TESTATE AMOEBAE (THECAMOEBIANS) AS INDICATORS OF AQUATIC MINE IMPACT

by

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ACADEMIC DISSERTATION

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Front cover: The tower and tailings of the Pyhäsalmi mine:
A view from Lake Pyhäjärvi. Photo: Susanna Kihlman, GTK.

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ABSTRACT

The environmental impacts of a single mine often remain local, but acidic and metal-rich acid mine drainage (AMD) from the waste materials may pose a serious threat to adjacent surface waters and their ecosystems. Testate amoebae (thecamoebian) analysis was used together with lake sediment geochemistry to study and evaluate the ecological effects of sulphidic metal mines on aquatic environments. Three different mines were included in the study: Luikonlahti Cu-mine in Kaavi, eastern Finland, Haveri Cu-Au mine in Ylöjärvi, southern Finland and Pyhäsalmi Zn-Cu-S mine in Pyhäjärvi, central Finland. Luikonlahti and Haveri are closed mines, but Pyhäsalmi is still operating. The sampling strategy was case specific, and planned to provide a representative sediment sample series to define natural background conditions, to detect spatial and temporal variations in mine impacts, to evaluate the possible recovery after the peak contamination, and to distinguish the effects of other environmental factors from the mining impacts. In the Haveri case, diatom analyses were performed alongside thecamoebian analysis to evaluate the similarities and differences between the two proxies. The results of the analyses were investigated with multivariate methods (direct and indirect ordinations, diversity and distance measure indices). Finally, the results of each case study were harmonized, pooled, and jointly analyzed to summarize the results for this dissertation.

Geochemical results showed broadly similar temporal patterns in each case. Concentrations of ions in the pre-disturbance samples defined the natural baseline against which other results were compared. The beginning of the mining activities had only minor impacts on sediment geochemistry, mainly appearing as an increased clastic input into the lakes at Haveri and Pyhäsalmi. The active mining phase was followed by the metallic contamination and, subsequently, by the most recent change towards decreased but still elevated metal concentrations in the sediments. Because of the delay in the oxidation of waste material and formation of AMD, the most intense, but transient metal contamination phase occurred in the post-mining period at Luikonlahti and Haveri. At Pyhäsalmi, the highest metal contamination preceded effluent mitigation actions. Spatial gradients were observed besides the temporal evolution in both the pre-disturbance and mine-impacted samples from Luikonlahti and Pyhäsalmi. The geochemical gradients varied with distance from the main source of contaminants (dispersion and dilution) and with water depth (redox and pH). The spatial extent of the highest metal contamination associated with these mines remained rather limited. At Haveri, the metallic impact was widespread, with the upstream site in another lake basin found to be contaminated.

Changes in thecamoebian assemblages corresponded well with the geochemical results. Despite some differences, the general features and ecological responses of the faunal assemblages were rather similar in each lake. Constantly abundant strains of *Difflugia oblonga, Difflugia protaeiformis* and centropyxids formed the core of these assemblages. Increasing proportions of *Cucurbitella tricuspis* towards the surface samples were found in all of the cases. The results affirmed the indicator value of some already known indicator forms, but such as *C. tricuspis* and higher nutrient levels, but also elicited possible new ones such as *D. oblonga* 'spinosa' and clayey substrate, high conductivity and/or alkalinity, *D. protaeiformis* 'multicornis' and pH, water hardness and the amount of clastic material and *Centropyxis constricta* 'aerophila' and high metal and S concentrations. In each case, eutrophication appeared to be the most important environ-

mental factor, masking the effects of other variables. Faunal responses to high metal inputs in sediments remained minor, but were nevertheless detectable. Besides the trophic state of the lake, numerical methods suggested overall geochemical conditions (pH, redox) to be the most important factor at Luikonlahti, whereas the Haveri results showed the clearest connection between metals and amoebae. At Pyhäsalmi, the strongest relationships were found between Ca- and S-rich present loading, redox conditions and substrate composition.

Sediment geochemistry and testate amoeba analysis proved to be a suitable combination of methods to detect and describe the aquatic mine impacts in each specific case, to evaluate recovery and to differentiate between the effects of different anthropogenic and natural environmental factors. It was also suggested that aquatic mine impacts can be significantly mitigated by careful design and after-care of the waste facilities, especially by reducing and preventing AMD. The case-specific approach is nevertheless necessary because of the unique characteristics of each mine and variations in the environmental background conditions.

Keywords (GeoRef Thesaurus, AGI): environmental geology, lakes, pollution, acid mine drainage, lake sediments, geochemistry, metals, Arcellacea, Thecamoeba, diatoms, Kaavi, Ylöjärvi, Pyhäjärvi, Finland

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CONTENTS

LIST OF ORIGINAL ARTICLES

This dissertation is based on following articles, which are referred to in the text by their Roman numerals.

PI Kauppila, T., Kihlman, S. & Mäkinen, J. 2006. Distribution of arcellaceans (testate amoebae) in the sediments of a mine water impacted bay in Lake Retunen, Finland. Water Air and Soil Pollution 172, 337−358.

PII Kihlman, S. M. & Kauppila, T. 2008. Mine water-induced gradients in sediment metals and arcellacean assemblages in a boreal freshwater bay (Petkellahti, Finland). Journal of Paleolimnology 42, 533−550.

PIII Kihlman, S. & Kauppila, T. 2010. Tracking the aquatic impacts of a historical metal mine using lacustrine protists and diatom algae. Mine Water and the Environment 29, 116−134.

PIV Kihlman, S. & Kauppila, T. 2012. Effects of mining on testate amoebae in a Finnish lake. Journal of Paleolimnology 47, 1−15.

In addition to the original papers, this dissertation includes previously unpublished material analyzed by the author.

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AUTHOR'S CONTRIBUTION TO THE PUBLICATIONS

PI
The study was planned by Tommi Kauppila. Fieldwork and sampling were carried out by Tommi Kauppila, Jari Mäkinen and Ari Parviainen. Susanna Kihlman performed the thecamoebian analysis and Tommi Kauppila the data analysis. Jari Mäkinen performed the geochemical factor analysis. Tommi Kauppila, Susanna Kihlman and Jari Mäkinen interpreted the results and wrote the article.

PII
The study was planned by Tommi Kauppila and Susanna Kihlman. Fieldwork and sampling were carried out by Tommi Kauppila and Susanna Kihlman with the assistance of Ari Parviainen and Jari Mäkinen. Susanna Kihlman performed the thecamoebian analysis. Susanna Kihlman and Tommi Kauppila performed the data analysis. Susanna Kihlman and Tommi Kauppila interpreted the results and wrote the article.

PIII
The study was planned by Tommi Kauppila. Fieldwork and sampling were carried out by Tommi Kauppila and Mikael Englund. Susanna Kihlman performed the thecamoebian analysis and Tommi Kauppila performed the diatom analysis. Susanna Kihlman and Tommi Kauppila interpreted the results and wrote the article.

PIV
The study was planned by Tommi Kauppila and Susanna Kihlman. Fieldwork and sampling were carried out by Susanna Kihlman with the assistance of Kari Savolainen and Jari Mäkinen. Susanna Kihlman performed the thecamoebian analysis and the data analysis. Susanna Kihlman and Tommi Kauppila interpreted the results and wrote the article.

1 INTRODUCTION

Mining always affects its surrounding environment to some degree, but the effects of an individual mine may remain rather local. The intensity of mine impacts depends on many different factors such as the characteristics of the ore, production methods, management actions, size, geometry, location and the characteristics of the surrounding environment (Lottermoser 2007). Lately, awareness of the assessment, management and mitigation of these environmental issues has increased (e.g. Ripley et al. 1996, BRGM 2001, Lottermoser 2007, Heikkinen et al. 2008, European Commission 2009).

The most relevant mine related environmental pressure at the moment is probably metal-rich acid mine drainage (AMD) (e.g. Wolkersdorfer & Bowell 2004, 2005a, b) and its potential impact on surface waters and the biota. In Europe, the need to develop environmental assessment is now even more essential, because of the EU Water Framework Directive (WFD) (European Commission 2000), which aims to achieve a good chemical and ecological status for surface waters when compared to the natural reference state before significant anthropogenic influence. There is also increasing interest in further exploiting the ore deposits in Fennoscandia, possibly posing more environmental threats to watercourses. Many of these deposits are situated in areas where mining has already ceased, and because of the exposure to historical emissions that have affected the water chemistry and ecosystems, these areas are especially challenging for environmental assessment. The social aspect of aquatic mine impacts should also be taken into consideration, especially in Finland, where we have many old closed metal mines, many of them left unmanaged, and many surface water systems that have locally significant roles in the water supply and as areas for recreational activities (e.g. fishing).

The nature of mine loading and the bioavailability of the contaminants are affected by many physical, chemical and biological factors and processes in different phases. Furthermore, the unique characteristics of each mine and its surrounding environment make it important to understand and define the pre-disturbance environmental conditions, and evaluate the aquatic impact case specifically. Because of this complexity, new methods are needed to assess, evaluate, and monitor the environmental effects of mining and management actions.

1.1 Mine impacts on the aquatic environment

1.1.1 Acid mine drainage (AMD)

Sulphidic mine waste repositories, i.e. tailings and waste rock piles, are often the main sources of AMD. AMD may start after waste deposition has ceased and sulphide minerals in the material become exposed to oxidation and water, which are the main factors in the generation of AMD besides the mineralogy of the waste material. However, physical attributes such as the grain size and temperature of the source material also affect the intensity of AMD.

Sulphides are stable under strongly reducing conditions, but when they are exposed to oxidizing conditions they destabilize, leading to a series of complex chemical weathering reactions. While some of the mineral-weathering processes are acid producing, i.e. releasing hydrogen ions (the oxidation of sulphides), others are acid buffering or neutral (reactions with gangue minerals such as carbonates and silicates or exchangeable cations) (Lottermoser 2007). The precipitation and dissolution of secondary minerals also affect the pH in both directions, but the neutralizing effects

often remain temporary. The balance between these chemical reactions determines whether the material will produce acidic or neutral discharge. Sulphide oxidation is an autocatalytic reaction, and once AMD generation has started, it can be very difficult to stop and it can continue for hundreds of years (Price 2003). Low pH waters liberate heavy metals (e.g. Fe, Cu, Pb, Zn, Cd, Co, Cr, Ni, Hg) and sulphates and additionally accelerate the leaching and release of other elements. Neutral mine drainage, however, can also be harmful and enriched with heavy metals (Heikkinen et al. 2009).

The unique characteristics of each mine lead to high variation of effluent quality between sites, but the quality may also vary spatially within the same source site (Heikkinen et al. 2009). However, despite the possible spatial variation, the final drainage waters from a sulphidic waste facility mostly represent a mixture of fluids from within the heterogenic pile, although different rates of solubility and weathering reactions of minerals can cause temporal chemical variation in the discharge (Lottermoser 2007).

1.1.2 Aquatic impacts of AMD

Once transported to the surface water system, mine-derived compounds and elements do not automatically affect the aquatic biota and do not necessarily cause damage to the environment. Differentiating pollution from contamination requires information on bioavailability and toxicity in addition to simple chemical analyses of concentrations (Chapman 2007). Contamination can be determined as a situation when some substance is present either in an environment where it should not be, or with elevated concentrations when compared to the background values. Despite the possible high concentration of contaminants, elements may not be in a bioavailable form or taken up by organisms, and thus not have adverse effects on biota. Even if the contaminants are in a bioavailable form, they do not necessarily lead to toxicity, because many metals are essential for life and are required by organisms. On the other hand, because of bioaccumulation (the accumulation of substances in an organism) and biomagnification (the increase in concentration of a substance in a food chain), even small concentrations in the environment can cause problems. The response of receptors (e.g. thecamoebians) to stressors (e.g. metals) depends on time and the route of exposure. Through these dose-response relationships, a number of effects can be studied on different organizational levels (e.g. population). A dose-response curve, a simple X-Y graph plotting the receptor and level of exposure (e.g. concentration of pollutant) on the same graph, can be used to determine the threshold dose. The threshold dose is the point when the response of the organism is above zero. However, mines are also often situated in geochemically anomalous areas, in which biota may be adapted to these diverging environmental conditions. This can mean improved tolerance and a raised threshold for an ecological response.

AMD is a multifactor pollutant and have both indirect and direct effects on the ecosystem (Gray 1997). Both the acidity and the released heavy metals may be harmful to aquatic life in their own right, and often their effects are not distinguishable. Metal mobility and availability to aquatic organisms is a complex issue controlled by speciation and diverse pathways. These factors are dependent on many interrelated chemical (i.e. precipitation, adsorption, solution reactions), biological, and environmental (advection, dilution, dispersion, sedimentation) processes, but also vary in relation to the exposed biota and its characteristics (Salomons 1995, Chapman et al. 2003). Major variables affecting these processes are pH, alkalinity, cation composition, anions and the dissolved organic content (Salomons 1995). The metallic mine impact finally accumulates and concentrates in aquatic sediments. The ecotoxicology of sedimentary metals in this ecologically important compartment is dependent on sediment–metal binding and release, which are again dependent on the chemical phases (acid volatile sulphides, particulate organic carbon, Fe and Mn oxyhydroxides), complexation by ligands, and oxidation (Chapman et al. 1998).

1.1.3 Lake sediment studies in the evaluation of aquatic mine impacts

Lake sediment studies provide a useful tool to evaluate and monitor the impacts of a mine on aquatic biota by offering an archive of the deposited material from periods for which other records do not necessarily exist. Down-core sediment studies with combined chemical and palaeoecological analyses make it possible to track the series of changes that can be linked to the evolution of metal inputs from the mine and its environment (Cattaneo et al. 2004, Couillard et al. 2004, Laperriere et al. 2008, Parviainen et al. 2012) and be used to evaluate system recovery when mitigation steps are taken (Tropea et al. 2010). They are very useful in establishing reference conditions and restoration targets for lakes (Bennion et al. 2011, Kauppila et al. 2012), and also offer the possibil-

ity to define site-specific background conditions for such anomalous environments as mining areas (Parviainen et al. 2012). For example, elevated concentrations in pre-mining lake sediments may result from the geochemistry of local bedrock and till (Mäkinen et al. 2009).

