but the variation between the replicates was large. In one of the test vessels 16 midges emerged out of the 20 from start compared to only 7 out of 20 in another. This implicates that the reason for the low emergence rate in the other vessels might not have been due to the high metal concentration in the sediments, per se, but might have been caused by other factors like pH, ammonia concentration and/or oxygen level.

Sampling of sediments with a core sampler disturbs the sediment. The profundal sediment is under natural conditions relatively structured and immobile. When this changes during the sampling the physicochemical gradients change. For example changes in the redox potential, dissolved oxygen and particle size distribution may occur. The sampling might also interact with processes concerning carbon, nitrogen and sulfur cycling. These effects may also influence the sediment toxicity (Burton and Scott, 1992).

In the sediments of L. Östersjön and L. Freden the metal concentrations (table 3) are similar, but still the emergence rate of the midges parted considerably from each other (fig 1). The Chironomids were probably affected by a combination of factors in the sediments and, additionally, they also have a capacity to detoxify several pollutants (Simkiss *et al*, 2001), which might confound the results. Chironomid larvae exposed to copper, nickel and zinc in the sediments have been shown to be able to regulate the accumulation of the metals in their tissues (Suedel *et al*, 1996). Since many pollutants are more concentrated in the sediments than in the water phase, benthic animals are more exposed to them, which can cause sublethal effects or adaptations to the prevailing conditions. Populations that exist in areas with mining and industrial activities have to adapt to the high pollutant levels, e.g. it has been shown that midges of *C. riparius* that live in such environments are genetically adapted to high concentrations of pollutants (Postma, 1995). In such metal contaminated sites a decrease in biodiversity is often found, which is due to the fact that it is only a few species that have the possibility to adapt to the prevailing conditions. For the larvae *C. riparius* morphological abnormalities has been discovered, especially mouthparts deformation. In a test by Meregalli *et al.* (2000) mouthpart deformities for Chironomids were related to estimated sediment toxicity. It is believed that examining mouthpart deformities on the larvae instead of using endpoints like growth and survival is a better way to estimate the presence of contaminants in the sediment (Meregalli *et al.*, 2000). Mouthpart deformities can be a sublethal toxicity affect, which means that the organism is affected of the pollutant in some other way then by death (Abel, 1989).
In L. Saxen the development rate of the midges was low (fig 3). A low development rate can be due to sublethal stress. The more stress the lower development rate for the midges. Mouthpart deformities were not investigated in this experiment, but could be useful in future

experiments. In an earlier investigation of Chironomid larvae from L. Norra Barken, L. Stora Aspen and L. Östersjön mouthpart deformities were found (Wiederholm and Dave, 1989). This implicates that the lakes are influenced by high metal levels. Toxicity tests with *C. riparius* larvae also have shown that different larval instars differ in sensitivity to metal stress. The first instar larvae are up to 1000 times more sensitive to metal toxicity than the fourth instar larvae (Postma, 1995).

The total emergence in all test vessels varied a lot (fig 1), especially those exposed to L. Saxen sediments. In one of these vessels only seven midges out of the 20 added emerged. The last time pH was measured in this vessel it was only 5.11, which could have affected the larvae negatively. On the other hand in L. Östersjön the pH was even lower, down to 4.71, but that did not seem to affect the emergence of the midges so much (appendix 1). Consequently, low pH alone did not seem to have any affect on the midges. *C. riparius* larvae are known to tolerate very low pH (Postma, 1995). In the controls, where the pH was around seven all the time the midges did not emerged to a larger extent than in the other vessels. Generally, pH in the water body of the investigated lakes is approximately neutral, but during conditions with high decomposition the pH may drop considerably close to the sediments (Sonesten and Goedkoop, 2002). The large differences in the pH between different vessels are probably a result of a varying degree of decomposition in the vessels. Metal solubility in water is influenced by the pH, generally the metal mobility increase with a decreased pH (Sonesten and Goedkoop, 2002). For example, copper gets more toxic at lower pH as the exposure of free copper ions increase (Suedel *et al*, 1996).

