DISTRIBUTION OF ARCELLACEANS (TESTATE AMOEBAE) IN THE SEDIMENTS OF A MINE WATER IMPACTED BAY OF LAKE RETUNEN, FINLAND

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Abstract. This study employs chemical fractionations of sedimentary metals and analyses of sediment arcellacean (thecamoebian) faunas to study the ecological effects of mining wastewaters in a boreal lake bay that receives metal-rich waters from a Cu mine. Sediment chemistry and arcellacean species compositions were analyzed from both surface sediment samples and a sediment profile to investigate spatial and temporal changes in mine water pollution and biota. Based on the results, geochemical gradients in the area are caused by dispersal and dilution of metal-rich, low-pH mine waters entering the lake; transport and focusing of fine grained metal precipitates and sulphate in the deep areas of the bay; increase in pH due to sulphate reduction and mobilization of redox-sensitive elements from deep water sites; and precipitation of the mobilized metals at shallower sites. Arcellacean species compositions change systematically with increasing distance from the source of pollution and species diversity as well as concentrations of tests in the samples increase as well. Fe:Mn ratio and adsorbed Al explained variation in surface sediment arcellaceans with statistical significance. Fe:Mn ratio is an indicator of the overall geochemical environment (Eh, pH), while the toxicity of Al in aquatic environments is well known. Changes in arcellacean species and geochemistry in the long core suggest that before the mine closure in 1983, mine waters differed in nature from the present acid drainage and metals such as Cu, Co, Zn and Ni may have affected arcellaceans at that time.

Keywords: arcellaceans, Finland, metals, mining, pollution, protozoa

1. Introduction

Arcellaceans (thecamoebians) are microscopic protozoans that mainly live in fresh waters and in other moist habitats such as peat bogs. They form saclike tests (shells) to protect the amoeboid cell. Most of the arcellaceans, a subgroup of the order Arcellinida, build their tests by agglutinating foreign particles (diatoms, mineral grains) with mucopolysaccharides (Patterson and Kumar, 2000a). These tests have a high preservation potential and occur abundantly in recent lacustrine sediments (Kumar and Patterson, 2000). While rhizopods in peat bogs have been used as indicators of moisture, pH and plant components (Tolonen, 1986; Warner and Charman, 1994; Ellison, 1995; Booth, 2002), lacustrine arcellacean remains have been shown

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to be potential indicators of industrial and mining-related pollution (Patterson *et al.*, 1996; Asioli *et al.*, 1996; Reinhardt *et al.*, 1998; Kumar and Patterson, 2000). The tests preserve well even in low pH environments often associated with mine drainage (Patterson and Kumar, 2000a) and their high abundance facilitates the use of small sample volumes (Kumar and Patterson, 2000). Since arcellaceans live at the interface between sediment and water, only a very thin sample (2–3 mm) of the topmost sediment is needed for analysis (Reinhardt *et al.*, 1998). This potential for high-resolution sampling makes it possible to not only analyse the pre-impact species compositions but also to follow recovery using sediment samples.

This study employs analyses of sediment chemical fractions and arcellacean species compositions to study the ecological effects of mining wastewaters and loading from other sources (e.g., forestry) on the Petkellahti Bay in Lake Retunen, eastern Finland. Metal-rich effluents from the Luikonlahti Cu (+Co, Ni, Zn; later talc) mine and processing plant, located some 800 m away from the lake, affect the chemistry of the bay (Räisänen and Juntunen, 2004). The aims of the study are to (1) use sediment chemical properties from the entire embayment to describe the overall geochemical environment that affects the biota in the area; (2) study the effects of the observed environmental gradients on arcellacean species composition and (3) attempt to identify, employing multivariate numerical methods, the most important environmental variables that effect arcellaceans.

2. Materials and Methods

2.1. Study site

Petkellahti Bay is located on the eastern shore of Lake Retunen and receives most of its inflowing waters from the Myllyoja stream (Figure 1). The drainage area of the bay is 9.4 km^2 in size and the Luikonlahti copper mine is the single most important factor affecting water quality in the Myllyoja stream (Tyni, 1997). The mine was in operation from 1968 to 1983 with a total of 7 Mt of ore extracted during this period. On average, the ore contained 0.99% Cu, 0.61% Zn, 0.11% Co and 17.22% S (Eskelinen *et al.*, 1983). In addition, soapstone was quarried for talc production between 1979 and 1983, after which it has been transported to the Luikonlahti production plant from other quarries (Räisänen, 2003; Räisänen and Juntunen, 2004). At present, the weathering of sulphide-bearing waste rocks and seepage waters from the tailings area mainly cause metal loading from the mine. The pH of the seepage waters is on average 3.6 with high concentrations of metals. However, surface runoff from the tailings area is collected into settling ponds outside of the Petkellahti Bay drainage area.

In addition to the mine, other human and natural factors affect water quality in the bay. For instance, the water level in the Palolampi pond in the upper reaches of

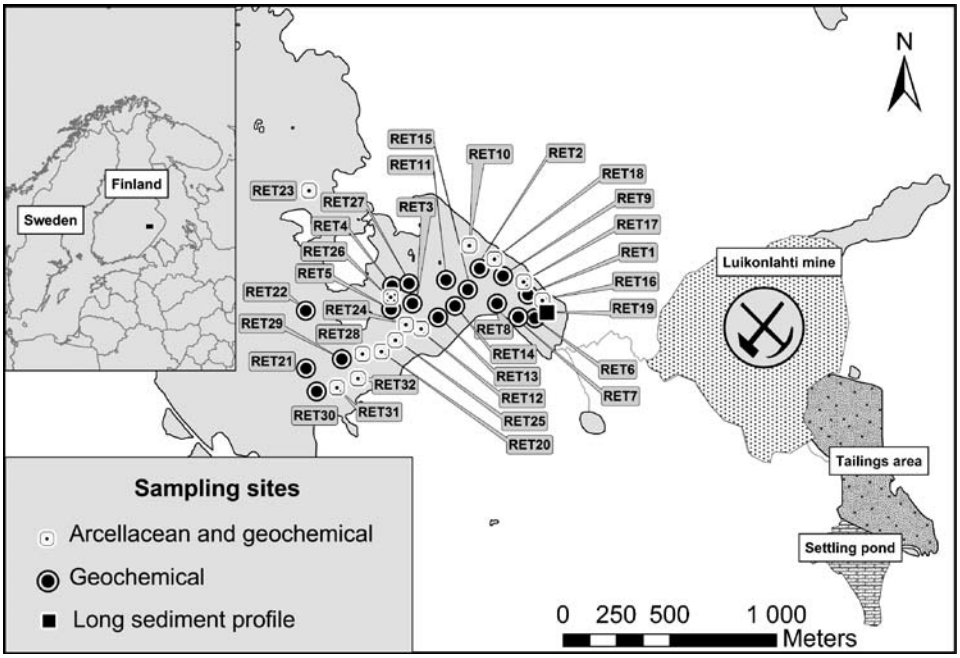

Figure 1. Study area and its location in Fennoscandia. Myllyoja stream flows from the mine to Petkellahti Bay. Coring site categories based on laboratory analyses are also shown: geochemistry + arcellaceans, geochemistry only, long core.

the Myllyoja stream was lowered by 3 m after the onset of mining. In addition, the drainage area has been subjected to various forest management actions and ditching of wetlands. Furthermore, glacial tills in the area contain elevated concentrations of sulphur and metals because of the characteristics of the local bedrock (Koljonen, 1992).