Geochemical signals work well as a proxy for the quality of mine effluent (Couillard et al. 2004, Parviainen et al. 2012), but they are not always solely sufficient to evaluate mine impacts, because geochemical profiles may also change after deposition. However, the use of fossilizable biological proxies to complement the chemical results changes this. In the case of the Hitura mine, Finland, diatoms still recorded the known reductions in mine water loading, while the geochemical record had been changed by post-depositional mobility of elements (Kauppila 2006). Alteration of geochemical profiles may originate from physical and biological mixing of the sediment and from post-depositional diagenetic mobility and its effects (e.g. redox reactions) on elements (Farmer 1991, Boyle 2001a). Element solubility is affected by the oxidation state, and some elements with several oxidation states, such as Fe, Mn, As, Co, Cr, and V, are more sensitive to redox-related changes and migrate easily with gradients in redox conditions (Boudreau 1999). In contrast, many of the common mine-induced contaminants, such as Cu, Cd, Pb and Zn, have only one oxidation state in lake sediments. They are also likely to migrate with changes in metal binding phases, but this is found significant only at extremely low sediment mass accumulation rates (Boyle 2001b). However, even when metals are relatively stable and bound to particles, their distribution in the sediment column does not represent a detailed historical record of inputs, because the mixing in the surficial zone smooths the record to some degree (Boudreau 1999).

Nevertheless, the geochemistry of aquatic sediments is an important proxy for metal loading, especially when defining the baseline for mine impact studies. However, in order to evaluate the actual pollution impact of the metal contamination, the use of fossil remains such as testate amoebae or diatoms is crucial. Used together, these methods not only reconstruct the lake history, but also help to distinguish natural perturbations from anthropogenic ones.

1.2 Testate amoebae as environmental indicators

Thecamoebians (testate amoebae) are a taxonomically artificial group of unicellular, mainly freshwater protozoans. The group includes forms from 2–3 classes and 2–3 orders, but only a small fraction of the order Arcellinida appears to be common in the fossil state in lacustrine sediments, so the fossils are often referred to as arcellaceans. They live in benthic environments in all types of freshwater bodies (i.e. lakes, rivers, ponds) and in a variety of sufficiently moist habitats such as mosses, soils and tree bark, but a few forms also tolerate brackish conditions. Thecamoebians reproduce every 2–11 days, mainly by asexual binary fission to form a replicate of the parent cell (Ogden & Hedley 1980). They mainly feed on bacteria, algae and fungi, but some forms are thought to prey on other protozoa. Even though found worldwide from polar regions (Beyens et al. 1995, Dallimore et al. 2000, Mattheeussen et al. 2005) to tropical environments (Dalby et al. 2000, Roe & Patterson 2006), there is still some uncertainty concerning the cosmopolitanism and ubiquitous nature of these organisms, mostly because of the wide size range of the species and usage of morphotypes, and excessive splitting of taxa (Mitchell et al. 2008). An important part of the effective, passive dispersal of thecamoebians is their ability to encyst and thus survive unfavourable environmental conditions such as desiccation, freezing and a lack of food or oxygen (Ogden & Hedley 1980, Dallimore et al. 2000).

Thecamoebians have a soft amoeboid cell, and to protect it they form simple sac-, or cap- like tests by secreting a siliceous, proteinaceous or calcareous shell autogenous test) or by agglutinating foreign particles such as mineral grains and diatoms glued with mucopolysaccharides (xenogenous test) (Patterson & Kumar 2000a). The tests can also be a mixture of the two types. After the original division and building, the test does not grow and the organism is unable to repair it if it becomes damaged (Scott et al. 2001). In lacustrine settings, most of the fossilized tests are agglutinated, and the nature of these glued particles (xenosomes) appears to be linked to the quality of the local substrate and thus the availability of different materials for building the test (Medioli & Scott 1983). Identification of fossilized specimens is based on the morphology of the only preserved part of the organism, the shell. This inevitably leaves out some taxonomically significant information about the pseudopodia, the flowing extension of the cytoplasm passing through the shell aperture of the living organism. To complicate matters further, thecamoebian populations are highly variable because of asexual reproduction,

which has led to serious taxonomic confusion and probable oversplitting of the species during the years. Despite the long history of thecamoebians (Medioli et al. 1990, Fiorini et al. 2007, van Hengstum et al. 2007, Bassi et al. 2008), most palaeontological studies of testate amoebae focus on the Quaternary and especially on the Holocene, where they are used as palaeobioindicators in lakes and peatlands (Charman 2001, Patterson & Kumar 2002).

In peatlands, thecamoebian studies have mainly concentrated on various environmental, palaeohydrological and -climatic studies (Tolonen 1986, Warner & Charman 1994, Booth 2002, 2008). Thecamoebians have also been used to reconstruct sea-level changes in salt marshes (e.g. Scott & Medioli 1978, Gehrels et al. 2001, Charman et al. 2002, Roe et al. 2002) and for flood characterization in fluvial environments (Medioli & Brooks 2003). In lacustrine environments, testate amoebae have been used in various palaeolimnological and palaeoclimatic studies. They have served as a proxy for different environmental factors, including land-use change (Patterson et al. 2002, Reinhardt et al. 2005), eutrophication and chemical fertilizers (e.g. Scott & Medioli 1983, Patterson et al. 1985, Medioli & Scott 1988, Boudreau et al. 2005, Reinhardt et al. 2005), lake water pH (Ellison 1995, Escobar et al. 2008), and other natural, climatic and human-induced environmental changes (e.g. McCarthy et al. 1995, Burbidge & Schröder-Adams 1998, Dallimore et al. 2000, Torigai et al. 2000, Boudreau et al. 2005, McCarthy et al. 2012). Some research has concentrated on their biogeographical distribution (Collins et al. 1990, Neville et al. 2010b).

Testate amoebae have additionally been shown to be good indicators of urban, industrial and mine-derived pollution and lake-bottom acidity (Asioli et al. 1996, Patterson et al. 1996, Reinhardt et al. 1998, Kumar & Patterson 2000, Patterson & Kumar 2000a, b, Roe et al. 2010). Recently, they have also been used to assess the rehabilitation and reclamation success of constructed wetlands in oil sands (Neville et al. 2011). Not only are certain species able to thrive in these stressed environments, often with low pH and high metal loading, but there are also infraspecific strains distinguishing sub-environments not detected at the species level (Asioli et al. 1996, Reinhardt et al. 1998). These taxonomically informal strains, morphotypes within the species population, have developed in response to various environmental features and can thus be considered as ecophenotypic variants. The living habitat in the water–sediment interface, the high reproduction rate (Ogden & Hedley 1980), small size and usually high abundances enables the use of very small, thin sediment samples.

These features, together with their environmental sensitivity, make thecamoebians good, rapidly responding (Neville et al. 2010a) environmental indicators suitable for high-resolution studies (Reinhardt et al. 1998). This possibility for high-resolution sampling also enables studies tracking the effectiveness of recent remediation efforts and the recovery of polluted environments (Patterson et al. 1996). In addition, compared to other proxies such as molluscs, thecamoebian tests preserve well, even in the very low pH environments (Swindles & Roe 2007) often associated with mining. Sedimentary remains are also well suited to distinguishing the spatial distributions of thecamoebians and defining differences between different areas of the lake bottom (Scott & Medioli 1983).

1.3 Objectives of the study

The objective of this dissertation is to integrate the results of four case studies of three different sulphidic metal mines investigating the impacts of mining and other anthropogenic and natural events on aquatic environments. Each case study was individually planned and local conditions were carefully considered.

Aims for the individual studies were as follows:

1. The first study of the Luikonlahti Cu-mine aimed to use surface sediment sample chemistry to describe the overall geochemical environment affecting biota in the embayment next to the mine, to examine the effects of the observed environmental gradients on thecamoebian species composition, and to identify the most important environmental variables by employing multivariate numerical methods.
2. The second study of Luikonlahti was built on previous results, and a temporal dimension was added to the spatial one. Two short sediment cores and deeper 'bottom' samples corresponding to the surface sample transect were analyzed to represent the time before the mine impact. Known changes in the production mode of the facility offered an opportunity to investigate the environmental impacts of different mine production phases. The aims were

to identify the natural and mine-impacted geochemical and faunal gradients in the bay area, to examine and compare temporal changes in the sediment cores and to identify possible causes of faunal changes using numerical methods where applicable.

3. A study of the closed Haveri Cu-Au mine aimed to determine the characteristics, timing and extent of different mine water inputs to the lake based on geochemical analyses of two sediment profiles and to compare the responses of thecamoebians and diatoms to observed mine water contamination. Numerical methods were used to separate the effects of different environmental variables such as metals, arsenic, pH and nutrients.
4. A study of the Pyhäsalmi Zn-Cu mine aimed to track the mine-induced spatial and temporal gradients of metals and thecamoebians, to identify the relationships between geochemical variables and palaeoecological results, and to study the possible recovery of the biota in two lake basins next to the mine. Multivariate methods were used to evaluate site-specific impacts of the mine on thecamoebians to distinguish between environmental factors affecting the faunal assemblages

In this synopsis, the geochemical and palaeoecological results of these four individual studies are analyzed together to detect and assess possible congruencies between the case studies, to identify the factors affecting the common features, and to develop methods for mine-site specific evaluation of the aquatic mine impacts. Besides the further development of the use of testate amoebae as mine impact indicators, it also aims to gather general information on these organisms and their distribution in mine-impacted environments in Finland.

2 MATERIALS AND METHODS

2.1 Study sites and sampling

Three diverse sulphidic mines in Finland were studied to assess their environmental impacts on aquatic biota (Fig.1). The general sampling strategy at each site was to provide a sample series to detect any possible mine impacts, variations in their spatial and temporal extent and distinguish them from the effects of other environmental variables. Case-specific sampling strategies were planned to take into account features such as the nature and timing of mining, which were slightly different at each site. Three types of corers were used: the Limnos gravity corer in PI, PII, PIV (Kansanen et al. 1991), a Kullenberg type of piston corer in PI (Putkinen & Saarelainen 1998) and a Kajak-type gravity corer in PIII (Renberg & Hansson 2008). In papers II and III, sediment cores were dated with the 1986 Chernobyl 137Cs fallout peaks, often detectable in Finland; otherwise, we used mine-related geochemical proxies for relative dating. Limnological measurements and lake physiography data found in the following site descriptions are taken from the OIVA database of environmental and geographical information, 30.5.2012. The database is maintained by Finland's environmental administration.

2.1.1 Luikonlahti (PI, PII)

The Luikonlahti mine is a Cu-Co-Ni-Zn mine in Kaavi, eastern Finland (62°56'N, 28°42'E) on the shore of Petkellahti Bay, a part of Lake Retunen (Fig. 1). The lake area covers 264,851 ha with an average depth of 4.9 m and a maximum depth of 21.2 m. The catchment of the lake mainly consists of sandy till with some smaller areas of fine-grained material. Chlorophyll–*a* concentrations in Petkellahti bay have fluctuated between 5–20 µg/l following the peak (45 µg/l) in 1990, but now seem to be settled around 5–10 µg/l (2005−2012). According to Organization for Economic Co-Operation and Development (OECD), the chlorophyll–*a* limit of lakes in a eutrophic state is 8 µg/l.

The mine was in operation during 1968–1983, but mining-related activities predate the actual mining period in the area. A total of 7 Mt of ore were extracted during the active period. On average, the ore contained 0.99% Cu, 0.61% Zn, 0.11% Co and 17.22% S (Eskelinen et al. 1983). The processing plant was also used to process talc ore during 1979–2006. Talc processing produced Ni concentrate as a by-product, but the facility has been shut down since 2007. Operation of the mill is planned to start again after renovation in 2012. The Luikonlahti mine is the single most important factor affecting the water quality of the Myllyoja stream, which is the main water source of the bay. Present metal loading is mainly caused by the weathering of sulphide-bearing waste rocks and

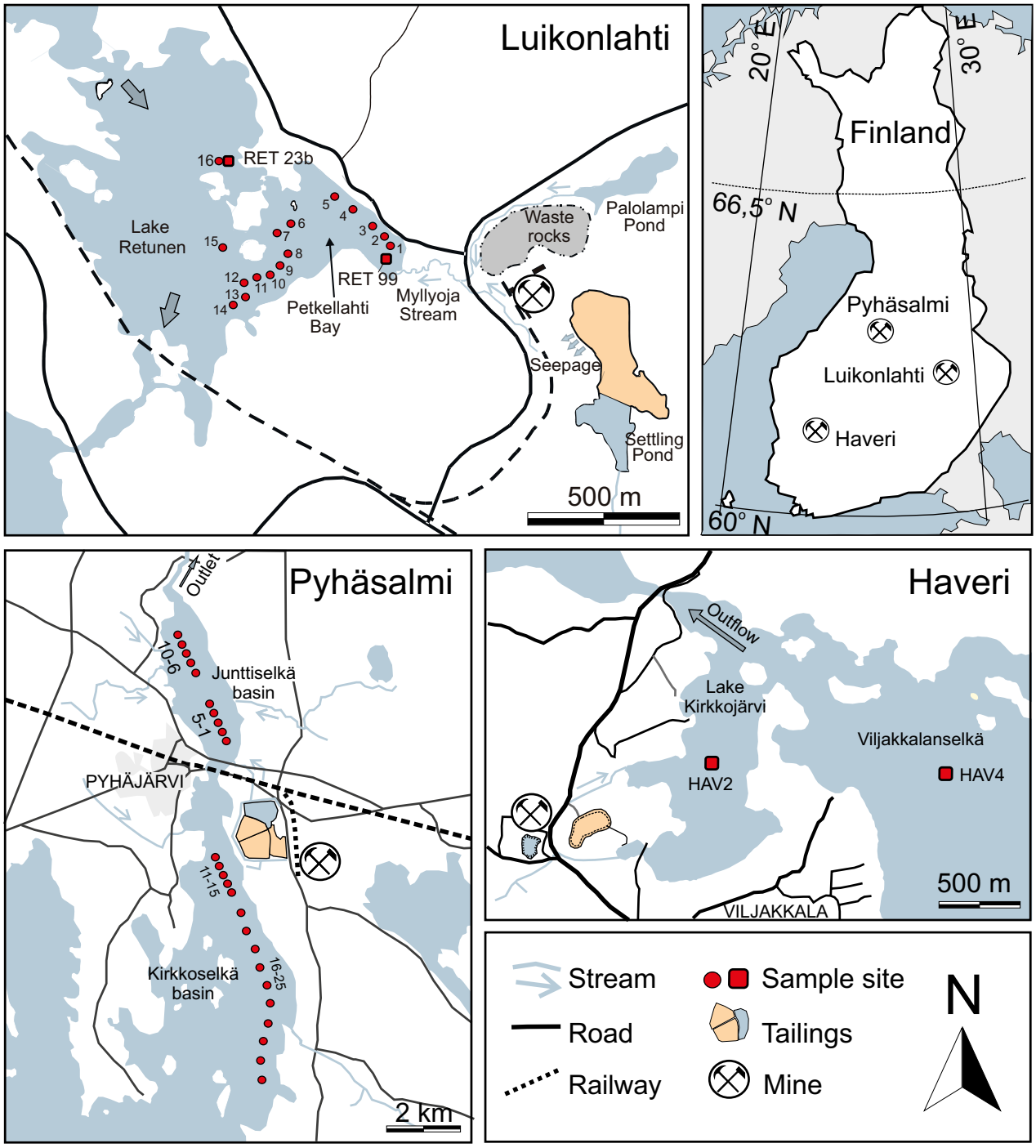


Fig. 1. The locations of Luikonlahti, Haveri and Pyhäsalmi mines in Finland, and the detailed sampling maps of the study sites. Note the two separate basins in Lake Pyhäjärvi, Junttiselkä and Kirkkoselkä, that were sampled near the Pyhäsalmi mine.

low pH seepage waters from the tailings area, but other human activities, e.g. forest management and ditching of the wetlands, have also affected water quality in the bay. Furthermore, because of the local bedrock, glacial tills in the area contain elevated concentrations of S and metals (Koljonen 1992). However, loading from the mine has diminished in recent years.