During the experiment the ammonia concentration increased (fig 5). Ammonia is known to be toxic to organisms, but ionized ammonia (NH_4^+), which is the most common in freshwaters, is not as toxic to organisms as unionized (NH_3) (Abel, 1989). In the sediments ammonia is often strongly adsorbed to the particles in the sediment (Wetzel, 2001). To know how much free ammonia there is in the water, the pH and the temperature have to be considered. Ammonia is more toxic at high pH. If the pH in the water is 8.5 and the temperature is 20˚C the total ammonia concentration should not exceed 0.22 mg l^{-1} ($NH_3 + NH_4^+$) to avoid toxic effects. In water with a pH of 6.5 and a temperature of 20˚C the total ammonia concentration should not exceed 20 mg l^{-1} (Abel, 1989). The highest level in the experiment was around 12 mg l^{-1} (NH_4-N), which implies that even if the ammonia concentration was very high, it probably did not alone affect the midges to a large extent.

L. Saxen and L. Freden had the highest C/N ratio (fig 6). A high ratio may be indicative that the sediment is less nutritious compared to sediments with a low ratio. The lowest emergence rate was found for L. Saxen and L. Freden, i.e. the least nutritious sediments. The *C. riparius* larvae are deposit feeders so the growth rate of the larvae is directly related to the amount of available food in their natural environment (Postma, 1995). In this experiment the larvae was fed with a pulverized fish food, TetraPhyll®. If the larvae had not been fed they might have been more affected by the metals in the sediment because then they had to rely on the food that existed in the sediment. Bioavailability is the fraction of the contaminants present that the organism can take up and the bioavailability depends on different characteristics of the sediment like organic matter content, clay content and pH (Meregalli *et al*, 2000). Feeding the larvae can influence the bioavailability of pollutants in that sense that the organisms eat the food they are fed with instead of the sediment, but on the other hand not to feed them at all might make them starve which also affects the sensibility to pollutants (Ankley *et al*, 1993).

At the termination of the experiment the sediment was checked for remaining larvae, dead or alive. Only a few larvae were found. The larvae are very quickly decomposed so the few that were found probably had died recently.

Littoral sediment toxicity test

There was a large variation in the survival of *G. pulex* in the different test vessels, except in L. Saxen where all Gammarids died (fig 7). L. Saxen has the highest metal concentration in the profundal sediment, of all the lakes in the experiment. No metal analyzes were made on the littoral sediments, but most certainly also these sediments contained high metal levels, which probably affected the Gammarids. It has been reported that the juvenile stages of *G. pulex* are more sensitive to pollutant stress than adult animals (Blockwell *et al*, 1998). The age of the Gammarids in the experiment is unknown so it is possible that many of the animals were juveniles. Gammarids are moulting (Hargeby and Petersen, 1988) and maybe that has affected the sensitivity for the animals to the toxicity of the sediments.

The littoral sediments were not sieved so other animals than Gammarids existed in the vessels (table 5). In L. Saxen, where all the Gammarids died, a lot of Oligochaetes were found, still alive at the end of the experiment. They feed on microscopic organisms and are mostly found in running polluted waters (Olsen and Svedberg, 1999). Oligochaetes are often described as insensitive to contaminants (Chapman *et al.* 1999). Oligochaetes are not predators on Gammarids, but they feed up to four times their own weight every day so the Oligochaetes could have competed with the Gammarids for food. The Gammarids are shredders, they tear leaves and other coarse detritus into smaller fragments, but in the L. Saxen vessels there were no leaves. So even if the Gammarids normally do not eat the same thing as the Oligochaetes they may have done that in the test vessels in lack of coarse detritus. In two of the controls there was a lot of the leech, *Erpobdella octoculata* (table 5). This leech is a predator on e.g. Gammarids (Olsen and Svedberg, 1999), which indicates that the low survival in the control might be due to the leeches. In one of the L. Freden vessels one leech was found, but no Gammarids. Maybe one leech was enough to kill all the 20 Gammarids? In one test vessel from L. Stora Aspen mayfly larvae (Ephemeroptera) was found (table 5). L. Stora Aspen had the highest mean survival of Gammarids (fig 8), but in the vessel where mayflies were found only eight Gammarids survived. The mayfly larvae can be a predator on Gammarids (Chinery, 1993).