Metal loading from the mine has decreased during the last ten years. Between 1993 and 2001, the average pH and Fe and Mn concentrations in the stream were 4.3, 7.3 mg l^{-1} and 1.9 mg l^{-1}, respectively, whereas in 2002–2003 the corresponding values were 6.5, 5.6 mg l^{-1} and 1.4 mg l^{-1}. The present metal and sulphur concentrations in the stream are Co 34 μg l^{-1}, Cu 11 μg l^{-1}, Ni 130 μg l^{-1}, Zn 257 μg l^{-1} and S 120 mg l^{-1} (Räisänen, 2003). Despite the reductions in loading from the stream, metal concentrations of hypolimnetic water samples from the deep areas of the bay have increased during the last two decades. Between 1984 and 1987, Fe concentrations varied between 2.0 and 5.0 mg l^{-1} and electric conductivity values were 40–55 mS m^{-1}. In contrast, Fe concentrations of 5.0–17.0 mg l^{-1} and conductivity values of 65–90 mS m^{-1} were measured between 2000 and 2003, suggesting internal metal loading (Mikkonen and Häikiö , 2004; Ronkainen, 2004).

2.2. Coring

Lake Retunen was cored from the ice in March 2004. To study the pollution gradient in the bay, 32 short cores (max 50 cm) were taken with a Limnos corer (Kansanen *et al*., 1991) to cover the whole embayment (Figures 1 and 2). In addition, a long core was taken with a modified Kullenberg corer (PP corer; Putkinen and Saarelainen, 1998) near the inlet of the Myllypuro stream (filled box in Figure 1). The short cores were subsampled with the top-bottom approach, in which a modern ('top') sample and a pre-impact ('bottom') sample are taken from each core. At Petkellahti, a thin (2 mm) layer of fine-grained mineral matter that was found in all cores aided the division of the cores into polluted and unpolluted samples. The mineral layer was considered to mark the lower limit of the top (polluted) sediment section, assuming it was deposited in conjunction with the onset of mining (e.g., due to the drainage of Palolampi pond). Therefore, the top samples never extended below this layer and the lower (bottom) subsamples were taken 10 cm below the clayish marker layer at all coring sites. The depth at which the mineral layer was found varied with distance from the mouth of the brook and water depth. The depth of the mineral layer was 48 cm near the mouth of the brook, 12–15 cm in the deeper areas of the bay and 3–5 cm at shallow water sites.

Fourteen top sediment samples were selected for arcellacean determinations from the top-bottom pairs (white boxes in Figure 1). The coring sites were 3–6 m deep to minimize the effect of water depth and depth-related secondary gradients on arcellaceans. The sites form an irregular transect that extends away from the source of pollution. In addition, the RET 23 site, west of the bay, was analyzed as a reference site and one deep site (RET 12, water depth 9 m) was analyzed for comparison. The RET 23 site is also somewhat deeper than the sites from Petkellahti Bay (7.2 m). When the sampling sites were arranged with increasing distance from the mouth of the stream to illustrate the possible effects of mine water pollution, RET 12 was included as the closest site and RET 23 as the farthest site.

To investigate temporal changes in mine water pollution, sediment chemistry and arcellaceans were also analyzed from a long sediment core taken with the PP corer close to the stream mouth (Figure 1). The core extended 366 cm and was divided into 17 continuous subsamples based on changes in sediment appearance. Eleven of these subsamples were used for chemical determinations between 0–192 cm and ten were selected for analyses of arcellaceans (0–292 cm).

2.3. Determination of arcellaceans

Weighed subsamples for micropaleontological determinations ($\sim$1 g fresh weight) were first sieved using distilled water and a 1000 μm sieve was used to remove coarse organic matter. Subsequently, a 56 μm sieve was used to retain arcellaceans while removing clay and silt-sized particles. In all sievings, care was taken not to mechanically break the tests. The smaller sieve mesh size is in the size range often used in thecamoebian research (30–63 μm; Beyens and Meisterfeld, 2001). However, smaller thecamoebians do exist (down to 10 μm; Beyens and Meisterfeld, 2001) and these will be lost during sieving, causing systematic bias in the results. After sieving, the samples were divided into eight portions with a wet splitter, as described by Scott and Hermelin (1993). The samples so obtained were studied immersioned in water with Nikon SMZ-1B and Wild M3Z stereomicroscopes with zoom ranges of $8 \times -35\times$ and $6.4 \times -80\times$, respectively. In most cases, at least 250 arcellaceans were identified (234–384), with the exception of the lowermost, clay-like sample of the sediment profile (272–292 cm), where no intact arcellaceans were found.

A total of 27 arcellacean species and strains were identified from the samples using Medioli and Scott (1983) as the reference for the species. The identification of *Lesquereusia spiralis* is based on Reinhardt *et al.* (1998) and this and other references (e.g., Asioli *et al.*, 1996) were used to name the local arcellacean strains encountered. Authors for the species also follow Medioli and Scott (1983) and Reinhardt *et al.* (1998).

2.4. Chemical analyses

Samples for chemical analyses were stored at $-20\,^{\circ}$C in the laboratory before freeze-drying. The dried samples were homogenized for chemical determinations with ICP-AES from ammonium acetate and nitric acid leachates and determinations of S, C and N using a Leco analyzer (S) and a CN analyzer. Sample pH was determined potentiometrically in a 1 M KCl solution.

The 1M ammonium acetate solution extracts chemically adsorbed elements from solid surfaces (Al_a, Ba_a..., subscript a = adsorbed). The solution was buffered to pH 4.5 and the solid:solution ratio was 1:60, a ratio that extracts nearly the maximum amount of ions adsorbed to solid surfaces (Räisänen and Carlson, unpublished material). Depending on the sample type, this 2 h extraction liberates elements with cation exchange capacity and those complexed on solid surfaces and dissolves carbonates (excluding magnesite) and hydroxide precipitates such as poorly crystalline ferrihydrite.

In the nitric acid leach (US EPA method 3051; US EPA, 1994), samples are dissolved in a closed Teflon® container in a microwave oven. The extraction breaks down trioctahedral micas (e.g., biotite), (most of) talc, 2:1 and 1:1 clay minerals, sulfides, carbonates (magnesite), titanite and most salts such as apatite. The extraction does not dissolve quartz, feldspars, amphiboles or pyroxenes unless these are weathered. In addition, etching of fresh mineral surfaces liberates some elements such as Ca, Na and K. The concentrations of adsorbed elements were subtracted from the nitric acid leached concentrations to give an estimate of element concentrations in the crystalline phase (Al_c, Ba_c ..., c = crystalline).