Geochemical analyses were performed on 32 short sediment cores retrieved from the embayment, and 16 of these were selected for palaeoecological analyses based on water depth at the coring site. Each core was subsampled to give a modern, mine-impacted 'top' sample and a pre-disturbance 'bottom' sample. This approach was used to detect the spatial extent of mine loading in the bay and the possible natural gradients existing before the mine impacts. Samples were selected based on a thin, light mineral layer visible in all cores, which was assumed to mark the onset

of mining, or more specifically the drainage of the Palolampi pond and other land clearance in the area. The depth of the mineral layer varied within the bay, and because recent top samples were taken above it, up to the surface, top sample thicknesses varied with the coring location. The second study (PII), however, showed that the mineral layer dates the end of the mining period instead of the beginning, and the mine-impacted samples thus represent the post-mining period. The 10- cm-thick 'bottom' samples were taken 10 cm below the marker layer in all cores. In addition to this 'top–bottom' transect, one low-resolution sediment core and two short sediment cores with 1-cm sample intervals were retrieved and analyzed to obtain records of the temporal evolution of the mine impacts. One of the short cores was taken from a mine-impacted site and the other was considered as a reference site outside the Petkellahti Bay.

2.1.2 Haveri (PIII)

The closed Haveri Cu-Au-mine is located between two lake basins in Ylöjärvi, SW Finland (61°43'N, 23°14'E). The studied basins, Kirkkojärvi and Viljakkalanselkä (Fig. 1), are small and separated basins of the Kyrösjärvi lake system with a total area of 9606.61 ha. The area is generally rural with cultivated land and forests and the soils mainly consist of fine-grained material (clay, silt). This part of the lake system is currently meso-eutrophic with an epilimnetic total phosphorus (TP) concentration of 20–24 lg/L and a pH of 6.6–7.4, measured at the strait between Lake Kyrösjärvi and Lake Kirkkojärvi in 1996–2002. Trophic level seemed to have elevated from 1992, when total phosphorus in Viljakkalanselkä basin was 14 µg.

The most active period of mining was during 1942–1962, but small-scale Fe mining occurred in the area in the 18th to 19th centuries. Tailings have been piled on a cape protruding into the basin called Lake Kirkkojärvi. The main sulphide minerals in the ore and in the tailings are pyrrhotite, chalcopyrite, magnetite and pyrite, of which pyrrhotite is the most oxidizable and chalcopyrite the most stable. The uppermost layers of the sulphidic tailings have oxidized and produce metal-rich AMD (Parviainen 2009), which affects the stream water quality discharging to the lake.

Two short sediment cores were studied. Kirkkojärvi bay has three gentle sloping depressions, and the coring site HAV2 was situated in the deepest one (7.6 m, diameter ~300 m) in the middle of the bay. It was located near the tailings pile to represent a 'mine-impacted site', while the deeper HAV4 site in the Viljakkalanselkä basin was located in the wide mid-basin with a depth of 22 m, further 'upstream' from the mine, as a reference site. The reference site was also used to evaluate nutrient enrichment in the area and to distinguish its effects from the mine impacts. Both cores were sectioned into continuous slices, HAV2 into 1-cm slices, while HAV4 was sectioned into 1-cm slices down to 10 cm and into 2-cm slices further down. This sampling strategy made it possible to follow the temporal changes in the geochemical nature of the mine loading and the corresponding ecological shifts.

2.1.3 Pyhäsalmi mine (PIV)

The Pyhäsalmi Zn-Cu-S mine is located in the town of Pyhäjärvi, central Finland, on the shore of the relatively large (12178.5 ha) and shallow (mean depth 6.27 m, max depth 27 m) Lake Pyhäjärvi (63° 24 N, 25° 58 E). Two of Lake Pyhäjärvi's lowest basins, Junttiselkä and Kirkkoselkä, were included in the study. The catchment of both basins mainly consists of ditched peatland and cultivated fine grained till. The lowest, smaller and quite closed Junttiselkä basin has higher nutrient and humus concentrations and it has suffered from oxygen depletion and internal loading of metals and nutrients. During 2000–2012, epilimnetic total phosphorus (TP) concentrations in Junttiselkä have fluctuated (12–50 µg/l), but the average is 25 µg/l. In Kirkkoselkä basin, the TP average is 13 µg/l (6–23 µg/l). The same difference in the trophic level of the basins is seen in chlorophyll-*a* concentrations: Junttiselkä 6–24 µg/l (~12 µg/l) and Kirkkoselkä 2–9 µg/l (~5 µg/l). The pH in both basins has been quite stable and neutral, ~6–7, being slightly lower in Junttiselkä.

The mine has been in operation from 1962 and is still under production. Metal-rich mine loading to the lake was at its highest in the 1970s and 1980s, and the main outlet for mine waters was located by the Kirkkoselkä basin next to the tailings. Currently, liming is used to precipitate the metals in the tailings area, which has reduced the metal concentrations of recent effluents. Modern mine loading thus mainly consists of Ca and S, which makes the wastewaters dense and nearly saturated with gypsum ($CaSO_4$). The loading site has also changed from Kirkkoselkä to the Junttiselkä basin.

Sampling was aimed to cover both the temporal and spatial extent of the mine loading in the basins. Short sediment cores were retrieved from 25 sample sites in a transect: 10 'downstream' and 15 'upstream' from the mine (Fig. 1). The sam-

pling interval was 2 cm on each core. Exploratory XRF analysis of Cu and Zn was used to select three sample levels from each core to represent phases immediately before the mine, during peak metal loading and the present situation. These three subsampling levels form stratigraphically correlated temporal horizons of each time period to provide a spatial profile of the mine impacts for each loading phase.

2.2 Geochemical analyses

A microwave-assisted HNO_3 digestion method (3051; US EPA 1994) was used for geochemical analyses on freeze-dried samples in all cases. In addition, the mine-impacted 'top' samples of the Luikonlahti case (PI) were analyzed using 1M ammonium acetate leach. All analyses were performed in accredited testing laboratories of the Geological Survey of Finland (PI, PII) and Labtium Ltd (PIII, PIV). HNO_3 extraction breaks down sulphides, most salts (e.g., apatite), carbonates, trioctahedral micas, 2:1 and 1:1 clay minerals and most of the talc, and provides a record of mine-derived elements. It does not, however, dissolve major silicates such as quartz, feldspars, amphiboles or pyroxenes. The method can extract certain fractions that are not bioavailable and the sediment concentrations should not be interpreted to have been biologically available at the time of deposition. However, the stability of the concentrations makes them more suitable to use as proxies of the mine water loading than those obtained with weaker extractions such as ammonium acetate leach. The 1M ammonium acetate solution used at Luikonlahti (PI) extracts chemically adsorbed elements from solid surfaces. 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. It was considered to be a more biologically relevant fraction, and was thus chosen to represent possible bioaccessible fraction of elements.

For specific element determinations in all case studies, both ICP-MS and ICP-AES were used, depending on the element. Leco and CN analyzers were used to determine sulphur, carbon and nitrogen concentrations. In addition, in the Pyhäsalmi case (PIV), exploratory XRF analysis of Cu and Zn was performed with an X-met® 3000 TXS+ analyzer (Oxford Instruments).

Organic and water contents were measured by loss on ignition. Samples were first weighed, then dried overnight at 105 °C and finally ignited for two hours at 550 °C.

2.3 Thecamoebian analyses

2.3.1 Sample procedures

Thecamoebian analyses were carried out with ~1–3 g samples of fresh weight sediment. The water content of the samples was taken into account when weighting them. The samples were sieved with distilled water through 500 and 56 µm meshes in order to remove coarse organics and silt and clay-sized particles, but to retain testate amoebae. Sieved samples were divided into 8 aliquots using a wet splitter described by Scott and Hermelin (1993) to optimize the number of tests for counting while retaining statistical significance. Most of the time a few samples were enough, but the reference core of Haveri case (PIII), in particular, required systemically more samples, despite the sample sizes being in the upper part of the scale (~3 g). Mechanical stress was avoided so as not to break the tests. The smaller mesh size we used falls in the range often used in thecamoebian research (30–63 µm), but smaller thecamoebians do exist (down to 10 µm; Beyens & Meisterfeld 2001). These were lost during sieving, causing systematic bias in the results in each case. At least ~200–250 specimens per sample were counted immersed in water. In a few samples thecamoebians were not abundant enough, resulting in a lower final count. Specimens were identified and counted using stereomicroscopes: Nikon SMZ-1B 8×−35× (PI), Wild M3Z (6.4–809) (PII) and Olympus SZH (7.5–64) (PII, PIII, PIV). Some specimens from Pyhäjärvi (PIV) were also photographed using a JEOL JSM 5900LV scanning electron microscope.

2.3.2 Identification and the species problem

Identification of thecamoebians was mainly based on the wide cluster mode of classification of Medioli and Scott (1983) (PI, PII, PIII, PIV), and the identification key of Kumar and Dalby (1998) (PII, PIII, PIV), but additional references were used as well (e.g. Asioli et al. 1996 PI,

PII, Reinhardt et al. 1998, Charman et al. 2000, Ogden & Hedley 1980, Leidy 1879). None of the papers were taxonomic in nature, and there was some taxonomic variability in identifications. To clarify this confusion, a taxonomic listing that also describes how the naming and definition of certain forms has evolved from paper to paper is provided (Appendix 1) together with sheets of the raw data (Appendix 2). Raw environmental data is available on request from the author. Most of the variability was associated with the definition of strains of *Difflugia protaeiformis*. Some strains of *Difflugia* species were left unidentified in the Luikonlahti case (PI), but were later identified as strains of *D. oblonga* (PII). Strains of *C. constricta* were not differentiated at Luikonlahti (PI, PII), and in the Pyhäsalmi case (PIV) *Centropyxis constricta* 'spinosa' and 'constricta' were combined.

2.4 Diatom analysis

In the Haveri case (PII), diatom analyses were carried out alongside thecamoebian analysis to examine the similarities and differences between these two proxies. Both proxies were subjected to same unconstrained and constrained (PCA, RDA, see below) multivariate methods. Krammer and Lange-Bertalot (1986, 1988, 1991a, b) were mainly used as a reference for identification. The responses of diatom assemblages to nutrients and pH were studied using inference models developed for total phosphorus (TP) concentrations and pH. TP was reconstructed with the inference model of Kauppila et al. (2002). A detailed description of sample procedures and the inference models used can be found in paper III.

2.5 Numerical methods

2.5.1 Ordinations

Thecamoebian results of each case study were summarized using indirect and direct multivariate statistical methods. All analyses and visualizations were carried out using the CANOCO 4.5 WIN and CanoDraw software package of ter Braak and Šmilauer (2002). Species data were first subjected to exploratory detrended correspondence analysis (DCA) to determine the length of the faunal gradient. On the grounds of this (<2 SD units), the linear-based method of principal components analysis (PCA) was chosen for indirect (unconstrained) ordinations. The corresponding linear-based redundancy analysis method (RDA) was used for direct (constrained) analyses instead of unimodal response-based methods. All ordinations were performed on percentage species data to even out differences between samples and improve their comparability.

Direct-gradient ordinations were used to define the relationships between microfossil assemblages and environmental variables in the data sets. The significances of the constrained axes were tested using a Monte Carlo-based test with 999 permutations, and the transect nature of the samples was taken into account by selecting the time series option and full model for permutations. RDA analyses were used to test both the "marginal" effects (single variables at a time) and "conditional" effects (other variables as co-variables). Environmental variables used in RDA varied according to the case. At Luikonlahti (PI), the ammonium acetate leached concentrations were used for the top sediment samples because these were considered to be the most biologically relevant of the fractions available, but also geochemical top–bottom enrichment factors (EF) of elements that were significantly correlated with the corresponding faunal change (PII).

The PCA axis sample scores from these analyses (i.e., the amount of faunal change) were compared with the concentrations of chemical variables based on linear correlations. Ordinations were constrained to single environmental variables one by one. Sample depth was employed as a co-variable in the analyses at Haveri (PIII) to detect any short-term changes that diverged from the continuous long-term trends.

In the Pyhäsalmi case (PIV), a different approach was used to select the environmental variables for analysis. Metal effects were modelled with summed exceedances of mine- derived metals (Cd, Cu, Pb, Zn) in peak loading samples with marked >100% increases over their respective threshold effect concentrations (TECs) for freshwater sediments. The TECs were based on NOAA Screening Quick Reference Tables (Buchman 2008). The resulting variable (sum metal toxicity) was included as an environmental variable in the RDA model alongside other geochemical variables that were selected to represent sediment quality (C), clastic input (Ti), present loading (Ca), redox conditions (Mn) and eutrophication

(C/N). RDA was constrained for each temporal horizon, but also separately for both basins with all three temporal horizons included.

2.5.2 Other indices

Thecamoebian data sets were also defined by species diversity indices, which were calculated using the PAST program version 1.68 (Hammer et al. 2001). Stressed environmental situations may reduce the diversity of the species composition and lead to the domination of only a few resistant and opportunistic taxa (Patterson & Kumar 2000a). The Shannon index (H), which ranges from 0 for samples with only a single taxon to higher values for samples with many taxa, each represented by few individuals, were used in all papers. A healthy thecamoebian fauna has Shannon diversity index > 2.5 (Patterson & Kumar 2000a). The Berger-Parker dominance index, which gives the proportion of the dominant taxon in the sample, was used in papers II, III and IV. Distance measures were used to study amount of change in faunal compositions. The amount of faunal change between top–bottom sample pairs (PII) and the pre-, peak- and post-sample horizons (PIV) were determined as Euclidean and chord distances calculated by the PAST program version 1.68 (Hammer et al. 2001). The same distance measures were also used in exploratory clustering with the paired group method in papers II and III.