The pH in the vessels was around 7-8 during the whole experiment, except at the first measurement when the pH in one of the vessels for L. Östersjön only was 5.85 (appendix 5). In this vessel all the Gammarids died. The *G. pulex* is very sensitive to low pH, studies have shown that a pH of 5.5 is enough to be lethal (Hargeby and Petersen, 1988). The low pH might have been enough to kill the Gammarids.

The mean weight of the Gammarids at the end of the experiment was higher for all the lakes compared to the weight of the Gammarids that was sacrificed at the start of the experiment (fig 10). This indicates that they fed and grew during the test. In L. Östersjön the mean weight was the highest. The sediment in L. Östersjön consisted of very soft material, mainly detritus with small twigs and gastropod shells (table 2), which could have been used by the animals as a complementary food source. In L. Stora Aspen where most Gammarids survived, but the mean weight was the lowest, the sediment consisted of clayey sand, which implies that it probably did not provide the animals with any additional food. If the Gammarids did not ingest the sediments they were less exposed to the metals and thereby less affected by them.

Fine materials like L. Stora Aspen sediments have a larger surface/volume ratio than do coarser materials. A large surface provides more binding sites and therefore enhances metal binding if the pH is neutral (Claesson, 2000). Since the pH in the experiment was close to neutral the metals could have been comparatively harder bound to the sediment particles in L. Stora Aspen and affected the Gammarids less than was the case for the other lakes.

In an experiment by Berenzen *et al.* (2001) it was shown that a concentration of 3 mg ammonia (NH_4^+) l^{-1} resulted in a clear decrease in Gammarid abundance. At higher concentrations all Gammarids died (Berenzen *et al*, 2001). The water in the littoral experiment was never analyzed for ammonia, but if the levels of ammonia were as high as in the profundal experiment (fig 5), the Gammarids might have been more affected then the Chironomids in the profundal experiment. Chironomids are generally more tolerate to contaminants than Gammarids and by using several different organisms in toxicity test the risk of making erroneous conclusions decrease (Suedel *et al*, 1996).

Sediments are very complex and the results of sediment toxicity tests are hard to evaluate. When natural sediments are brought to the laboratory many factors change due to decomposition of organic matter, there might be changes in ammonia concentration, pH and oxygen concentration. The increased temperature might e.g. cause a higher decomposition as the temperature in the profundal zone in lakes seldom reaches 20˚C. These changes in sediment and water chemical composition also affect the fate of the metals in the sediment and water. Changes in the chemical environment as well as metal speciation also affect the metal bioavailability and toxicity, which shows the complexity of the tests.

References

Abel P. D. 1989. Water pollution biology. Ellis Horwood Limited.

Ankley, G. T., Benoit D. A., Hoke R. A., Leonard E. N., West C. W., Phipps G. L., Mattson V. R. and L. A. Anderson. 1993. Development and Evaluation of Test Methods for Benthic Invertebrates and Sediments: Effects of Flow Rate and Feeding on Water Quality and Exposure Conditions. Environ. Contam. Toxicol. 25:12-19.

Berenzen N., Schulz R. and M. Liess. 2001. Effects of chronic ammonium and nitrite contamination on the macroinvertebrate community in running water microcosms. Wat. Res. Vol 35, 14:3478-3482.

Blockwell S. J., Taylor E. J., Jones I and D. Pascoe. 1998. The Influence of Fresh Water Pollutants and Interactions with *Asellus aquaticus* (L.) on the Feeding Activity of *Gammarus pulex* (L.). Environ. Contam. Toxicol. 34:41-47.

Burton G. A, Jr and K. J. Scott. 1992. Sediment toxicity evaluations their niche in ecological assessments. Environ. Sci. Technol. Vol. 26, No 11.