TABLE I

Factors 1–3, derived using top-bottom concentration ratios. E.v. = explained variation(%), loadings between −0.5 and 0.5 are not shown

	Factor 1	Factor 2	Factor 3
Al_{aR}	0.80		
Ca_{aR}	0.63	0.61	
Co_{aR}	0.76		
Cr_{aR}	0.78		
Cu_{aR}	−0.54		0.69
Fe_{aR}	0.76		
Mn_{aR}		−0.92	
Ni_{aR}	0.88		
S_{aR}	0.83		
V_{aR}	0.72		
Ba_{cR}		−0.58	0.64
Co_{cR}			0.90
Cu_{cR}		0.77	
Mn_{cR}		−0.71	
Ni_{cR}	0.74		
S_{cR}	0.81		
Zn_{cR}	0.84		
e.v. %	60	15	8

2.5. Statistical methods

Results of the geochemical analyses were studied using factor analysis of top-bottom concentration ratios. Ratios were used because the aim was to investigate the mine water derived pollution, that is, the changes in sediment concentrations due to impacts of mining. Ratios of top (c. 0–5 cm) and bottom (c. 20–30 cm) sample concentrations were calculated for all analyzed elements and all sampling sites except RET 6 for which no unpolluted bottom sample was available (e.g., $Al_{aR} = Al_{a\ top}/Al_{a\ bottom}$ . . ., R = ratio). To reduce the number of the concentration ratios, which exceeded the number of cases, only those in which a marked top-bottom difference was observed were included (coefficient of variation at least 0.54: Al_{aR}, Ca_{aR}, Co_{aR}, Cr_{aR}, Cu_{aR}, Fe_{aR}, Mn_{aR}, Ni_{aR}, S_{aR}, V_{aR}, Ba_{cR}, Co_{cR}, Cu_{cR}, Mn_{cR}, Ni_{cR}, S_{cR} and Zn_{cR}; Table I). Of these, Co_{aR}, Cu_{aR}, Fe_{aR}, and Mn_{cR} were log transformed prior to factor analysis because of their skewed distributions. The factor scores of the samples (first three factors) were further used in a hierarchical classification analysis employing the between groups linkage method. This grouping of samples facilitates group-wise comparisons between the actual concentrations and the concentration ratios (amount of top-bottom change).

The arcellacean species data were summarized using multivariate statistical methods. The species compositions of both the surface sediment samples and the sediment profile were presented as principal components analysis (PCA) ordination plots generated using the CANOCO 4 WIN software of ter Braak and Šmilauer (1998). The linear-based method of PCA was chosen because of the short gradient lengths in both species data sets. Similarly, the influence of the measured environmental variables on arcellaceans of the surface sediment samples was studied with redundancy analysis (RDA) instead of unimodal response-based methods. The ordinations were performed on percentage species data because the use of abundances resulted in ordinations that solely reflected the large variations in the numbers of individuals in the samples. The RET 12 surface sediment sample, taken from water deeper than the rest of the samples, was entered as a passive sample in the ordinations. Monte Carlo-based tests of significance, which are available in CANOCO, were used to study the ordinations produced when the different environmental variables were supplied in the RDA. For sediment chemical variables, employed as environmental variables, the ammonium acetate leached concentrations were used because these were considered to be the most biologically relevant of the fractions available. Environmental variables were $\log_{10}(x + 1)$ transformed prior to analyses to bring their numerical values to the same order of magnitude and to reduce the skewness in the distributions. In addition to ordinations, the PAST software of Hammer *et al.* (2001) was used to calculate Shannon diversity measures from the arcellacean species data (e.g. Patterson *et al.*, 2002; Boudreau *et al.*, 2005).

3. Results

3.1. Surface Sediment Chemistry

Major spatial trends in the chemical environment affecting sedimentary arcellaceans were investigated by grouping the coring sites according to their factor scores (F1, F2 and F3), derived using top-bottom concentration ratios. Table I shows the factors F1–F3 and Table II lists the geochemical groups (1–4; group 5 consisted of RET 28 only and was excluded) showing group medians of the factor scores as well as selected median concentrations. The results are presented as a map in Figure 2, showing the distribution of the geochemical groups in the bay. Sites that belong to group 1 (triangles) are located in the deep area of the bay while those of group 2 (boxes) are shallower but close to the Myllyoja stream mouth. Sites from groups 3 (circles) and 4 (stars) are located further away from the source of pollution.

Sulphur and chalcophilic metals have the highest loadings on F1 (Table I). Comparisons of the F1 loadings with median concentrations within the groups show that the top sediment samples in the deep areas of the bay (group 1) are enriched with S and metals (Table II). Of these, Ni, Zn and S are found both in the adsorbed and crystalline phases. In contrast, the loadings of Cu_{aR} on F1 are

TABLE II

Properties of the geochemical groups 1–4: medians of factor scores and selected median concentrations (top and bottom). _a = adsorbed, _c = crystalline

	Group 1		Group 2		Group 3		Group 4	
Factor 1	1.37		−0.72		−0.71		−0.59	
Factor 2	0.49		0.94		0.00		−0.78	
Factor 3	−0.30		−0.01		2.11		−1.02	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Al_a	4390	1190	2080	1210	1360	1650	1020	1170
Ba_a	144	210	126	268	206	273	195	169
Ca_a	11400	2550	5390	1590	5350	1840	2230	1580
Co_a	172	19.1	113	19	94.2	20.2	65.7	17.2
Cr_a	2.98	1	1.80	1.35	1	1.14	1	1.21
Cu_a	1	1.40	7.61	1.02	28.40	1.67	1.90	1.71
Fe_a	52500	20700	18700	16000	8360	19900	6470	5780
Mn_a	2930	4050	2110	4420	5620	4050	6460	1870
Ni_a	464	9.3	133	14.4	151	21.8	53.7	14.6
S_a	4410	604	1010	348	465	296	343	96.5
V_a	24.2	25.1	11.9	20.3	2	22	8.9	18.8
Zn_a	297	33	397	35.4	500	54.8	137	43.6
Al_c	22330	21651	22620	20650	21090	22020	23080	25230
Ba_c	85.6	148	145	157	266	140	136	133
Ca_c	4200	4440	4870	3720	3920	3730	3830	4030
Co_c	165	20.1	142.9	21	436	26.6	82.2	26.6
Cr_c	51.65	48.4	60.97	45.71	48.3	44.39	45.7	50.2
Cu_c	230	26.7	205.4	25.9	132.6	27.3	49.2	29.9
Fe_c	69400	61600	141500	69200	189560	103700	136220	99960
Mn_c	760	1320	2360	1330	11630	1410	3660	1140
Ni_c	339	33.9	199	36.7	260	41.9	86.9	42
S_c	27320	2357	2570	1852	1035	1504	1910	1537
V_c	58.4	70.4	78.7	63.6	81.7	68.3	85.5	75.1
Zn_c	1738	119.1	588	121.2	700	160.2	326	173.3
C_leco	15.2	7.97	10	6.85	8.25	6.36	7.6	7.29
pH	6.15	5.36	5.51	5.20	5.66	5.10	5.56	5.02
Eh	−178	−283	−153	−326	67	−324	−175	−290
n.	9	9	9	9	3	3	9	9

negative because in the deep area (group 1), top sediment samples have lower Cu_a concentrations than the bottom samples. However, Cu concentrations in the crystalline phase (Cu_c) are higher in the top samples than in the bottom samples.