3 RESULTS AND DISCUSSION

3.1 Geochemical gradients in the case studies

Temporal changes in the magnitude and composition of mine water loading were observed in all cases. The temporal geochemical results had similar general features, regardless of the study site in question. At Haveri and Pyhäsalmi (PIII and PIV), the first mine-related changes showed as an increased clastic input into the lakes, with higher concentrations of elements such as Ti, K, Na and Mg. This phase preceded the metallic contamination (PIII) and may be directly related to the beginning of mining and construction of the mine, but could also result from some other change in the regional land use. The mine-impacted sediment core HAV2 from Lake Kirkkojärvi at Haveri (PII) showed clearly increasing concentrations of clastic input-related elements before the metal contamination peaks. This feature was not detectable at the reference site HAV4. Instead, loss on ignition increased profoundly at this stage, probably because of ongoing changes in regional land use and eutrophication. At Pyhäsalmi (PIV), the land use-related changes were observed in a different manner because of the diverging sampling strategy. No sediment cores were analyzed throughout, but samples of the peak loading horizon already had clearly elevated concentrations of clastic input-related elements. In contrast, similar increases in mineral matter inputs were not recorded in the impacted RET99 core next to the Luikonlahti mine (PII). Instead, the onset of mining coincided with a decrease in Ti levels and fluctuation in Mg and Cr levels, if anything. However, S concentrations increased sharply before the highest metal loading (Fig. 2). Furthermore, the water content of the sediment increased, linking these changes to an increase in the organic matter inputs. In these samples, concentrations of mine-related metals also seem to have slightly increased, a feature additionally found in the Haveri sediment cores before the peak loading phase.

A phase of peak metal contamination followed these first geochemical changes at each mine site. In the impacted Haveri core (PIII) it showed as two consecutive metal peaks, with Cu and Ni peaking first and other metals such as Ag, As, and Zn following a few years later. S, Pb, Co and Cd also increased, but not as sharply as the other metals. The same feature was shown in the reference core, but the two peaks were merged because of the thicker subsamples (lower temporal resolution). In the mine-impacted core from Luikonlahti (PII), geochemical features of the peak metal loading were notably different. Co and Cu peaked sharply at the beginning of the 1980s and have since decreased, but not to their background levels. In contrast, Ni and Zn reached their peak concentrations in the top 10 cm of the core. In the Luikonlahti reference core, concentrations of metals and S were highest in the 10 topmost centimetres (PII).

Spatial trends were studied in more detail in the Luikonlahti (PI, PII) and Pyhäsalmi cases (PIV). At Pyhäsalmi, the spatial extent of the highest metal peaks remained limited to a few sites in the Kirkkoselkä basin, close to the tailings area, even though concentrations in the peak loading phase

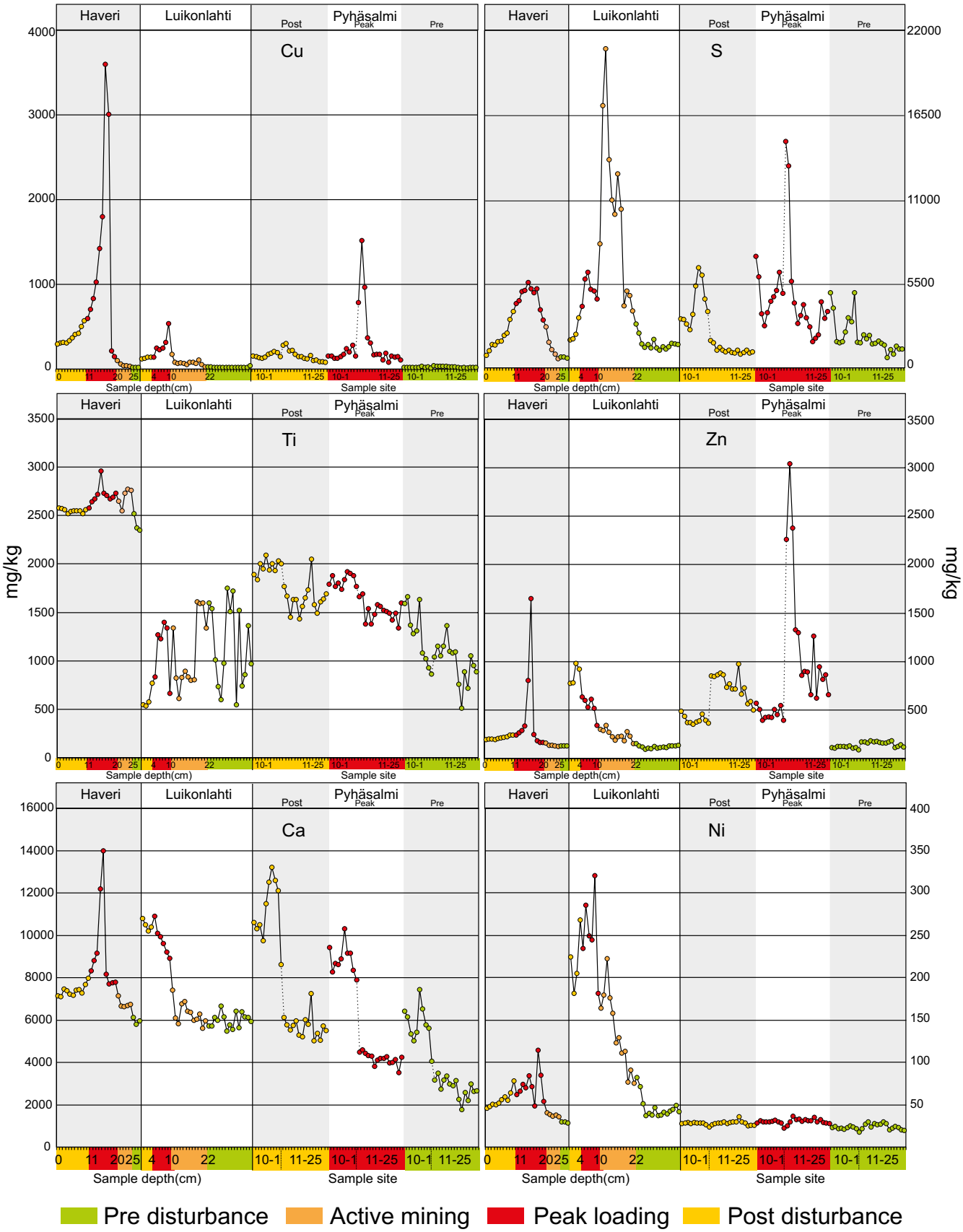


Fig. 2. Geochemical results for various elements (Cu, S, Ti, Zn, Ca and Ni) in the profiles of mine-impacted sediment cores from Haveri and Luikonlahti and Pyhäsalmi sediment sample transects, all placed horizontally. Samples in the sediment profiles of Luikonlahti and Haveri are divided into four groups: pre-disturbance (green), active mining (orange), peak loading (red) and post-disturbance (yellow). Pyhäsalmi samples are in three transect-forming groups: pre-disturbance (green), peak loading (red) and post-disturbance (yellow).

samples were in general higher than in the pre-disturbance samples in both basins under study. At Luikonlahti, the modern top sediment samples illustrated a more complex picture of the elemental distribution in the investigated bay area. The spatial pattern of contamination and the geochemical gradients varied with distance from the source (dispersion and dilution), water depth (redox and pH) and time (from clastic inputs towards colloids and low pH). In the Luikonlahti case, too, it seemed that the strongest metal contamination was confined to the four closest sites from the stream mouth, the source of mine loading.

Despite the similarities, the geochemical fingerprint in the mine-impacted samples (and thus the nature of environmental forcing on biota) was substantially different in each case. Figure 2 points out some of these differences between the case studies. First of all, Ti, an element that has been widely used to represent the mineral matter supply, illustrates the clear difference between the Haveri cores and sediments in the other lakes. This element also clearly illustrates the succession in the Pyhäsalmi case, towards increased clastic sedimentation, but also shows the difference between the two lake basins. Ti concentrations in Luikonlahti sediments were lower but strongly fluctuating, following the concentrations of other mineral matter-related elements (i.e. K, Na, Mg).

There were also major differences in the peak concentrations of sulphur and metals between the mine sites. Peak concentrations of S were several times higher at Luikonlahti than in the other cases. Only some of the peak loading samples from Pyhäsalmi had such high levels of S. In addition, there was considerable variation in the peak concentrations of metals. Zn concentrations were highest in the Pyhäsalmi peak samples, Cu concentrations at Haveri, and the highest Ni concentrations were found at Luikonlahti. At Haveri, the Ni concentrations were somewhat elevated, but at Pyhäsalmi they were constantly low. Some other metals that are not shown in Figure 2 were also distributed unevenly: Pb and Cd peaked in the samples from the Kirkkoselkä basin at Pyhäsalmi and remained elevated after the changes in mine water loading. Co peaked in the Luikonlahti peak contamination samples, while at Pyhäsalmi the elevated Co concentrations were related to redox conditions in the Kirkkoselkä basin rather than to mine-derived contamination.

A PCA on the geochemical variables summarizes the clear division between the sites and samples (Fig. 3). The Haveri samples showed only

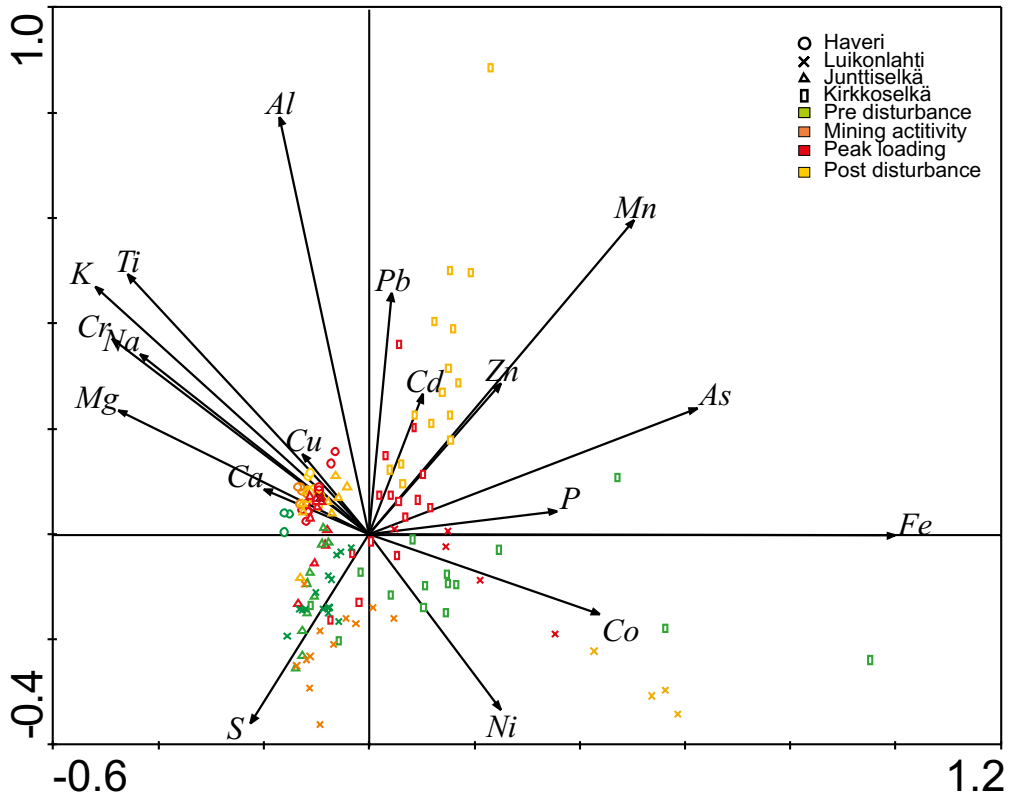


Fig. 3. A PCA diagram of the geochemical distribution of Pyhäsalmi sediment from Junttiselkä and Kirkkoselkä lake basins and the mine-impacted sediment cores of Haveri and Luikonlahti. Haveri and Luikonlahti samples are divided into four groups: pre-disturbance (green), active mining (orange), peak loading (red) and post-disturbance (yellow). Pyhäsalmi transects have three sample horizons: pre, peak and post.

little variation, whereas the Kirkkoselkä samples of the Pyhäsalmi case showed a clear succession along the second axis from the pre-disturbance conditions to the present, post-disturbance situation. The Junttiselkä samples remained in the left half of plot with the temporal horizons mixed together. Only the pre-disturbance samples separated slightly. The Luikonlahti samples are spread along the first axis, but almost every sample plotted in the lower half of the diagram with Co, Ni and S.

3.2 Distribution of thecamoebians

Basic features of the faunal assemblages were quite similar in all of the lakes studied, and an abbreviated taxonomy with the authors or descriptions of found forms is provided in Appendix 1. A large part of the assemblages consisted of different forms of *Difflugia oblonga*, especially in the undisturbed samples and in less environmentally stressed environments such as in the Kirkkoselkä basin in the case of Pyhäsalmi (PIV). In the sediment cores from Luikonlahti (PII) and Haveri (PIII) their proportion decreased towards the surface, mostly because of the increase in the proportion of *Cucurbitella tricuspis*. Despite this, forms of *D. oblonga* were always abundant. The species has been connected gyttja and it has been found to be ubiquitous when the substrate is sufficiently organic (McCarthy et al. 1995). The diverse group of strains of *D. oblonga* could possess an even more specific indicator potential, but the strains are often challenging to distinguish from each other and leave the identifications open to subjective interpretation. In the present studies, at least one of the strains of *D. oblonga* showed clear signs of indicator value (PIV): The strain 'spinosa', a form that has been reported in previous studies by Patterson et al. (1985, 2002) and Boudreau et al. (2005), was spatially and temporally distinctly distributed in the Pyhäsalmi case. It was clearly connected to the increased clastic input as well as to an increase in Ca concentrations, thus being related to a clayey substrate, high conductivity and/or alkalinity. Previous studies from the Greater Toronto Area, Canada (Roe et al. 2010), subtropical Florida (Escobar et al. 2008) and Ontario, Canada (Reinhardt et al. 1998) support this suggestion. In contrast, increasing abundances of *C. tricuspis* suggested a general increase in the nutrient level and trophic status of each of the study lakes. *C. tricuspis* is a species known to indicate eutrophication (Scott & Medioli 1983, Patterson et al. 1985, Asioli et al. 1996, Boudreau et al. 2005, Reinhardt et al. 2005, Roe et al. 2010). This succession was shown most clearly in the sediment cores of the Luikonlahti and Haveri cases (PII, PIII), but also in the Pyhäsalmi case (PIV), especially in the presently eutrophic Junttiselkä basin.