Chapman K. K, Benton M. J, Brinkhurst R. O and Scheuerman P. R. Use of the Aquatic Oligochaetes *Lumbriculus variegatus* and *Tubifex tubifex* for Assessing the Toxicity of Copper and Cadmium in a Spiked-Artificial-Sediment Toxicity Test. Environmental toxicology 14(2): 271-278.

Chinery M. 1993. Insekter i Europa. Bokförlaget Bonnier Alba AB, Stockholm.

Claesson P. 2000. Undersökning av metallsituationen i Kolbäcksåns tillflöden i Fagersta. Uppsala universitet. *Ekotoxikologiska avdelningen* Nr. 74.

County Administration Board. 1998. Kolbäcksån Försurning, övergödning och syrgasförhållanden. Miljöenheten, Nr 9.

Forsberg C and K. Pettersson. 1998. Lake Erken, 50 years of limnological research. Archiv für Hydrobiologie, Advances in limnology 51. E. Schweizerbart´she Verlagsbuchhandlung, Stuttgart.

Hargeby A and R. C. Petersen, Jr. 1988. Effects of low pH and humus on the survivorship, growth and feeding of *Gammarus pulex* (L.) (Amphipoda). Freshwater biology 19:235-247.

Johansson K. 1997 Metallhalter i svenska sjöar och vattendrag. Sjöar och vattendrag, årsskrift från miljöövervakningen 1997.
Klaasen C. D., Amdur M. O and J. Doull. 1986. Toxicology, the basic science of Poisons, third edition. Mcmillan Publishing company, USA.

Lasu R. 2001. Genomgång av befintliga miljödata I Kolbäcksåns vattensystem. Luleå tekninska universitet. 2001:197 CIV.

Meregalli G., Vermeulen A. C and F. Ollevier. 2000. The use of Chironomid Deformation in an *in Situ* Test for Sediment Toxicity. Ecotoxicology and Environmental Safety 47:231-238)

OECD guidelines for testing of chemicals. 2001. Proposal for a new guideline 218. Sediment-Water Chironomid Toxicity Test Using Spiked Sediment. www.oecd.org

Olsen L-H and U. Svedberg. 1999. Smådjur i sjö och å. Bokförlaget Prisma, Stockholm.

Postma J. F. 1995. Adaptations to metals in the midge *Chironomus riparius*. Aquatic Ecotoxicology, Department of Fundamental and Applied Ecology, University of Amsterdam, the Netherlands.

SEPA, Swedish Environmental Protection Agency. 2000. Bedömningsgrunder för miljökvalitet, Sjöar och vattendrag, rapport 4913. Almqvist & Wiksell, Uppsala.

Simkiss K., Davies N. A., Edwards P. A., Lawrence M. A. M. and M. G. Taylor. 2001. The use of sediment analogues to study the uptake of pollutants by chironomid larvae. Environmental pollution 115:89-96.

Sonesten L. and W. Goedkoop. 2002. Kolbäcksån, recipientkontroll 1999. Institutionen för miljöanalys, SLU, Uppsala.

Sonesten L., Goedkoop W., Herlitz E. and A-M Wiederholm. 2000. Kolbäcksån, recipientkontroll 1999. Institutionen för miljöanalys, SLU, Uppsala.

Suedel B. C., Deaver E. and J. H Rodgers, Jr. 1996. Experimental Factors That May Affect Toxicity of Aqueous and Sediment-Bound Copper to Freshwater Organisms. Environ. Contam. Toxicol. 30:40-46.

Wetzel R. G. 2001. Limnology, Lake and River Ecosystem, third edition. Academic press, USA.

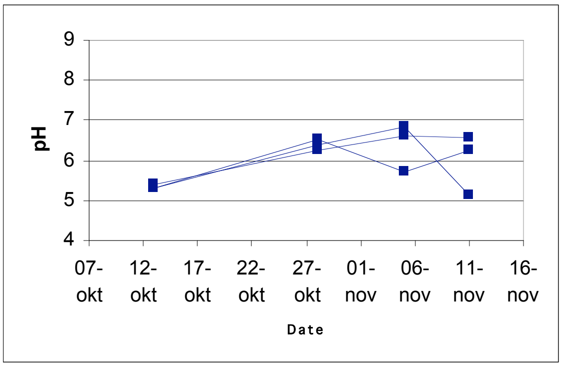
Weyhenmeyer G. 1999. Lake Erken Meteorological, physical, chemical and biological data and a list of publications from 1933 to 1998. Scripta Limnologica Upsaliensa 1999 B:16.