Factor 2 sample scores are highest at the inner bay sites, close to the Myllypuro stream mouth (geochemical groups 1 and 2; Table II). For individual elements, Mn has the highest negative loadings on F2. The top-bottom ratio of Ba bound to the

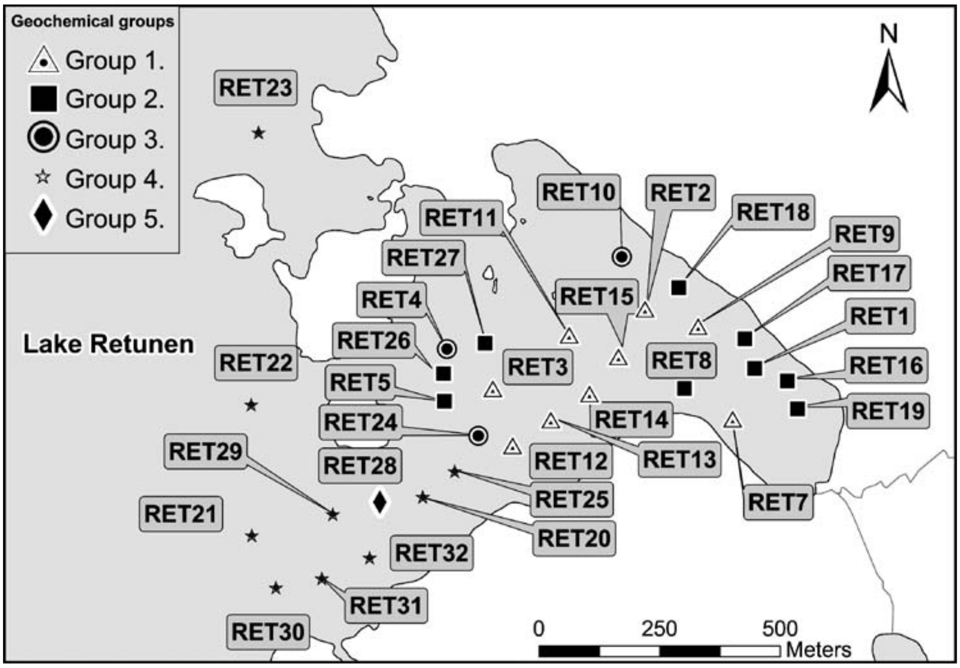

Figure 2. Spatial distribution of the geochemical groups in Petkellahti Bay. No group was assigned to RET 6 because of the polluted bottom sample.

crystalline phase (Ba_{cR}) also has negative loadings on F2 while those of Cu_{aR} and Cu_{cR} are positive. Mean concentrations show that Mn is depleted in top sediments at group 1 and 2 sites but is enriched at sites belonging to groups 3 and 4 (Table II). In contrast, highest Cu_a and Cu_c concentrations are found close to the stream mouth in group 1 and 2 samples.

C and Fe were not included in the factor analysis but as major elements are important in sediment chemistry. The high proportions of C in groups 1 and 2, located close to the source of pollution, are noteworthy. The proportion of labile Fe_a is high in group 1 samples (43%) but the total concentration of Fe is not elevated in the top subsamples (Table II). In contrast, Fe is mostly found as Fe_c in group 3 and 4 samples, further away from both the stream mouth and the deep area, with low proportions of Fe_a(4%).

In addition to describing the overall chemical conditions and gradients in the area, conditions at the sampling sites for arcellaceans were studied in more detail. The ammonium acetate-leached (‘bioavailable’) concentrations in these shallow water top sediment samples parallel the trends observed using top-bottom concentration ratios for all sampling sites. Figure 3 shows the ammonium acetate leached concentrations of selected elements and the Fe:Mn ratio in the arcellacean samples arranged with increasing distance from the source of pollution. The value of the

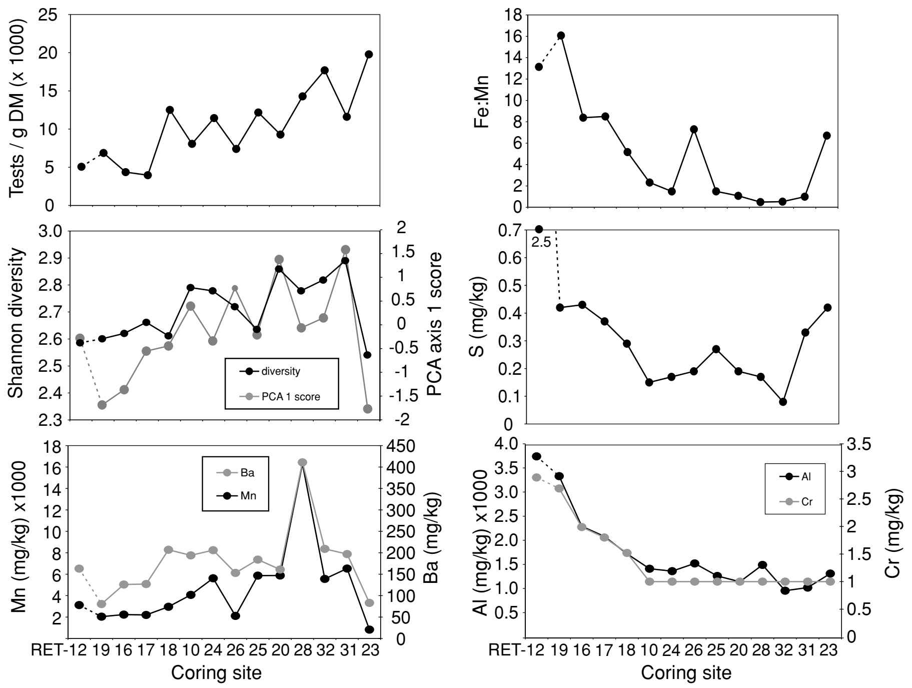

Figure 3. Features of the arcellacen faunas, Fe:Mn ratio and ammonium acetate-leached concentrations of selected elements in the top sediment arcellacean samples, arranged with increasing distance from the source of pollution.

Fe:Mn ratio, a widely used indicator of reducing conditions, decreases outwards in the bay, as do the concentrations of S, Al_a and Cr_a. In contrast, as already suggested by their negative loadings on F2, top sediment concentrations of Mn_a and Ba_a are highest further away from the Myllypuro stream mouth.