Another important cluster of thecamoebians included strains of *Difflugia protaeiformis*. In these studies, the most promising environmental indicator form was the strain 'multicornis' (referred as *D. protaeiformis* 'strain A' in PI, and *Difflugia fragosa* in PII) . At Luikonlahti (PII), it was linked to increased levels of Ca, Ni and Zn and decreased concentrations of the clastic input-related elements K, Na, and Mg. Such conditions developed after the mine switched to talc processing (PII). At Haveri (PIII), the strain was connected to the beginning of the mining activities and increasing clastic input, including Ca. At Pyhäsalmi the form increased in abundance in the peak loading samples and was statistically significantly related to Ca, and thus to water hardness and pH. Other forms of *D. protaeiformis* were also abundant in the present cases. Asioli et al. (1996) connected *D. proteiformis* [sic] 'proteiformis' with an environment rich in organics and sulphides and low in oxygen, while the form 'rapa' was connected to strongly polluted environments. These forms, however, do not fully match with our classification (see taxonomic listing Appendix 1). Despite the slight variation in the taxonomy, the occurrence of strains 'acuminata' and 'claviformis' are probably linked to sediment quality in terms of organic content (PIV) and the strain 'amphoralis' may have some indicator value for metal contamination. The latter correlated with the metal sum toxicity at Pyhäsalmi (PIV), was part of the mine-impacted species assemblage in Lake Retunen (PII) and occurred in the metal- (Co and Pb) and nutrient-rich group at Haveri (PIII)

Different forms of centropyxids made up a considerable part of all of the species assemblages. Forms of *Centropyxis aculeata* were more abundant than *Centropyxis constricta*, but the latter seems to have more specific indicator value. Centropyxids are a group of thecamoebians that have been considered to be opportunistic and tolerant of extreme conditions. They have been found to withstand various stressed environmental conditions such as metal-rich waters (Patterson et al. 1996, Reinhardt et al. 1998), high concentrations of naphthenic acids from oil sand mining (Nev-

ille et al. 2011), slightly brackish environments (Scott & Medioli 1983, Patterson et al. 1985), lakes contaminated by road salt (Roe et al. 2010), oligotrophic, turbid and low production environments (Burbidge & Schröder-Adams 1998), and low oxygen concentrations (Reinhardt et al. 1998, Roe et al. 2010). In all the cases in this study (PII, PIII, PIV), *C. constricta* 'aerophila' seemed to be connected with high metal and S concentrations, although it still occurred in low numbers. Some variability in identification complicates the discussion regarding *C. aculeata*, but the form seemed to be connected with metal maxima in the peak loading samples in the Pyhäsalmi case (PIV). *C. constricta* 'aerophila' and *C. aculeata* 'discoides' were also found to be the most opportunistic in a seasonal study on an oil sands reclamation wetland in northern Alberta (Neville et al. 2010a). However, the quality of the substrate and perhaps also water turbulence affected the species, since it appeared in greater numbers near the outlet on the Junttiselkä basin and close to the Tikkalansalmi strait. The suggested connection of *C. aculeata* with metals and mining operations could also be found in the results from Luikonlahti and Haveri.

In addition to the common, ubiquitous species and strains, the thecamoebian assemblages included species with minor proportions at all mine sites. These less abundant species included *Pontigulasia compressa, Lesquereusia spiralis, Difflugia urceolata, Lagenodifflugia vas, Difflugia bidens* and *Arcella vulgaris*. Some of these forms showed potential indicator value, while others appeared to be mostly indifferent to environmental gradients occurring in these studies. Of the environmentally sensitive species, *D. bidens* has previously been connected to high terrigenous inputs (Patterson et al. 1985) and clear cutting of forest (Scott & Medioli 1983). The same was noticeable in the Pyhäsalmi case (PIV), where *D. bidens* was almost absent from the pre-disturbance samples, but appeared in peak loading samples and increased in numbers in recent sediments, together with mineral matter. In contrast, *L. spiralis* was present in every case, being only slightly less abundant in 'background' sediment cores from deep water in the cases of Haveri and Luikonlahti (PII, PIII). Patterson and Kumar (2000b) connected this species with coarser, sandier substrate, and the same connection was suggested in all the cases of the present study. The species was found in higher proportions in the basin with a higher clastic sedimentation rate at Pyhäsalmi (PIV) and increased in numbers when the mining activities and changes in the land-use began and the transport of coarser matter into the lake increased (PII, PIII, PIV). This is supported by the observations of lower numbers of *L. spiralis* in the deeper water samples, where the sediment grain size is usually finer. Besides these indicator species for substrate quality, *L. vas* appeared to be affected by pH and Ca in the Luikonlahti sediment core (PII), but this remains inconclusive.

In addition to the distribution of individual species and strains, the species diversities of the faunal assemblages showed similar patterns from case to case. Especially the increase in the abundance of *C. tricuspis* caused lower diversity at Luikonlahti and Haveri (PII, PIII). On the other hand, in the reference core of Haveri, diversity increased in the peak loading samples instead of the opposite. The reference core had initially lower species diversity, and when opportunistic thecamoebian forms appeared in the assemblage with metal contamination, diversity increased. The core was retrieved from deeper water, which may be the reason for the generally lower diversity. This relationship between water depth and diversity has been found in lacustrine diatoms within a basin (Kingsbury et al. 2012).

3.3 Relationships between thecamoebians and environmental variables

Numerical analyses were employed to study the aquatic mine impacts and to distinguish between the effects of different environmental factors. RDA and linear correlations between thecamoebian PCA axis sample scores (i.e. the variation in faunal assemblages) and the corresponding geochemical concentrations were used to find the relationships between faunal features and environmental variables. The observed relationships between testate amoebae and geochemical variables are summarized in Table 1. Generally, at Luikonlahti, the most important environmental factors seemed to be related to the overall geochemical conditions, such as pH and redox conditions, whereas the Haveri results showed the clearest connection between metals and amoebae. At Pyhäsalmi, the strongest relationships between faunal assemblages and environmental variables were linked with the present Ca and SO_4-rich loading, redox conditions, trophic status of the lake and substrate composition.

The results from Luikonlahti pointed to effects of factors such as redox conditions, substrate quality, Al, Cr, Cu, and Zn on thecamoe-

Table 1. Summarized relationships between environmental variables and thecamoebians of all the case studies; marginal effects (RDA), conditional effects (RDA) and correlations (with PCA axis scores).

	Marginal Effects (RDA)	**Conditional effects (RDA)**	**Correlations with PCA axis scores**
Luikonlahti:			
Surface transect:	Al, Cr, Mn, Fe and Ba * Fe:Mn, S, C, P,	Fe:Mn, Al and Mn EFs: Al, Cr, and Zn	Not analyzed
Impacted core (Ret99):	Not analyzed	Not analyzed	*Axis 1:* Ca, Zn, Fe, Fe:Mn, Co, Cu, and Ni *Axis 2:* Al
Other:	Top-Bottom pairs (EFs): Cu, -Pb, C, N Al, Cr, Zn		
Haveri:			
Impacted core:	Co, Pb, P, LOI, Cd, U	Not analyzed	*Axis 1:* Co, P, LOI, Al, B, Fe, Mn, Pb *Axis 3:* -S, -Mo, -Ni, -Cu
Reference core:	Ag, Cu, As, Mo, and Zn	Not analyzed	*Axis 1:* Mg, K, Ti, –LOI, –Ba, –P, – Cd, –Sr, –Sb, –Mn, –Co, –Pb *Axis 2:* Zn, Ag, Cu, Mo, Ni, Bi, S, As.
Pyhäsalmi:			
Pre disturbance:	Mn, Ca and C:N	Mn	Not analyzed
Peak loading:	Ca, Ti		
Post disturbance:	C:N, Ti, Ca Mn		
Junttiselkä:	C, Ti, Ca, Mn, C/N, metal sum tox	-	Not analyzed
Kirkkoselkä:	Ti, C:N, Ca	C:N, Metal sum tox, C	Not analyzed

* NH4Ac-leached

bians. RDA marginal effects for the Luikonlahti surface sediment sample transect showed statistically significant relationships between faunal changes and six environmental variables. NH_4Ac-leached Cr, Al, and S, as well as the Fe:Mn ratio, decreased away from the source of pollution, while the concentrations of Ba and Mn increased. Conditional RDA analysis reduced the number of statistically significant variables to three: Fe:Mn, Al and Mn. In the second study from Luikonlahti (PII), concentrations of S, C, P, and the NH_4Ac-leached Al, Fe, and Mn also showed a significant correlation with faunal distribution in the modern sediment transect. However, none of the easily leachable ('bioaccessible', potentially able to interact with and be absorbed by organisms) concentrations of the major mining related heavy metals (Cu, Ni, Zn, Co) had statistically significant relationships with the species assemblages. Besides these bioaccessible geochemical concentrations in the top sediments, bottom-to-top enrichment factors (based on HNO_3 extractions) of Al, Cr, and Zn were correlated significantly with the surface sediment faunal assemblages. In the top–bottom sample pairs, the bottom-to-top faunal changes and the corresponding top–bottom enrichment factors of Cu, C, N, Al, Cr, Zn and Pb correlated significantly. Furthermore, despite the attempt to standardize the water depth at coring sites, the remaining variation may have affected the transect results. In the impacted RET99 core, the variables with constantly increasing values from the start of the mining period (Ca, Zn) showed the highest correlations with PCA axis 1 scores. Fe and the Fe:Mn ratio were also highly correlated with axis 1 scores for the core samples, suggesting the importance of redox conditions. The mining-related metals Co, Cu, and Ni correlated with axis 1 scores, but the pH-related metal Al was the only variable having a statistically significant correlation with PCA axis 2.

In the mine-impacted core from Haveri, the highest and statistically significant correlations were found between faunal assemblages (PCA axis 1) and the variables that remained elevated after the post mining metal concentration peak at 15 cm (Co, P, LOI, Al, B, Fe, Mn, and Pb). None of the variables correlated significantly with PCA axis 2, but S, Mo, Ni, and Cu, the elements of the first geochemical peaking phase, had significant negative correlations with Axis 3 sample scores. RDA analysis limited the significant correlations to Co, Pb, P, LOI, Cd and U. These were again

elements with elevated concentrations in the upper part of the core, where the eutrophic C. tricuspis dominates the species assemblages. Nutrient enrichment may thus have affected the result of the analysis without any link to mining effluents. In the reference core of Haveri, over half of the analyzed elements were statistically significantly correlated with faunal PCA axis scores, but the variables could be divided into three groups. The first group, comprising the mineral matter-related elements Mg, K, and Ti, had a positive correlation with PCA axis 1, whereas the second group with loss on ignition, several heavy metals, and P correlated negatively. Organic content had the strongest relationship with faunal changes, and all these environmental variables increased towards the surface of the core. The third group consisted of sharply peaking elements at 10–12 cm, i.e. Zn, Ag, Cu, Mo, Ni, Bi, S and As, and correlated positively with the second faunal axis. The only elements that had significant marginal effects on faunal assemblages in RDA were Ag, Cu, As, Mo, and Zn. These results suggest that eutrophication and changes in land use are the dominant environmental factors affecting thecamoebian assemblages, and mine-induced changes remained minor further afield. Thecamoebian and diatom responses to the mine-induced changes were largely similar, with some differences in relation to metals and to the phase after the highest metal loading. Both records showed dependence on the nutrient enrichment in the water bodies and on the composition of mine water inputs. Elements with statistically significant marginal effects on both proxies were partly the same, but the thecamoebian response seemed to be more limited. Co, Pb, Cd and U had significant marginal effects on both proxies, but diatoms responded to other elements as well. Diatom records also showed that shifts in algal assemblages during the peak metal loading were not caused by the effects of pH or nutrients (phosphorus).

At Pyhäsalmi, the relationships between testate amoebae and environmental variables were studied with RDA. In the full pre-disturbance dataset, with both lake basins included, Mn, Ca and C:N attained statistical significance. In the peak impact sample transect, only Ca and Ti showed statistical significance. Metal sum toxicity was not statistically significantly related to thecamoebian assemblages, despite the clear trend in geochemistry. In the post-disturbance horizon, statistically significant variables included C:N, Ti, Ca, and Mn. Conditional testing radically limited the number of significant variables: only Mn in the pre-disturbance samples remained significant. In the basin- specific analysis of the Junttiselkä basin, all variables except sediment quality showed statistically significant marginal effects on species data, but none of the conditional effects were significant. At Kirkkoselkä, Ti, C:N and Ca had statistically significant marginal effects, but only C:N had an independent signal in the faunal data. In addition, metal sum toxicity and sediment quality had significant conditional effects on thecamoebians in this basin located by the mine tailings area. For the complete dataset, metal sum toxicity did not have a statistically significant effect on biota, but other environmental variables, substrate quality, redox conditions and eutrophication were more important factors.

PCA based on faunal assemblages was also used to summarize the pooled results of all case studies (Fig. 4). There was relatively high, natural variation between the case studies, even with harmonized taxonomy. However, the species assemblages showed similar faunal successions in relation to human disturbance in all case studies (Fig. 4a). Natural, pre-disturbance samples (shown in green) plot on the left on the first axis, but are spread along the second axis (Fig. 4b). Pre-disturbance samples from the Kirkkoselkä basin (Pyhäsalmi) plot on the upper left, while the Haveri and Luikonlahti samples plot down in the left quadrant. The Junttiselkä samples lie somewhere in between. The combined PCA also suggests that the beginning of the mining activities may not cause a clear faunal response, since samples representing these periods at Luikonlahti and Haveri plot with the pre-disturbance samples (Fig. 4c). Metallic peak loading samples, instead, are found in the middle of the diagram with the mine-related environmental variables (Fig. 4d). Post-disturbance samples from Haveri, Luikonlahti and most of the Junttiselkä samples (Pyhäsalmi) plot with varying metal concentrations on the right half of the diagram, along the first axis (Fig. 4e). In contrast, post-disturbance samples of the rather nutrient-poor Kirkkoselkä basin plot in the lower left quadrant of the diagram. These results suggest that most of the observed succession is due to the increase in a well-known eutrophic indicator, C. tricuspis, especially in the Haveri and Luikonlahti cases and in the Junttiselkä basin of Pyhäsalmi. Post-disturbance samples of the still fairly oligotrophic Kirkkojärvi basin, in contrast, grouped together with the pre-disturbance samples from Haveri and Luikonlahti, suggesting that they have been largely unaffected by the nutrient increase. These results thus clearly show that human-induced eutrophication often overshadows the mine-related changes in faunal assemblages.