Wiederholm T. and G. Dave. 1989. Toxicity of metals polluted sediments to D*aphnia magna* and *Tubifex tubifex*. Hydrobiologia 176/177:411-417.

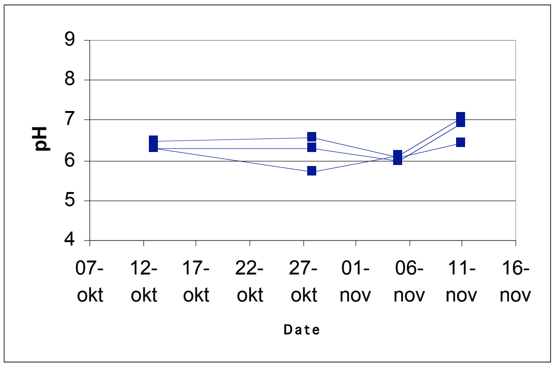
Wästlund D. 1999. The role of sediment characteristics and food regimen in a toxicity test with *Chironomus riparius*. Department of Environmental Assessment, Uppsala, 1999:3.

Appendix 1.

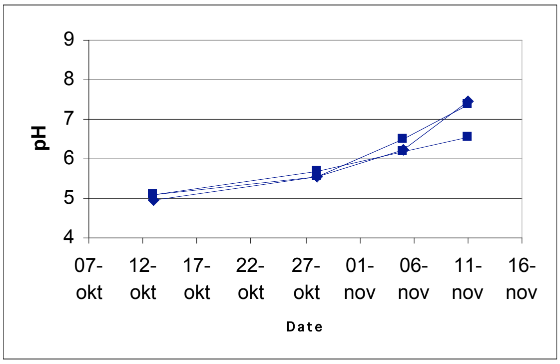
Changes in pH in the test vessels (fig a-f) during the profundal sediment experiment for the different lakes and buffer addition to the test vessels (table).