3.2. Chemistry of the sediment profile

Temporal changes in loading were investigated with a down-core study. Geochemistry of the sediment profile, which was taken close to the Myllyoja stream mouth, changes above the clay marker layer in the 29–48 cm sample (Figure 4). Deeper in the profile, concentration levels fluctuate slightly following changes in sediment quality (organic content) but above the clay layer at 49–48 cm, HNO_3 and ammonium acetate–leached concentrations of elements such as Zn, Ni and Cu increase. S_{leco} concentrations and sediment organic content are also high between 48 and 13 cm but decline again in the two topmost samples. The 13–9 cm sample contains another thin clay layer (see Figure 4) and concentrations of Cr, Cu, Co and Ni, for example, increase in this mineral-rich layer or immediately above it (analysis results from a thinly sectioned short core are not shown). In the core, Cr is highly

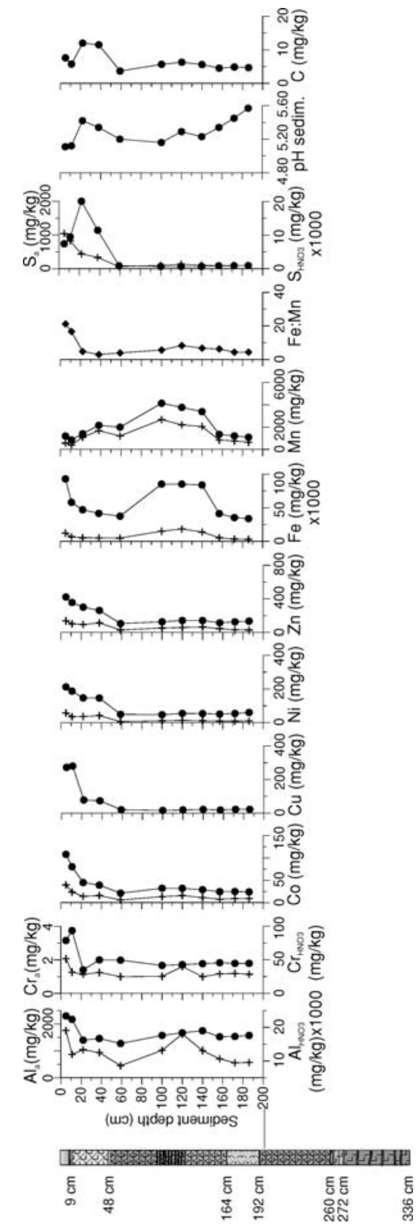

Figure 4. Distribution of selected elements in top 192 cm of the long sediment core. Crosses = ammonium acetate leaches (*x* axis on top), dots = HNO_3 leaches.

correlated with Ti, an element associated with mineral matter, and negatively correlated with C. In addition, a peak in ^{137}Cs activity was found in the core between 10 and 8 cm (max 225 Bq kg^{-1}), suggesting that sediments above 8 cm have been deposited after the 1986 Chernobyl nuclear accident.

S_{leco} concentrations are high in sediments deposited during the operation of the mine with the highest concentrations between 48 and 13 cm. Metal concentrations are also high in this section, but the highest concentrations of Co, Cu, Ni and Zn are measured in the uppermost section (above 9 cm), deposited after mine closure. Sulphate (S_a) concentrations are also high in this top sediment unit, sediment pH is low and the concentrations of metals related to fine-grained mineral matter (Cr, K and Mg) increase towards the sediment surface. In contrast, the easily mobilized Mn and Ba are depleted in the topmost section.

3.3. Arcellaceans

A total of 14 arcellacean species were identified from the Lake Retunen samples and with the strains the total number of forms is 27 (Table III). Several strains of *Difflugia oblonga, D. protaeiformis* and *Centropyxis aculeata* were identified. Strains have been found to convey useful information on aquatic subenvironments whereas variation in species alone may not allow inferences on environmental factors such as low oxygen levels, organic matter and pollution levels (Asioli *et al*., 1996; Reinhardt *et al*., 1998; Patterson and Kumar, 2000b).

Fifteen arcellacean forms were commonly encountered, including *Centropyxis aculeata* (Ehrenberg, 1830) 'aculeata', *Difflugia tricuspis* (Carter, 1856), *Difflugia globulus* (Ehrenberg, 1848) and *Difflugia protaeiformis* (Lamarck, 1816). From the latter, four strains were identified, three of which are based on Asioli *et al*. (1996): *'protaeiformis'*, *'crassa'* and *'rapa'*. The fourth strain (*Difflugia protaeiformis* 'strain A'), resembles strain 'protaeiformis', but has 2–3 spines at the end of fundus. Several strains of the commonly occurring *Difflugia oblonga* (Ehrenberg, 1832) were also identified, mostly according to Reinhardt *et al*. (1998): 'oblonga', 'linearis', 'tenuis', 'bryophila', 'glans' and 'lanceolata'. The last two of these occurred in low numbers, however. In addition, *Difflugia oblonga* 'strain A' is a small, short-necked and coarse form of *D. oblonga* . Other common strains, not identified to species level, include *Difflugia* strain A, *Difflugia* strain B and Test A.

Other arcellacean taxa that occurred regularly in the samples but in lower numbers included *Difflugia urceolata* (Carter, 1864), *Lagenodifflugia vas* (Leidy, 1874), *Lesquereusia spiralis* (Ehrenberg, 1840), *Pontigulasia compressa* (Carter, 1864) and *Centropyxis constricta* (Ehrenberg, 1843). Less common were tests of *Difflugia bidens* (Pénard, 1902), *Difflugia corona* (Wallich, 1864), *Difflugia fragosa* (Hempel, 1898) and *Arcella vulgaris* (Ehrenberg, 1832).

The most abundant arcellaceans in the inner bay samples (sites RET19-RET10 and RET 12; Figures 1 and 5) include *D. protaeiformis* 'protaeiformis', *D. oblonga* 'oblonga' and *D. tricuspis* . The proportion of *D. tricuspis* decreases steadily towards the outer bay, but the species is abundant at the deeper RET12 site. Also, the abundance of *D. protaeiformis* 'protaeiformis' decreases with increasing distance from the stream mouth but, unlike *D. tricuspis* , is lowest at RET12. *D. oblonga* 'oblonga' becomes more abundant outwards in the bay while

TABLE III

Arcellacean species and strains identified from the Petkellahti samples. Taxon codes are used in the ordination plots (Figure 7)

Taxon	Author/description	Code
Arcella vulgaris	Ehrenberg, 1832	*ARCEvulg*
Centropyxis aculeata	Ehrenberg, 1830	*CENTacul*
'discoides'		*CENTacuD*
Centropyxis constricta	Ehrenberg, 1843	*CENTcons*
Difflugia		
'Strain A'	short neck, rounded fundus, large clasts might cover the shape	*DIFFstrA*
'Strain B'	shape pyriform, clear neck absent, fundus not so rounded	*DIFFstrB*
Difflugia bidens	Pénard, 1902	*DIFFbide*
Difflugia corona	Wallich, 1864	*DIFFcoro*
Difflugia fragosa	Hempel, 1898	*DIFFfrag*
Difflugia globulus	Ehrenberg, 1848	*DIFFglob*
Difflugia oblonga	Ehrenberg, 1832	
'Strain A'	small, short-necked and coarse	*DIFFobSA*
'bryophila'	variably flask-shaped test characterized by relatively large clasts	*DIFFoblB*
'glans'	test ovoid with rounded fundus	*DIFFoblG*
'lanceolata'	elongated test, long neck	*DIFFobLa*
'linearis'	test flask-shaped with tiny aperture	*DIFFobLi*
'oblonga'	elongated to oblong with simple aperture	*DIFFoblO*
'tenuis'	similar to 'oblonga', except that the neck is absent and the fundus is almost subconical	*DIFFoblT*
Difflugia protaeiformis	Lamarck, 1816	
'crassa'	test shorter and somewhat globose	*DIFFproC*
'protaeiformis'	cylindrical test, narrow and elongated	*DIFFproP*
'rapa'	test smaller and globose	*DIFFproR*
'strain A'	resembles strain 'protaeiformis', but has 2–3 spines at the end of fundus	*DIFFprSA*
Difflugia tricuspis	Carter, 1856	*DIFFtric*
Difflugia urceolata	Carter, 1864	*DIFFurce*
Lagenodifflugia vas	Leidy, 1874	*LAGEvas*
Lesquereusia spiralis	Ehrenberg, 1840	*LESQspir*
Pontigulasia compressa	Carter, 1864	*PONTcomp*
Test A	relatively small, simple cylindrical test, neck absent, fundus rounded	*TESTspeA*