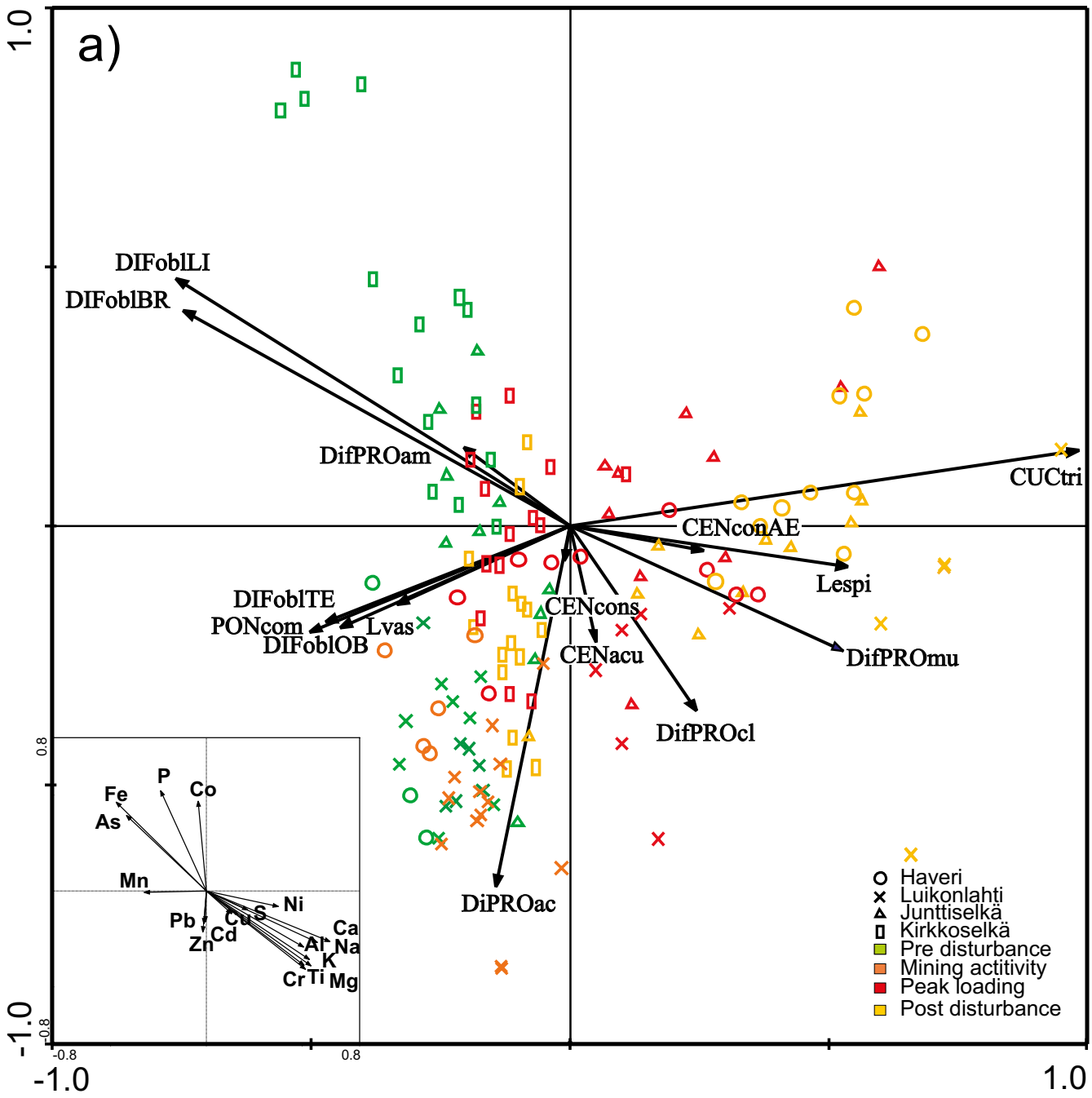
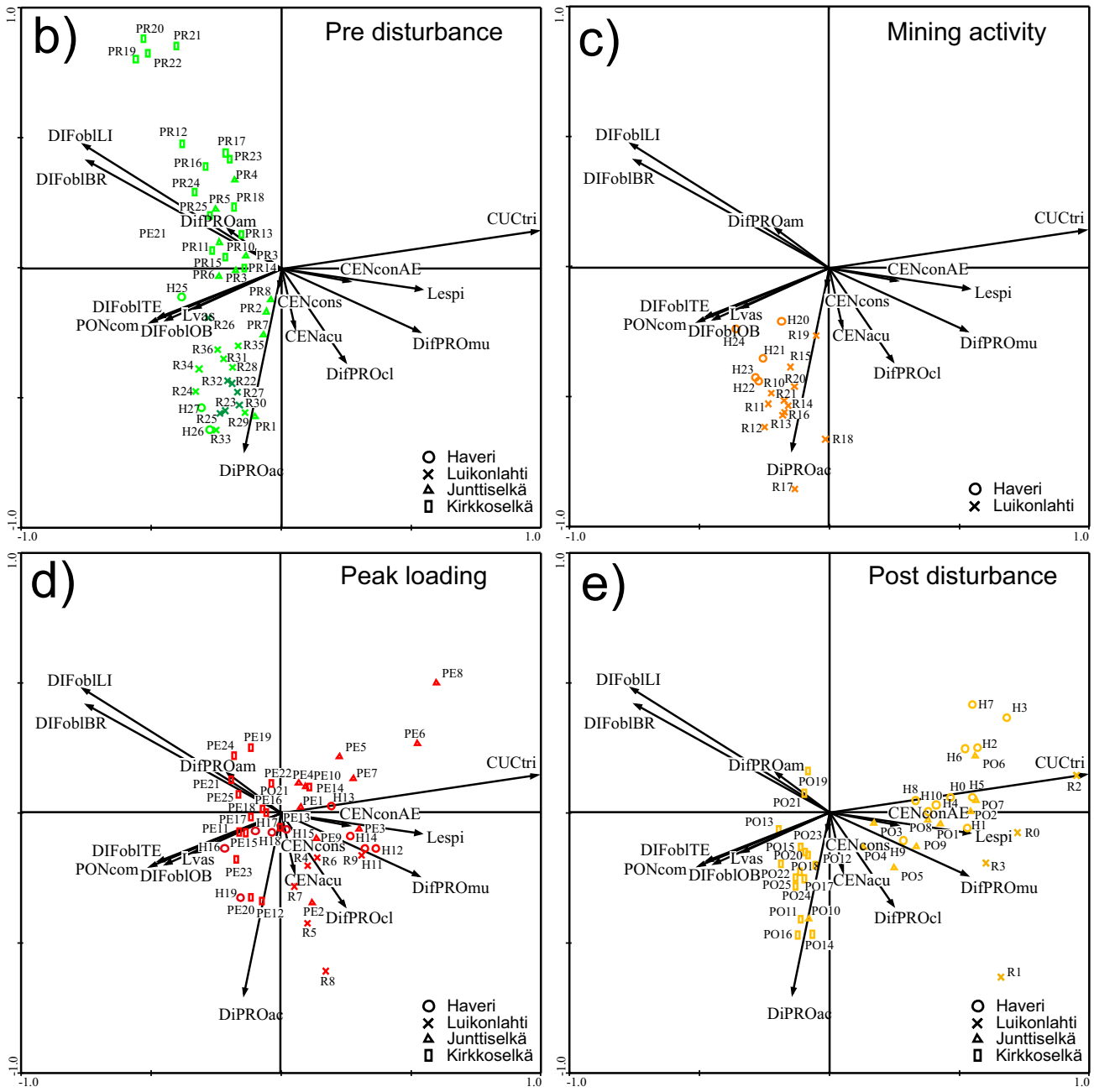


Fig. 4 a, b, c, d, e. PCA diagrams of harmonized thecamoebian assemblages of Pyhäsalmi (Junttiselkä and Kirkkoselkä basins) and the mine-impacted sediment core samples of Haveri and Luikonlahti. Samples are divided into four groups: pre-disturbance (b), active mining (c), peak loading (d) and post-disturbance (e). Pyhäsalmi transects have three sample horizons: pre, peak and post.


b)
Pre disturbance
c)
Mining activity
d)
Peak loading
e)
Post disturbance
DIFoblLI
DIFoblBR
DifPROam
CUCtri
CENconAE
Lespi
DIFoblTE
PONcom
Lvas
DIFoblOB
CENcons
CENacu
DifPROmu
DifPROcl
DiPROac
Haveri
Luikonlahti
Junttiselkä
Kirkkoselkä
1.0
-1.0

3.4 Application of testate amoeba analysis and sediment geochemistry in case-specific mine impact studies

3.4.1 Defining the baseline

All the studies together affirmed the importance of knowing the background conditions in mine impact studies, especially the geochemistry in the area. Mines are often situated on geochemical anomalies that may have affected the aquatic environment even before any mining actions. Tills in the Luikonlahti area have elevated concentrations of S and metals (Koljonen 1992). At the same time, sediment cores also showed that opportunistic centropyxids, especially *C. aculeata*, that have previously been connected to contaminated substrates in higher pH environments (Patterson et al. 1996, Reinhardt et al. 1998) formed an important part of the natural faunal assemblages in Lake Retunen (PI, PII), even before the peak metal loading phase. This may be a sign of a 'naturally stressed' environment and faunal adaptation, which was also observed in James Lake in Ontario, Canada, but with low pH and *A. vulgaris* (Patterson & Kumar 2000b). James Lake was contaminated by weathering of pyrite-rich rocks long before mining started in the area. At Pyhäsalmi, pre-disturbance samples from the Junttiselkä basin had higher proportions of *C. constricta* than those from the Kirkkoselkä basin, referring to a more stressed environment. At Haveri, however, Ti concentrations and other clastic input-related elements such as K and Na (not shown) were at a completely different level from the start than in our other study cases (Fig. 2), suggesting a more minerogenic composition of the sediments. This did not show up in the faunal distribution of the pre-disturbance samples (Fig. 4a). Even though substrate quality is a major determinant of thecamoebian assemblages, it suggests that the substrate still was not minerogenic enough to affect the species assemblage.

While choosing the background samples, other possible stress factors should also be taken into consideration. At Pyhäsalmi, the lake level was lowered in the 1930s by ~1m, which has probably caused severe environmental stress to the aquatic biota. In order to distinguish the ecological mine impacts from the effects of the other stressors, it was important to target the pre-mining samples to the time period after the lowering of the lake level, but before the onset of the mine. Even if there are no known specific events such as this on the record, it is good to keep the point of comparison as near as possible, to minimize the effects of other, more gradual natural or anthropogenic changes that could skew the results and interpretations. Background sample selection also plays an important part in the after-care plans, through the assignment of realistic restoration targets for water bodies after disturbance. In the case of James Lake (Patterson & Kumar 2000b), the natural acidity of the lake questions the rehabilitation actions to raise the pH with limestone barriers. Sometimes, baseline definition is impossible because environmental conditions have changed too much from the pre-disturbance state. In the case of the Hitura mine in Finland, lake management actions drastically changed lake conditions after mine loading had already started, making the baseline conditions that prevailed before the mine invalid for setting the restoration targets (Kauppila 2006).

Besides temporal variation, spatial variability should also be taken into account. At Luikonlahti, where the top–bottom approach was used with unimpacted–impacted sediment sample pairs, a natural faunal gradient in the pre-mining sample transect was observed. Because of this, the spatial gradient found in the corresponding, mine-impacted surface samples could not directly be considered to be mine induced. However, the sampling arrangement employed at Luikonlahti provided a sampling site-specific comparison and assessment of faunal change. Further evidence of the significance of within-lake or intra-basin spatial variation is that we did not manage to obtain suitable reference cores or modern reference samples that could be used for straightforward comparison. At Luikonlahti, the reference core turned out to be inherently different and thus not suitable for direct comparison with the mine-impacted core. At Pyhäsalmi, the unaffected reference samples taken further away from another basin were also incomparable with the ones taken from the Kirkkoselkä basin. The reference coring site appeared to be sandy, wind-stressed, and the faunal assemblages were profoundly different, and was therefore omitted from the study. Even the two basins included in the Pyhäsalmi study, Junttiselkä and Kirkkoselkä, were discussed separately because of the fundamental differences between them. At Haveri, however, the sediment core retrieved for the comparison with the mine-impacted one and to evaluate the possible eutrophication worked rather well. Even then, the idea of the reference core did not fare: the species diversity was lower probably because of the deeper water, and despite the upstream

location from the mine, a metallic impact was clearly detectable.

3.4.2 Detecting the changing mine impacts

Chemical changes in the sediments were rather easily detectable and linkable to the mining activities. The peak metal loading phase was very clear in each case study, but the ecological response of thecamoebians was often more complex and remained rather minor. Because the difference between chemical contamination and pollution is complex to determine (Chapman 2007), it should be kept in mind especially in cases of metal contaminants and palaeolimnological studies with the possibility of post-depositional migration of contaminants in the sediment column. At Pyhäsalmi (PIV), metal concentrations strongly peaked in the peak loading horizon in the northern part of the Kirkkoselkä basin, predicting a strong ecological response. However, the faunal changes, while detectable, remained rather minor, with only a few opportunistic centropyxid forms increasing slightly. This suggests that despite the high contamination, metals either have not been bioavailable to testate amoebae or the amoebae have developed mechanisms to deal with the ambient metal levels. The results also further attested that the HNO_3 leach is not the most suitable method to represent the bioaccessible fraction of metals. However, the HNO_3 leach was chosen as a proxy for the mine effluent loading, because when a milder ammonium acetate leach (NH_4Ac) was used to leach modern sediments in the first study from Luikonlahti, the mine-related heavy metal concentrations (Cu, Ni, Zn, Co, Cd) did not coincide or correlate with thecamoebian assemblages, either.

One of the biggest challenges of mine impact studies is to differentiate the mine water effects on thecamoebians from the effects of other, coexistent environmental variables. In the present studies, the most dominant faunal feature was the increase in the proportions of *C. tricuspis*, a species linked to high nutrient levels and eutrophication. This was particularly evident in the sediment cores from Luikonlahti and Haveri (especially the reference core HAV4), but the same succession was also seen in the Pyhäsalmi and Luikonlahti sample transects when the recent samples were compared with their pre-disturbance counterparts. *C. tricuspis* is a well-known indicator of eutrophication, but other secondary environmental factors may also have affected the biota, and these effects may be difficult to separate from the effects of mine water loading. Different stress factors, for example pH and metals, may cause similar faunal changes, making the effects difficult to distinguish if they are contemporaneous. Combined effects may also be synergistic or antagonistic, and thus difficult to separate.

In all of the present mining cases, the changes from the natural background conditions started with increasing amounts of mineral matter-related elements such as K and Ti. Dating suggested that these changes were connected with the mining activities, but the inputs could also be related to changes in the general land use in the area, such as cultivating and ditching of the surrounding wetlands. The exact cause of the increased mineral matter inputs therefore remains unclear. Other land uses in mining regions may also mobilize mining-associated metals (Parviainen et al. 2012). The increase in mineral matter (Ti) with time was an especially outstanding feature at Pyhäsalmi. A change in mineral matter contents from the predisturbance samples to peak loading samples occurred in both basins, but continued in the post-disturbance samples from the Junttiselkä basin. Due to the lack of a stratigraphically studied sediment core, it is impossible to conclude whether the first switch was related to mining or other land use in the area, or probably both. Junttiselkä and Kirkkoselkä basins are fundamentally different, Junttiselkä being smaller, shallower, quite closed and with a large catchment. It thus receives more mineral matter and nutrients to begin with. The faunal results, however, suggested that mine pressure on aquatic ecology may remain rather limited during the active mining phase.