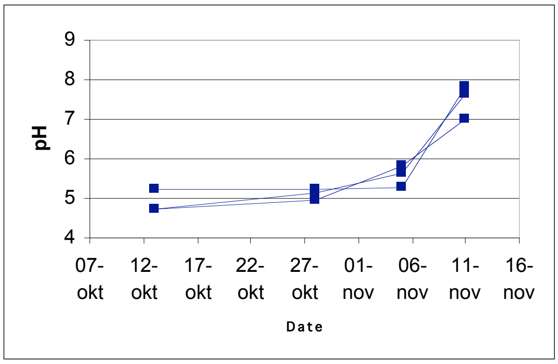
a, L. Saxen



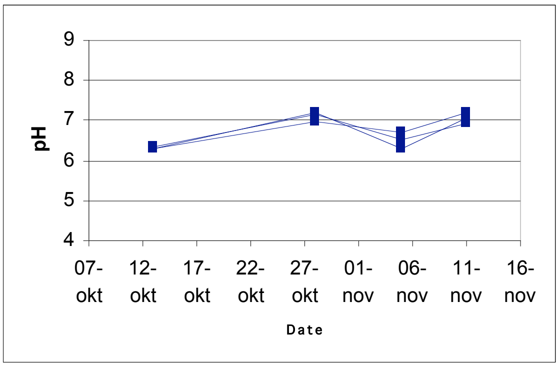
b, L. Norra Barken



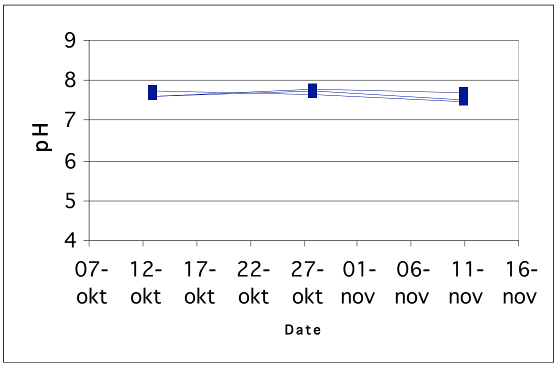
c, L. Stora Aspen



d, L. Östersjön



e, L. Freden

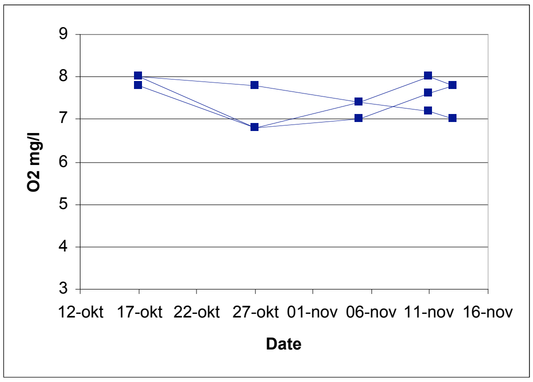


f, L. Erken (control)

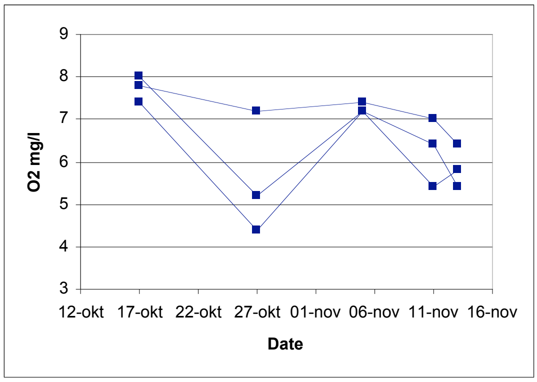
Lake	Buffer (ml), $NaHCO_3$ (1.2 µmol/l)		
	17-oct	30-oct	31-oct
Saxen 1	1.8		
Saxen 2	6.4		
Saxen 3	6.1		
N. Barken 1			0.6
S. Aspen 1	3.7	1.0	0.7
S. Aspen 2	2.5	1.0	0.5
S. Aspen 3	2.6	0.7	1.4
Östersjön 1	1.3	2.0	3.4
Östersjön 2	1.6	2.3	4.4
Östersjön 3	1.6	3.7	4.7

Appendix 2.

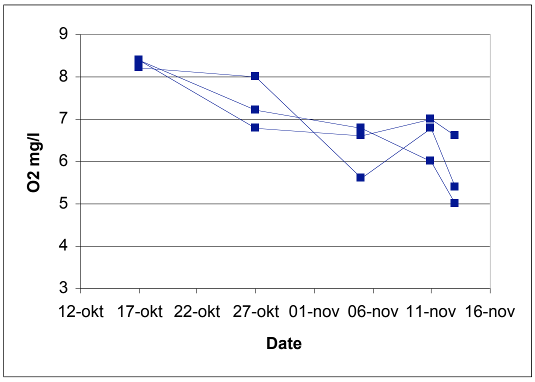
Oxygen concentration fluctuations in the test vessels during the profundal sediment experiment for the different lakes.