TABLE IV

Percentage of explained variation in arcellacean assemblage data for variables with statistically significant marginal effects on surface sediment arcellaceans. The $\lambda_{axis1}/\lambda_{axis2}$ ratio is also shown as well as the explained variation with the associated *p*-value when the other variables were used as covariables in the (partial) analysis

Variable	% explained	*p*-value	$\lambda1/\lambda2$	% expld partial	*p*-value partial
Fe:Mn	22.1	0.010	0.80	17.5	0.020
Al	20.6	0.010	0.81	16.4	0.030
Cr	19.2	0.025	0.71	6.3	0.385
S_{leco}	19.0	0.035	0.67	4.5	0.625
Mn	18.3	0.020	0.64	17.4	0.025
Ba	15.9	0.045	0.54	9.2	0.160

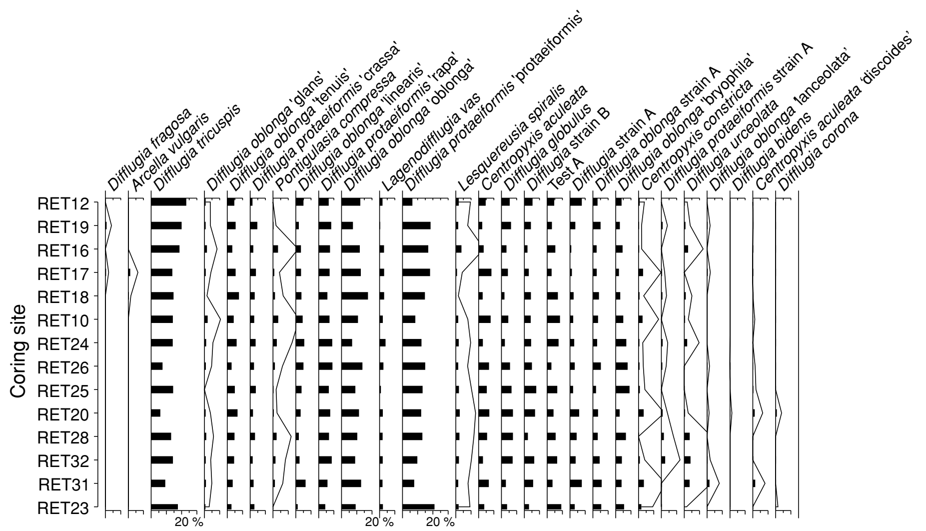

Figure 5. Distribution of arcellaceans in top sediment samples arranged with increasing distance from the Myllypuro stream mouth.

the abundances of centropyxids fluctuate and is most abundant in RET17 and RET10.

The relative abundance of *D. protaeiformis* 'protaeiformis' diminishes only slightly in samples from the shallow water area south of the islands (RET24-RET20, Figures 1 and 5) compared to the inner bay sites. In contrast, *D. oblonga* 'bryophila' is more abundant than in the inner bay samples and the fluctuations in *C. aculeata* 'aculeata' stabilize. Moderate amounts of the otherwise rarely occurring *C. aculeata* 'discoides' and *Difflugia corona* were encountered in RET20. In RET28-RET31, *Difflugia urceolata* increases markedly and *D. protaeiformis* 'strain A' is abundant in RET28 and RET32. The changes are minor, however, compared to RET23, which was taken outside of the bay in somewhat deeper water (7.2 m). At this site, *D. protaeiformis* 'protaeiformis' increases markedly as does *D. tricuspis*, while the proportions of *D. oblonga* 'oblonga' and *D. protaeiformis* 'rapa' decrease.

Overall, both the total number of arcellaceans and the Shannon diversity in the samples increase towards the outer bay (Figure 3). Nevertheless, the total number of arcellacean strains identified is ∼20 at all sites. RET23 is a notable exception to the increasing trend in species diversity. In this 'background' sample, species composition is similar to samples taken close to the Myllypuro stream mouth and the Shannon diversity is low but the number of individuals is high at this 'reference' site.

In the sediment profile, the most prominent changes in arcellaceans are concentrated in the four uppermost samples. The most notable feature is the decrease in the abundances of *D. oblonga* 'oblonga' , *D. oblonga* 'linearis' and *Difflugia* strain A + B (Figure 6) with associated increases in *Lesquereusia spiralis* and the strains of *D. protaeiformis* . In the topmost sample (0–9 cm), *D. tricuspis* increases markedly while *Centropyxis constricta* and *Lesquereusia spiralis*, both species that increased from the 48–29 cm sample upwards, decline.

3.4. Ordination

Figure 7 shows a PCA biplot of arcellacean species and surface sediment samples with an inset depicting trends in the values of selected environmental variables.

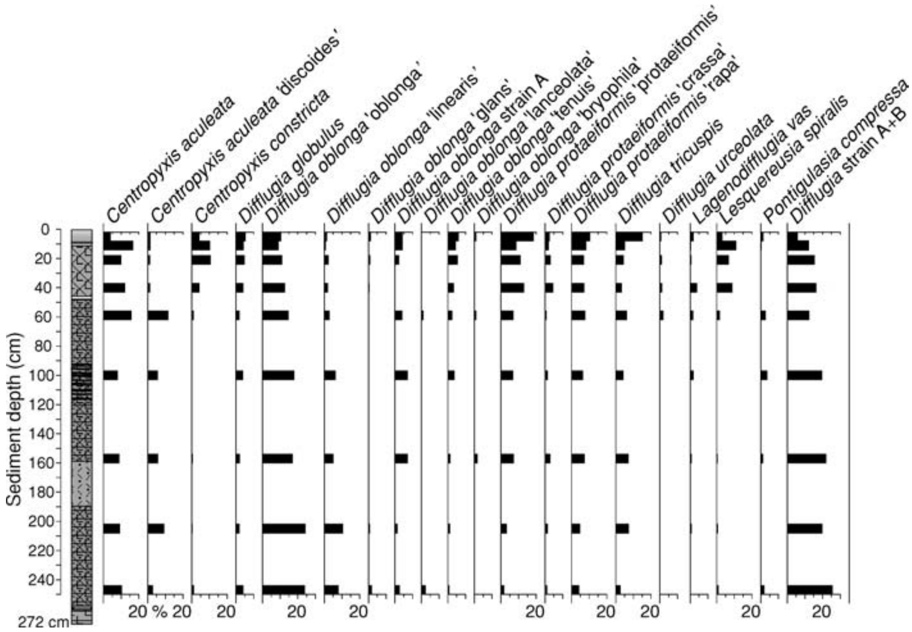

Figure 6. Distribution of arcellaceans in the long sediment core.