The Luikonlahti and Haveri cases showed that the most intensive metallic inputs typically date to the post-mining period, most likely due to the commonly observed delay in AMD generation after the cessation of tailings deposition. However, as the results of the Pyhäsalmi case showed, even high metallic inputs detected in the sediments may not cause a major ecological response. This is despite the commonly observed low pH that is often characteristic of AMD effluents. Diatoms have long been used to infer lake water pH, and the results suggest that the lake ecosystem may already react to a lowering of pH by 0.8–0.4 units (Ek & Renberg 2001). However, they have also been found to respond to metal contamination even without severe acidification (Cattaneo et al. 2004, Salonen et al. 2006). Concerning thecamoebians, the water column pH has been suggested to have an influence on the species assemblages (Escobar et al. 2008, Patterson & Kumar 2000b). At Luikonlahti, the horizontal species gradient in modern sediments and faunal changes in sediment cores were both connected to Al, and thus

to a possible AMD-related decrease in pH. At Pyhäsalmi, the continuous operation of the facility and deposition of fresh tailings may have prevented oxidation of the tailings surface and thus the evolution of more severe AMD with drastic impacts on the biota. Differentiating the possible effects of pH and metals at mine sites is, however, challenging. For instance, in the case of Luikonlahti, the results suggested a decrease in pH, but the toxicity of Al to aquatic organisms could not be ignored, either. Sediment quality may also have an influence on the severity of AMD impacts. Lac Dufault in Canada avoided severe and continuing acidification because of the buffering capacity of the sediments, which was seen as Ca depletion in a sediment profile of the lake (Couillard et al. 2004). The same type of feature could be observed in the Ca concentration profile in the mine-impacted Haveri core HAV2 (Fig. 2).

Besides environmental factors unrelated to mining, mine effluent quality itself may vary temporally and spatially. This was most clearly seen in the Pyhäsalmi and Luikonlahti cases. At Pyhäsalmi, mine water loading has changed from one with high metal concentrations towards the present loading that mainly consists of Ca and sulphates. These changes were also detected in the faunal distributions. At Luikonlahti, the acidic and metal-laden mine effluents discharging into Lake Retunen are a mix of drainage waters from the surroundings of the mine site and seepage waters from the tailings facility. However, the partly oxidized tailings are nowadays covered with more alkaline, magnesite-rich tailings from talc processing deposited on top of the sulphidic, acid-producing tailings (Räisänen & Juntunen 2004). A slight decrease in effluent acidity has occurred because of the introduction of this neutralizing tailings material (Heikkinen et al. 2009). Before this most recent change, however, thecamoebian analysis suggested an abrupt change to more acidic conditions with high metal concentrations in the mine-impacted sediment core at the depth of 10 cm. Faunal compositions were also consistent with the most recent change towards a higher pH of the effluents (PII), suggesting the significance of pH conditions.

The ecological impact of mining, its spatial and temporal range and especially how it manifests itself will also depend on the proxy used. At Haveri, two diverging ecological indicators were used: testate amoebae and diatoms. The former represent the sediment water interface and conditions on the lake bottom, whereas the latter represent the combined ecological signal from a variety of habitats. These two biological proxies also record changes in different ecosystem levels and taxonomic groups. The general view of the changes in the lacustrine environment surrounding the Haveri mine was largely similar for both proxies, but there were also differences between the records. The shifts in species assemblages nevertheless documented different phases of mine loading and other environmental stresses.

Besides defining the temporal limits and evolution of the mine loading, sediment studies and thecamoebian analysis offered a tool to assess the spatial extent of mine impact. In the Luikonlahti transect of top–bottom sample pairs, faunal distributions showed that the most severe impact was limited to the nearest sample sites from the pollution source, while the faunal changes progressively declined at sites further out in the bay and were almost undetectable outside of it. On the other hand, the Haveri and Pyhäsalmi cases demonstrated that metals had spread to 'net upstream' locations, affecting the faunal compositions there, against the original assumptions. Thecamoebians worked well in the spatial separation of mine impacts, probably because of their living habitat at the sediment–water interface, which prevents drifting.

3.4.3 After the peak loading phase: towards recovery?

Sediment core results from all case studies showed that the most intense metal inputs remained fairly short-lived. However, despite the decrease after the peak loading, concentrations remain elevated and have not returned to the pre-disturbance levels, a feature also found in other mine impact studies (Couillard 2004, Kauppila 2006). Numerous studies have found AMD to be most severe in the first few decades after sulphide oxidation begins (Demchak et al. 2004, Lambert et al. 2004), but it can also persist for centuries if conditions are favourable (Ek & Renberg 2001). The short duration of the intensive metal loading most likely indicates a decreased oxidation rate in the tailings surface that has affected acidity, leaching and mine drainage quality. Sulphide depletion in the oxidation layer progressively increases the distance that oxygen needs to diffuse to reach unoxidized tailings material (Alakangas et al. 2010). At Haveri, the upper part of the tailings was nearly depleted of primary sulphides, but some secondary precipitates were present with enriched trace elements such as Cu, Zn and As (Parviainen 2009). Such precipitates are known to bind elements effectively in acid mine waters (Lin & Herbert 1997), although the effect may remain tempo-

rary. In the lower part of the Haveri tailings pile, discontinuous cemented layers were also detected. These have been suggested to have an influence on the movement of dissolved metals through the tailings and also to act as a zone of metal accumulation (Blowes et al. 1991). At Pyhäsalmi, the drastic decrease in metal loading occurred because the mining company started treating the effluents by liming. This may also have prevented the impacts of low pH on the lake, contrary to what was suggested at Luikonlahti. Even though the most recent switch to talc processing and the resulting neutralizing wastes have probably eased the pH situation at Luikonlahti, the shift has also introduced Ni and Zn contamination. At Pyhäsalmi, in contrast, the current mining effluents that are dense and nearly saturated with gypsum have affected the stratification patterns in the Junttiselkä Basin and contributed to seasonal oxygen depletion in the hypolimnion.

Besides the chemical changes, there were some ecological changes that could be considered as recovery in all cases. However, as sediment metal concentrations remained elevated after the peak loading phase, the species compositions did not return to the pre-disturbance states. A simplified theory represents ecosystems as self-regulating and self-repairing systems in which natural processes bring the system back to equilibrium after disturbances. Conversely, the hysteresis theory implies that a disturbed ecosystem may not revert to its pre-disturbance state, even when the stress is completely removed. In practice, recovery is more complex and affected by the frequency and extent of the disturbances, and the heterogeneity of the ecological system. Environmental changes may pass thresholds causing recovery to different stable communities, and synergetic effects may lead to unpredictable trajectories (O'Neill 1999). In our study cases, despite the fact that not all mining-related disturbances have yet disappeared, the most important environmental factor was eutrophication, which has profoundly changed conditions in the lakes and probably led to a succession towards climax communities that differ from the pre-mining state. This third state has also been observed in other studies. In the Sudbury area, which has suffered from the impacts of severe acid rain, Tropea et al. (2010) suggested that biological recovery may be impeded by other environmental stressors such as climate warming, because diatoms did not to response to the observed chemical recovery.

As with mine impact detection, the ecological proxy employed affects how the possible recovery is perceived. At Haveri, diatoms and thecamoebians responded slightly differently to decreasing metal concentrations. The most probable reason for these differences can be addressed to the different living habitats of these organisms. Thecamoebians live at the sediment–water interface, whereas diatom results are a combination of species from different habitats. The influence of habitat was also observed within the diatom analysis results, when subtle differences were observed between the planktonic and non-planktonic diatom signals. The non-planktonic species compositions have not returned to their original state. Both proxies suggested that sediment characteristics and the contaminants in the sediments may have affected the biota, especially thecamoebians. In contrast, Escobar et al. (2008) found organic content to be the only sediment characteristic affecting the thecamoebian assemblages in subtropical Florida, and suggested water quality to be more a important environmental factor than sediment characteristics. However, whether it is the reflection of the water quality at the time of deposition or effects of the sediment contaminants, thecamoebians work well as a proxy for mine water loading.

3.5 Future prospects of the method

Despite the long history of thecamoebian analysis, it is still finding its place as an established method in environmental assessment. Besides their undeniable strengths as indicators to trace mining effects, there are still some weaknesses and limitations. Based on this and previous studies, thecamoebians respond to several environmental parameters, and one of the challenges is to differentiate the effects of these often simultaneous, interlinked factors. This uncertainty can be reduced by using multivariate methods to account for the effects of secondary environmental factors and to tease out the connections that may otherwise be too weak to detect. However, even numerical treatments may not be sufficient to separate the effects of the variable of interest from other coincident changes in lake-water quality, sediment chemistry or other variables. Careful design of the sampling strategy is therefore important to control substrate quality and other physical parameters that may affect the faunal assemblages. This study, however, showed great potential by not only finding multiple relationships between thecamoebians and mine induced effects, but also by

being able to connect three separate cases, despite their slightly different starting points.

A logical next step to develop the use of this method and assess its limitations would be the development of training sets and transfer functions to create predictive models, as has been done for diatoms and chrysophytes. This would require a large-scale systematic sampling program of modern surface sediments with comprehensive measurements of the corresponding environmental variables. In peatlands, the group is already used more widely to model various variables, such as the water table, soil moisture and pH (Booth 2002, 2008, Lamentowicz & Mitchell 2005, Swindles et al. 2009), and some steps have also been taken in this direction in lacustrine settings. Escobar et al. (2008) investigated the ecology of thecamoebians in subtropical lakes of Florida and Roe et al. (2010) studied the controls of the modern distribution of thecamoebians and their potential as water quality indicators in the urban Toronto area in Canada. Both studies found the group to be good water quality indicators with great potential for model development. In Florida, thecamoebians particularly responded to total alkalinity and pH, whereas in Canada the greatest potential as water quality indicators was linked to changes in lake trophic status driven by fluctuations in phosphorus and salt contamination.

4 CONCLUSIONS

Sediment geochemistry and testate amoeba analysis is a suitable combination of methods for case-specific studies of aquatic mine impacts. Sediment geochemistry provided a workable proxy of changing mine effluent inputs to surface water bodies with a corresponding ecological response, even if it is not an accurate predictor of the bioaccessible fraction in the sediments.

The importance of case-specific studies was evident at all mine sites: Natural faunal gradients were detected in pre-disturbance samples, suggesting that not every observable change in biota is necessarily mine-induced, and spatial variation within a water body should always be taken into account. The heterogeneity within a single lake basin was also seen in the difficulties in retrieving suitable reference cores. The same applies when designing after care, rehabilitation and monitoring actions; goals should be set based on data that are representative of the ecosystem in question.

The beginning of the mining activities had mainly minor impacts on the geochemistry and ecology of the sediments. Despite the intense metal inputs detected in the sediments during the peak loading phases, ecological responses also remained minor here. This suggests that metals may not have been in a bioaccessible form or not have affected testate amoebae.

The peak loading of metals occurred with a delay after mine closure, probably because the oxidation of sulphides in the tailings and waste rocks and the generation of AMD take time. The highest metal inputs also appeared to be short-termed, but metal concentrations also remained elevated after the peak loading phase. These observations support the possibility to reduce environmental damage by controlling the oxidation of tailings and waste rock piles to prevent AMD, the most severe mining-related threat to the aquatic environment.

After the peak loading phases, some recovery was detectable in both the geochemical and ecological results. Instead of returning to the baseline compositions, the geochemical properties and species assemblages trended towards a third state after the peak loading.

Eutrophication appeared to be the most important environmental factor in each case, effectively masking the effects of metals and other variables. Other environmental stresses and variables that seemed to have an influence on the faunal composition were substrate quality (clastic/organic), redox conditions, alkalinity and pH.

Numerical methods proved to be a useful tool when differentiating between the effects of different environmental variables. However it remains challenging to distinguish the effects of stressors such as pH and metals from each other when the effects are contemporaneous.

Despite the differences between baseline faunas in the case studies, the ecological responses of thecamoebians were rather similar in all cases, with the same species/forms indicating certain changes. This constancy lends support to the applicability of testate amoebae to track the ecological effects of mine water loading.

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Appendix 1. Taxonomy.

An abbreviated taxonomic listing of thecamoebian forms used in this thesis. The author mainly followed the identification key of Kumar & Dalby (1998). Differences in the identification between papers I−IV are described under a genus or a species name when required. A case specific nomenclature is provided under each form, to clarify further the evolution of the species concept of the studies. An explanatory figure in PIV (Kihlman & Kauppila 2012) or in other literature is referred if obtainable and was seen necessary.

Subphylum *Sarcodina*; Schmarda 1871
Class *Rhizopodea*; von Siebold 1845
Subclass *Lobosia* Carpenter 1861
Order *Arcellinida* Kent 1880
Superfamily *Arcellacea* Ehrenberg 1832

Family *Arcellidae* Ehrenberg 1832
Genus *Arcella* Ehrenberg 1832

Family *Centropyxidae* Jung 1942
Genus *Centropyxis* Stein 1859
PI and PII: Strains of *C. constricta* were not distinguished.
PIII: Three *C. aculeata* strains were identified: 'discoides', *C. aculeata* and bigger and flatter 'species 1'
PIV: Different forms of *C. aculeata* were counted as one. *C. constricta* 'aerophila' was only strain differentiated from specimens of the species *C. constricta.*

Centropyxis aculeata Ehrenberg 1832, Kihlman & Kauppila 2012 (PIV), Fig. 3, 18
Strain 'aculeata'
C. aculeata 'aculeata' Reinhardt et al. 1998, Plate 1, Figs. 1a−c
C. aculeata Kihlman & Kauppila 2010, PIII
C. aculeata Kihlman & Kauppila 2012, PIV
Strain 'discoides'
C. aculeata 'discoides' Reinhardt et al. 1998, Plate 1, Fig. 2
Centropyxis constricta Ehrenberg 1843, Kihlman & Kauppila 2012 (PIV), Fig. 3, 18
Strain 'aerophila' Kihlman & Kauppila 2012 (PIV), Fig 3, 18
C. aerophila, Ogden & Hedley 1980, 48−49
Cucurbitella constricta, Reinhardt et al. 1998, Plate 1, Fig. 6
Strain 'constricta'
C. constricta 'constricta' Reinhardt et al. 1998, Plate 1, Figs. 4a, b
Strain 'spinosa'
C. spinosa Ogden & Hedley 1980, p. 62, Plate 20, Figs. a−d

Family *Hyalospheniidae* Schulze 1877
Genus *Lesquereusia* Schlumberger 1845
Lesquereusia spiralis Ehrenberg 1840, Kihlman & Kauppila 2012 (PIV), Fig. 3, 18

Family *Difflugiidae* Wallich 1864
Genus *Difflugia* Leclerc in Lamarck 1816
Difflugia protaeiformis Lamarck 1816
PI and PII: Three strains of *D. protaeiformis* was identified, 'rapa', 'crassa' and 'protaeiformis' following Asioli et al. 1996. The strain 'crassa' used in PI–PII includes specimens of strains 'claviformis' and 'acuminata', used in PIII−PIV.
PIII: *D. protaeiformis* 'species 1' correspond 'claviformis' in PIV, *D. protaeiformis* strains 'claviformis' and 'acuminata' correspond 'acuminata' in PIV.
PIII and PIV: *D. protaeiformis* strain 'multicornis' was named for the first time in PIII, but was described later in PIV.