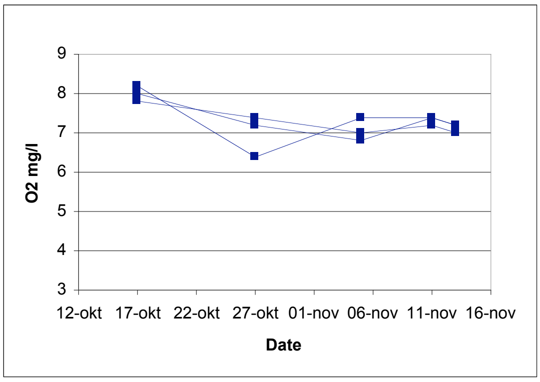
a, L. Saxen



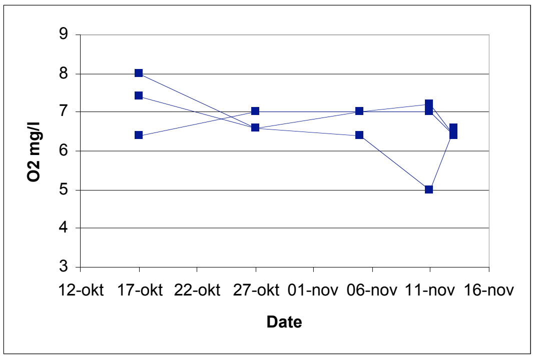
b, L. Norra Barken



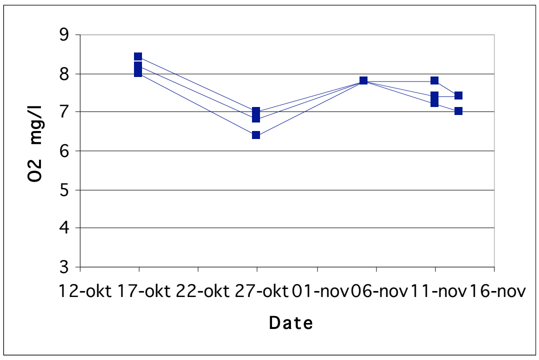
c, L. Stora Aspen



d, L. Östersjön



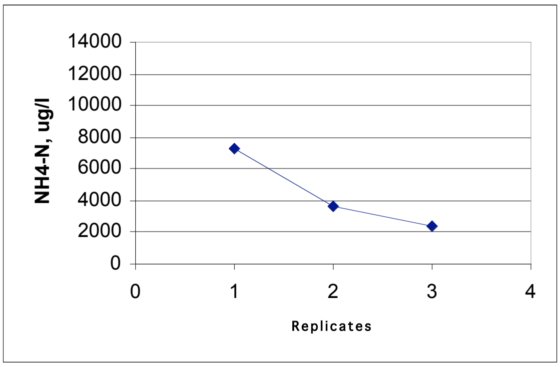
e, L. Freden



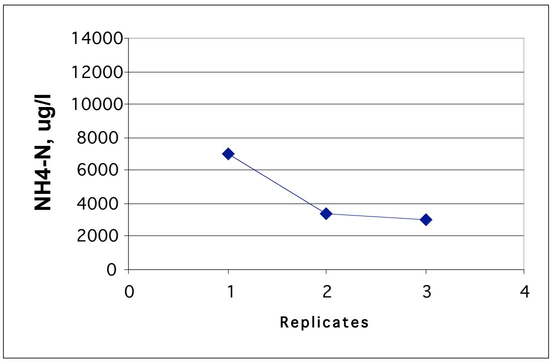
f, L. Erken (control)

Appendix 3.

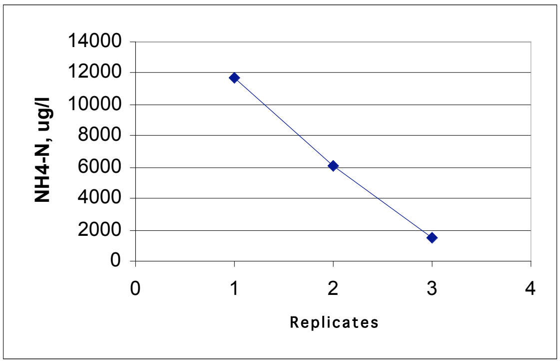
Ammonia (NH_4-N) concentration at approximately 3 cm water depth at the end of the experiment. The concentrations are given for the three replicates of each lake in the experiment with profundal sediment.