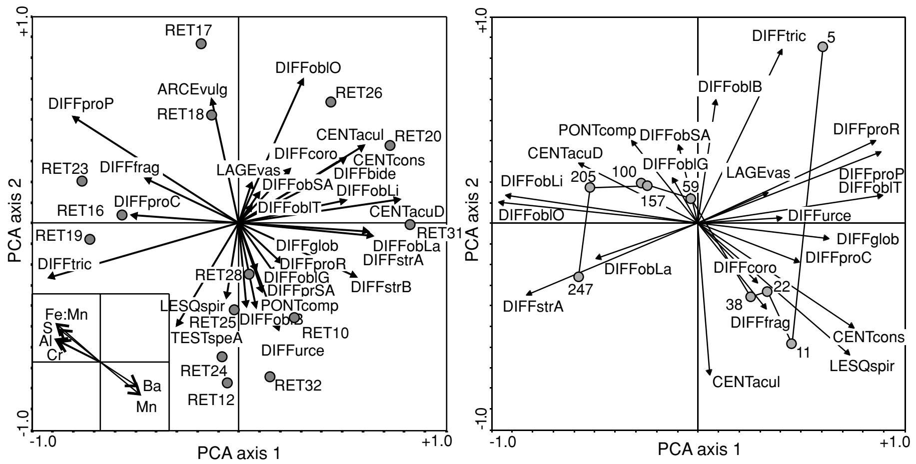

Figure 7. PCA plots of arcellacean species compositions in top sediment (left) and long core (right) samples. The inset shows trends in environmental variables selected based on their effect on arcellaceans (see text). For species codes see Table III.

Samples closest to the inlet of Myllypuro stream, the pathway of mine waters to the bay, cluster on the left side of the diagram, mainly in the upper left quadrant while samples from the outer bay plot in the lower right quadrant. The species arrows show that relatively few species and strains have their highest abundances in the RET 16, 17, 18 and 19 samples, located in the inner bay: *Difflugia tricuspis, Difflugia protaeiformis* “crassa”, *Difflugia fragosa* and *Arcella vulgaris*. In contrast, samples from other areas contain more arcellacean species and have high abundances of species such as *Centropyxis aculeata, C. aculeata* “discoides”, *Difflugia oblonga* “linearis” *and D. oblonga* “lanceolata”. When samples are arranged with increasing distance from Myllypuro stream, their PCA axis 1 scores generally increase, indicating a relatively systematic change in the arcellacean species composition (Figure 3). However, certain samples that plot on the right hand side of the PCA diagram contain high abundances of other strains, inducing variation in the arcellacean faunas of the outer bay samples. In addition, the reference sample taken outside of the bay (RET-23) plots together with the inner bay samples because of its high relative abundance of *Difflugia tricuspis* and *Difflugia protaeiformis* “protaeiformis”.

A PCA biplot of samples and species (Figure 7, right panel) summarizes the changes in arcellacean species composition in the sediment profile (Figure 6). In general, the samples change from high relative abundances of species and strains such as *Difflugia oblonga* “oblonga”, *D. oblonga* “lanceolata” and *Difflugia* strain A to high proportions of *Difflugia oblonga* “tenuis”, *D. protaeiformis* “protaeiformis”, *D. protaeiformis* “rapa”, *D. globulifera* and *Centropyxis constricta* . Superimposed on this general trend is an increase in the percentages of *Lesquereusia spiralis*, *Centropyxis constricta*, *C. aculeata* and *Difflugia fragosa* in the metal-rich 38, 22 and 11 cm samples, followed by a major increase in the relative abundance of *Difflugia tricuspis* in the uppermost sample (5 cm). Due to this latter shift in species abundances, the arcellacean species composition of the topmost sample of the core resembles those of the other surface sediments in the bay.

3.5. Relationships between environmental variables and arcellaceans

Multivariate statistical methods were used to study the relationships between environmental variables and arcellacean faunas. The inset in Figure 7 shows the trends in the values of selected geochemical variables in the surface sediment samples, superimposed on an ordination based solely on the arcellacean species compositions and with RET-12 entered as a passive sample. The variables shown in the figure are those six that produced a statistically significant ordination at the 5% level when supplied as sole environmental variables in RDA ('marginal effects'). In decreasing order of explained variance, these variables include: Fe:Mn ratio, Al, Cr, S_{Leco}, Mn, and Ba. The concentrations of Cr, Al and S as well as the Fe:Mn ratio decrease away from the source of pollution while the concentrations of Ba and Mn increase (Figure 3). However, the trends in the selected environmental variables do not completely coincide with the major trend in arcellacean species compositions, for example, the first PCA axis. Of the variables that did not explain variation in arcellaceans statistically significantly, water depth at the coring site appears to vary in a constant manner in the ordination diagram, with deeper sites in the upper part of the plot. This was despite the attempt to standardize the coring site water depth for the arcellacean samples.

With several environmental variables that showed statistically significant marginal effects on arcellaceans, an attempt was made to further reduce the number of variables using forward selection. In this procedure, environmental variables are added to the model one by one until no improvement is seen when adding further variables (ter Braak and Šmilauer, 1998). The purpose is to select a smaller set of variables that represents the effective environmental gradients equally well as the original variables. In the reduced data set, the six environmental variables are all correlated (see Figure 7) so that forward selection was completed with only a single variable included (significance tested with Monte Carlo resampling). However, three variables (Fe:Mn, Al and Mn) yielded a statistically significant constrained axis in an RDA with the other variables entered as covariables in the analysis, suggesting that there is an independent signal for them in the arcellacean species compositions. Fe:Mn and Al also had high ratios of explained versus residual variation ($\lambda 1/\lambda 2$) when used as single constraining variables in RDA (Table IV).

In the sediment profile, with a low number of arcellacean-chemistry sample pairs, the relationships between arcellacean faunas and chemical concentrations were studied with Pearson correlations using PCA sample scores to represent arcellacean faunas. The major direction of variation in arcellacean species compositions (PCA axis 1 scores) was significantly correlated (at the 5% level) with ammonium acetate leached concentrations of Co, Cu, Ni, Zn and S. In contrast, no significant correlations were found for axis 2 scores, which are mainly related to the recent major shift in species composition.

4. Discussion

4.1. Geochemical gradients in the study area

Surface sediment metal concentrations indicate that mine waters affect the whole Petkellahti Bay area. Several dispersion mechanisms appear to be involved in the formation of the spatial pattern of contamination. For instance, the thick contaminated sediment layers near the stream mouth (48 cm, Figure 4) suggest that clastic dispersion, or transport of metal-rich mineral particles has been important in this area. Vertical changes in the sediment profile indicate that this mode of dispersion was most intensive during the operation of the mine whereas after mine closure, grain size diminished and sedimentation shifted towards hydromorphic dispersal of colloids, sulphate and other fine-grained precipitates. This has resulted in higher sedimentary concentrations of metals such as Co, Cu, Ni and Zn but Mn and Ba have become soluble and depleted in the topmost sediments. Sediment sorting and focusing occurs in conjunction with these modes of dispersion so that most of the maximum metal concentrations were measured in the deep areas of the bay (geochemical group 1).

In addition to clastic and hydromorphic dispersal, metals are transported in a dissolved phase. This is seen in the distribution of Mn as the metal dissolves and moves outwards from the deep areas of the bay (group 1 sites), precipitating at shallower sites (sites of groups 3 and 4, see also Figure 4). Dissolution of Mn together with binding of calcophilic elements in the sulphide phase and a slight rise in pH, all point to sulphate reduction in the deep water areas.

Besides the Eh gradient between deep and shallow areas of the bay, a horizontal pH-Eh gradient is observed close to the stream mouth, as seen by the distribution of the Fe:Mn ratio and in the depletion of Mn and Ba and lowered pH at the top of the sediment profile. The pH gradient may be the result of mixing and dilution of the low-pH water of Myllyoja stream as it enters the lake while the low redox potential in the inner bay can be caused by organic carbon in the stream water and reduction of the abundant sulphate from the mining area.

4.2. Factors affecting arcellaceans

Surface sediment arcellacean species compositions change systematically with increasing distance from the inlet of Myllyoja stream. Amoeba concentrations change in a comparable manner, a feature that is only partly explained by the high matrix deposition rate close to the stream inlet. Species diversity (Shannon) in the samples also increases away from the source of mining wastewaters. Therefore, it appears that the present mine water inputs have an effect on arcellaceans.

Of the six variables with significant marginal effects on surface sediment arcellacean species compositions in RDA (Mn, Fe:Mn, Al, Cr, S_{leco}, Ba), three remained significant even with the other variables entered as covariables in the analysis (Mn, Fe:Mn, Al). The relationship between arcellaceans and Fe or Mn concentrations is not a direct one, because the concentrations of Fe_a and Mn_a in the top sediments are similar to those of uncontaminated sediments. However, the Fe:Mn ratio reflects changes in pH and Eh, indicating that variations in biota may be linked to processes in solution. A similar pattern is seen in the sediment profile where arcellacean species compositions change markedly in the upper part, in samples where Ba and Mn have solubilized because of low pH or redox potential. Oxygen depletion and low pH may have a direct effect on arcellaceans (Dalby *et al*., 2000; Beyens and Meisterfeld, 2001) but these changes in faunas may also be related to differences in metal bioavailability caused by pH and redox conditions.

While sediment pH had no statistically significant relationship with the surface sediment arcellacean faunas, the low pH of the mine waters entering the bay has likely had a direct or indirect effect on arcellaceans. Parallel to the findings of Kumar and Patterson (2000) in the mine water polluted James Lake in Canada, *Arcella vulgaris* is abundant in the impacted RET 17 and RET 18 samples from Petkellahti whereas centropyxids occur more abundantly further away from the source of pollution. However, in contrast to James Lake, *Difflugia protaeiformis* ‘protaeiformis’ is abundant at the impacted sites of the present study, a finding that is in accordance with Asioli *et al*. (1996) from Lake Orta, Italy. The association of this species with low pH may also explain its high abundance in the ‘background’ sample (RET 23), which had the lowest measured sediment pH of 5.0. Furthermore, centropyxid species, known to be able to withstand high metal concentrations unless the pH is too low (below 5.5 or even 6.2; Patterson and Kumar, 2002), occur in lowest relative abundances in sites located close to the source of pollution.

Although none of the heavy metals related to the Luikonlahti mine (Cu_a, Co_a, Ni_a and Zn_a) significantly explained variation in surface sediment arcellacean faunas, Al_a appeared to vary systematically with arcellacean species compositions. Toxicity of mobile Al to aquatic life is well known, especially due to the acid rain debate and such a mechanism is also possible here. Alternatively, very fine particle size Al precipitates (hydroxides and sulphates) may be transported long distances, making surface sediment Al concentrations a good indicator of the spatial extent of present mine water impacts in the area.

Changes in chemistry and biota in the long sediment core indicate that the nature of contamination and its impacts have been altered after mine closure. Mine waters apparently became more acid and rich in sulphate and metal-bearing colloids with the shift to drainage from the weathering waste rock and tailings piles (acid mine drainage). This is essentially the present type of pollution, represented by the surface sediment samples. However, earlier mine water pollution appears to have affected arcellaceans as well. In this case, the mining-related heavy metals may have played a role because ammonium acetate leached concentrations of Co, Cu, Ni, Zn and S in the sediment core were significantly correlated with the major direction of variation in arcellacean species composition. This most important change in arcellaceans is the shift from pre-mining species compositions to those observed in samples deposited during the operation of the mine.

5. Conclusions

The geochemical results indicate that mining wastewaters have affected all of Petkellahti Bay. The spatial pattern of contamination and the geochemical gradients in the area result from several different mechanisms: dispersal and dilution of metal-rich, low-pH stream waters that enter the lake; transport and focusing of fine grained metal precipitates and sulphate to the deep areas of the bay; increase of pH due to sulphate reduction in the deep areas; mobilization of redox or pH-sensitive elements from sites located close to the source of pollution or in deep water and precipitation of the mobilized metals at shallow sites in the outer bay. In addition, the nature of the pollution appears to have changed over time from predominantly clastic dispersion during the operation of the mine towards smaller particle size (colloids) and lower pH after mine closure. All of these geochemical features have been involved in creating the environmental conditions that affect arcellaceans in the study area.

Top sediment arcellacean species compositions, species diversity and numbers of individuals all change in a systematic manner with increasing distance from the source of pollution. The statistically significant effect of Mn and Fe:Mn on arcellaceans suggests that Eh and pH conditions affect biota in the area, either directly or by modulating metal bioavailability. Distributions of certain individual arcellacean species further point to the effects of pH, as does the 'polluted' species composition at the reference site with the lowest measured sediment pH of 5.0. Thus, species compositions similar to those of the most impacted sediments in the bay can also occur in sites that were presumably unaffected by mining activities.

Of the individual metals, ammonium acetate-leached Al concentrations were significantly related to top sediment arcellacean species compositions. The toxicity of Al to aquatic life is well known, but the relationship may also result from Al being an especially good indicator of the spatial extent of pollution in the area due to the transport of fine-grained Al precipitates. The relationships between major variations in arcellaceans faunas and Co, Cu, Ni, Zn and S concentrations in the sediment profile suggest that metal loading had an effect on arcellaceans during the operation of the mine. Overall, the results suggest that sedimentary arcellaceans are a group of organisms with strong potential in studies of industrial pollution.

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