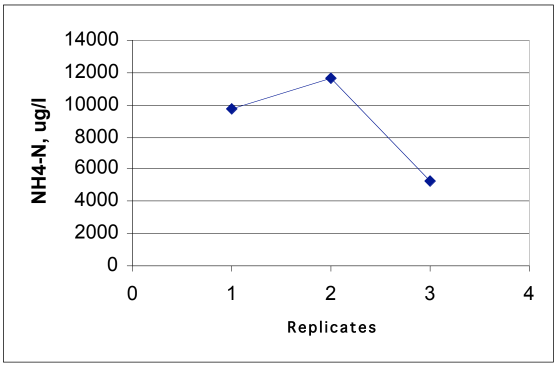
a, L. Saxen



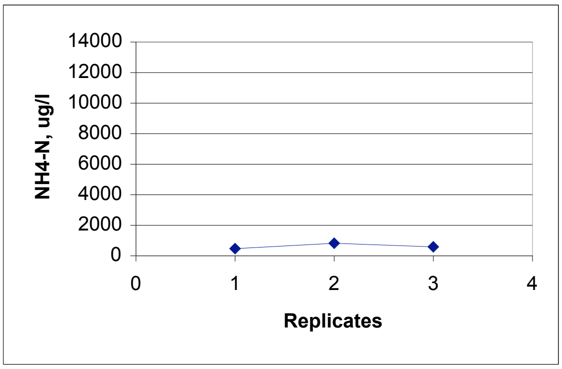
b, L. Norra Barken



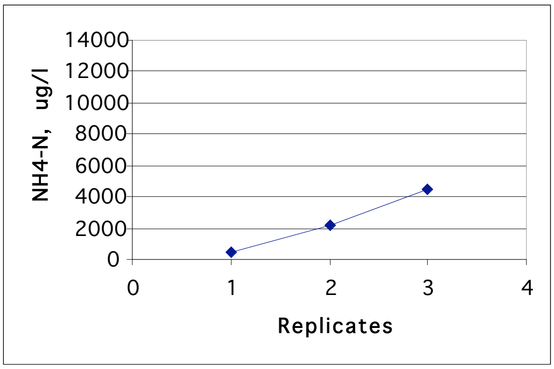
c, L. Stora Aspen



d, L. Östersjön



e, L. Freden



f, L. Erken (control)

Appendix 4.

The variation of oxygen concentration (mg/l) during the littoral sediment experiment in the test vessels from the different lakes.

Lake	Oxygen concentration, mg/l			
	03- oct	08- oct*	23- oct	27- oct
Saxen 1	8.9	*12.4*	4.4	8.2
Saxen 2	9.2	*12.2*	5.6	7.8
Saxen 3	9.2	*12.2*	6.4	7.8
N. Barken 1	9.1	*12.6*	4.0	7.8
N. Barken 2	9.0	*12.6*	4.8	7.4
N. Barken 3	9.1	*12.2*	4.2	8.0
St. Aspen 1	8.4	*9.8*	4.8	7.8
St. Aspen 2	9.0	*12.0*	2.6	7.8
St. Aspen 3	9.1	*12.0*	5.2	2.8
Östersjön 1	9.0	*11.8*	4.4	8.2
Östersjön 2	8.4	*10.2*	6.2	7.6
Östersjön 3	9.4	*12.6*	8.0	7.4
Freden 1	9.5	*12.6*	6.8	7.4
Freden 2	8.4	*10.4*	4.4	7.4
Freden 3	9.6	*9.6*	4.4	8.0
Control 1	8.5	*9.4*	4.2	8.0
Control 2	8.2	*10.8*	7.6	7.6
Control 3	8.4	*10.2*	6.8	7.6

*At the second measurement there were some problems with a defect battery.

Appendix 5.

The variation of pH during the littoral sediment experiment for the test vessels from the different lakes.

Lake	pH	
	02-okt	24-okt
Saxen 1	6.68	
Saxen 2	6.58	
Saxen 3	6.42	7.85
N. Barken 1	6.83	
N. Barken 2	6.88	
N. Barken 3	7.19	8.07
St. Aspen 1	6.68	
St. Aspen 2	6.84	
St. Aspen 3	6.92	7.44
Östersjön 1	6.49	
Östersjön 2	6.46	
Östersjön 3	5.85	6.26
Freden 1	6.47	
Freden 2	6.5	
Freden 3	6.41	7.65
Erken 1 (Control)	7.74	
Erken 2 (Control)	7.75	
Erken 3 (Control)	7.78	8.23