Strain 'amphoralis' Kihlman & Kauppila 2012 (PIV), Fig. 3, 20−21
D. proteiformis 'rapa' [sic] Asioli et al. 1996
D. protaeiformis 'amphoralis' Reinhardt et al. 1998, Plate 2, Fig. 4
D. protaeiformis 'rapa' Kauppila et al. 2006, PI
Strain 'claviformis' Kihlman & Kauppila 2012 (PIV), Fig. 3, 26
D. proteiformis 'crassa' and 'proteiformis' [sic] Asioli et al. 1996
D. protaeiformis 'claviformis', Reinhardt et al. 1998, Plate 2, Fig. 3
D. protaeiformis 'protaeiformis' and 'crassa' Kauppila et al. 2006, PI
D. protaeiformis 'crassa' and 'protaeiformis' Kihlman & Kauppila 2009, PII
D. protaeiformis 'species 1' Kihlman & Kauppila 2010, PIII
Strain 'acuminata' Kihlman & Kauppila 2012 (PIV), Fig. 3, 24−25
D. proteiformis 'proteiformis' [sic] Asioli et al. 1996
D. protaeiformis 'acuminata', Reinhardt et al. 1998, Plate 2, Fig. 5
D. protaeiformis 'protaeiformis' and 'crassa' Kauppila et al. 2006, PI
D. protaeiformis 'protaeiformis' Kihlman & Kauppila 2009, PII
D. protaeiformis 'claviformis' and 'acuminata'

Kihlman & Kauppila 2010, PIII
D. protaeiformis 'acuminata', *D.protaeiformis*, Kihlman & Kauppila 2012, PIV
Strain 'multicornis' Kihlman & Kauppila 2012 (PIV), Fig. 3, 22−23
D. acuminata Leidy 1879, Plate XII, Figs. 24−29
D. protaeiformis 'strain A' Kauppila et al. 2006, PI
D. fragosa (misidentified) Kihlman & Kauppila 2009, PII
D. protaeiformis 'multicornis' Kihlman & Kauppila 2010, 2012, PIII, PIV

Difflugia oblonga Ehrenberg 1832
PI and PII: Strains 'A' and 'B', a clear shape covered by coarser clasts in PI, were identified as strains 'bryophila' and 'tenuis', respectively, in PII.

Strain 'oblonga' Kihlman & Kauppila 2012 (PIV), Fig. 3, 1
Ogden & Hedley 1980, Plate 63, Figs. a−c
Reinhardt et al. 1998, Plate 2, Figs. 10a, b
Strain 'linearis' Kihlman & Kauppila 2012 (PIV), Fig. 3, 2−3
Reinhardt et al. 1998, Plate 2, Figs. 8a, b
Strain 'glans'
Reinhardt et al. 1998, Plate 2, Figs. 9a, b
Strain 'bryophila' Kihlman & Kauppila 2012 (PIV), Fig. 3, 4−5
Reinhardt et al. 1998, Plate 2, Figs. 9a, b
Strain 'tenuis' Kihlman & Kauppila 2012 (PIV), Fig. 3, 6
Reinhardt et al. 1998, Plate 2, Figs. 12a, b
Strain 'spinosa' Kihlman & Kauppila 2012 (PIV), Fig. 3, 18
Reinhardt et al. 1998, Plate 2, Figs. 11a, b

Difflugia bidens, Penard 1902, Kihlman & Kauppila 2012 (PIV), Fig. 3, 18
Difflugia urceolata Carter 1864, Kihlman & Kauppila 2012 (PIV), Fig. 3, 18
Difflugia globula Ehrenberg 1848
Difflugia corona Wallich 1864

Genus *Cucurbitella* Penard 1902
Cucurbitella tricuspis Carter 1856, Kihlman & Kauppila 2012 (PIV), Fig. 3, 18
Difflugia tricuspis Kauppila et al. 2006 (PI)

Genus *Pontigulasia* Rhumbler 1895
Pontigulasia compressa Carter 1864, Kihlman & Kauppila 2012 (PIV), Fig. 3, 18

Genus *Lagenodifflugia* Medioli & Scott 1983
Lagenodifflugia vas Leidy 1874

PI and PII: Test A, relatively small, simple cylindrical test, neck absent, fundus rounded. Remained unidentified, most likely belongs to *Difflugia* species. Requires revision.

Appendix 2. Raw thecamoebian data.

Luikonlahti top samples (% data)																	
Sample ID (Kihlman & Kauppila 2009)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Sample weight (g)	1.285	1.215	1.38	1.42	1.36	1.367	1.311	1.421	1.089	1.294	1.045	1.313	1.26	0.901	1.511	1.361	1.024
Water content (%)	84.0	87.8	83.9	88.8	85.9	86.2	n/a	87.8	88.4	87.7	84.2	89.1	n/a	87.7	85.8	n/a	87.2
Total counts (specs)	260	255	242	237	299	380	207	321	360	242	384	252	293	245	312	260	324
Counted subsamples (X/8)	2	2	2	3	2	2	1	2	2	1	2	1	1	1	1	1	1
Arcella vulgaris	0.00	0.00	0.00	1.27	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Centropyxis aculeata	4.62	2.35	3.31	8.44	2.68	7.89	1.93	6.85	2.78	5.37	7.03	5.56	6.83	2.86	6.73	8.46	3.70
Centropyxis aculeata 'discoides'	0.00	0.00	0.00	0.00	0.00	0.26	0.00	0.31	0.00	0.41	1.30	0.00	0.00	0.00	1.60	0.77	0.00
Centropyxis constricta	0.77	0.39	0.41	2.95	0.67	2.63	0.00	0.62	0.56	0.83	3.39	0.00	0.00	0.82	3.53	0.00	1.85
Difflugia bidens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.00	1.37	0.00	0.00	0.77	0.00
Difflugia corona	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.78	0.00	0.00	0.00	0.00	0.00	0.31
Difflugia globulus	5.38	6.27	3.31	4.22	2.01	4.47	2.42	5.61	2.22	6.61	7.55	7.54	3.07	7.35	2.88	3.85	3.70
Difflugia oblonga 'oblonga'	12.31	7.45	11.98	12.66	17.39	10.79	12.08	13.71	6.94	9.09	11.46	10.32	10.58	8.98	12.82	12.31	9.26
Difflugia oblonga 'linearis'	5.00	1.96	3.31	3.38	3.68	4.47	2.90	4.98	5.56	3.72	3.13	1.98	5.46	2.45	6.41	3.46	1.23
Difflugia oblonga 'glans'	0.77	0.78	1.65	0.84	0.33	2.11	0.48	0.93	1.11	0.00	0.78	1.19	0.34	0.82	0.96	0.00	0.62
Difflugia oblonga (small.short neck)	3.08	5.10	2.48	2.53	4.01	3.42	4.35	5.30	2.78	2.07	3.39	3.17	1.71	4.49	5.77	0.00	5.56
Difflugia oblonga 'lanceolata'	0.00	0.39	0.00	0.42	0.00	0.00	0.00	0.31	0.00	0.00	0.26	0.00	0.68	0.41	1.60	0.38	0.00
Difflugia oblonga 'tenuis'	4.62	5.49	3.31	5.49	7.69	4.21	6.76	2.80	6.11	4.96	6.77	4.76	13.99	4.49	3.53	9.62	2.78
Difflugia oblonga 'bryophila'	3.85	2.75	4.55	2.11	3.01	5.00	0.48	7.79	6.94	9.09	2.60	6.75	10.92	4.08	3.85	11.54	5.56
Difflugia protaeiformis 'protaeiformis'	6.54	18.43	16.94	18.14	14.72	8.42	19.81	14.95	12.50	13.22	12.24	13.10	7.85	9.80	7.69	9.23	20.99
Difflugia protaeiformis 'crassa'	1.54	4.71	2.07	3.80	3.01	2.63	3.86	2.49	3.61	3.72	1.82	3.17	3.41	1.63	2.24	1.92	2.78
Difflugia protaeiformis 'rapa'	6.54	8.24	7.44	7.17	5.69	7.11	5.80	9.03	8.89	6.61	7.55	6.75	5.80	8.57	5.77	7.31	4.32
Difflugia tricuspis	23.08	20.00	18.60	13.92	14.72	14.21	22.22	7.48	14.17	14.46	5.99	13.10	10.24	14.29	9.29	10.38	17.59
Difflugia urceolata	0.38	0.78	2.48	0.00	1.00	0.53	0.97	0.00	1.94	0.00	0.52	3.57	2.39	4.08	3.53	2.69	0.31
Lagenodifflugia vas	1.92	0.39	2.89	2.95	3.01	2.11	0.97	2.49	3.89	0.83	2.08	1.98	3.07	2.04	1.92	0.77	2.16
Lesquereusia spiralis	1.92	1.57	3.72	0.84	0.33	1.58	2.42	1.56	1.94	2.07	2.60	2.38	1.02	2.04	1.60	0.77	1.85
Pontigulasia compressa	0.00	0.39	3.31	0.84	1.34	3.42	2.42	1.25	2.50	0.41	0.52	2.38	1.02	1.63	1.28	0.38	0.00
Difflugia strain A	7.69	3.53	0.83	1.27	2.34	2.11	5.31	3.12	2.78	2.07	5.99	4.37	3.41	3.67	7.69	3.08	3.09
Difflugia strain B	5.38	4.31	2.07	2.95	4.35	4.21	1.93	4.98	4.44	7.85	7.03	2.38	2.73	6.53	5.77	3.85	3.09
TEST A	4.62	3.14	5.37	2.95	7.02	8.42	1.93	2.80	7.50	6.61	4.17	5.56	3.75	6.53	3.53	8.46	9.26
Difflugia protaeiformis 'species A'	0.00	0.78	0.00	0.42	0.67	0.00	0.97	0.62	0.83	0.00	0.78	1.59	0.34	2.45	0.00	0.00	0.00

Appendix 2. cont.

Luikonlahti bottom Samples (% data)																	
Sample ID (Kihlman & Kauppila 2009)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Sample weight (g)	1.138	1.279	1.291	1.248	1.114	1.160	1.061	1.048	1.115	1.141	1.193	1.184	0.809	1.040	1.168	1.192	0.959
Water content (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Total counts (specs)	289	272	330	309	232	257	230	359	246	327	451	441	266	241	449	437	214
Counted subsamples (X/8)	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1
Arcella vulgaris	0.00	0.00	0.00	0.00	0.00	0.00	1.74	0.00	0.00	0.00	0.00	0.00	0.38	0.00	0.00	0.69	0.00
Centropyxis aculeata	6.92	13.97	16.97	11.97	8.62	7.78	10.00	4.74	7.32	5.50	5.10	3.40	4.51	9.13	4.23	3.89	4.67
Centropyxis aculeata 'discoides'	1.04	3.31	3.03	2.59	2.59	1.95	1.30	0.56	2.03	1.83	0.89	1.13	0.75	1.24	1.11	2.06	0.00
Centropyxis constricta	2.08	1.47	2.73	1.94	2.59	1.17	0.87	2.23	2.44	0.92	1.33	3.85	0.38	2.90	2.23	0.69	0.47
Difflugia bidens	0.00	1.10	0.61	0.65	0.86	0.78	0.87	0.56	2.03	0.61	0.44	0.45	0.00	0.00	0.00	0.00	0.00
Difflugia corona	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia globulus	2.08	3.31	6.06	6.15	5.17	5.45	4.78	5.57	6.50	7.03	4.66	5.44	2.63	4.56	6.90	4.58	1.87
Difflugia oblonga 'oblonga'	16.96	15.81	7.88	11.00	12.07	13.62	10.00	14.21	9.35	10.70	15.08	15.42	16.54	15.35	16.70	15.33	12.62
Difflugia oblonga 'linearis'	1.73	3.68	5.76	3.24	3.02	1.95	6.09	2.23	4.88	1.22	2.88	2.27	6.02	3.73	5.12	6.64	5.61
Difflugia oblonga 'glans'	0.69	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.00	0.44	0.91	0.00	0.00	0.89	0.00	0.00
Difflugia oblonga (small.short neck)	0.69	1.10	3.33	5.18	4.74	2.72	3.04	3.06	4.88	2.75	4.21	4.54	3.01	8.30	6.24	2.29	8.88
Difflugia oblonga 'lanceolata'	0.00	0.00	0.00	0.00	0.00	0.39	0.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.92	0.00
Difflugia oblonga 'tenuis'	10.38	9.19	6.06	6.80	6.47	10.89	9.57	9.47	9.35	7.65	10.20	7.26	13.16	4.15	7.35	11.21	4.67
Difflugia oblonga 'bryophila'	4.15	4.41	5.45	6.80	6.03	7.39	8.70	6.69	9.35	6.73	9.09	5.22	17.29	7.05	4.45	10.53	3.27
Difflugia protaeiformis 'protaeiformis'	12.11	8.09	6.06	7.77	5.17	8.17	10.00	10.03	11.38	13.76	6.43	5.44	9.77	3.73	7.13	8.47	15.89
Difflugia protaeiformis 'crassa'	1.73	2.21	3.03	1.62	2.59	2.33	2.61	3.34	3.25	3.98	2.88	3.17	3.01	1.24	2.00	1.60	3.27
Difflugia protaeiformis 'rapa'	1.38	4.41	3.64	1.29	3.45	5.84	4.78	5.01	2.44	4.28	4.88	5.67	2.63	6.22	4.90	4.12	3.74
Difflugia tricuspis	15.57	4.78	7.88	7.77	9.48	6.61	7.39	11.98	7.72	9.17	8.65	7.71	6.39	11.20	9.58	9.38	10.75
Difflugia urceolata	0.00	0.37	0.30	0.00	0.00	0.39	0.43	0.00	1.22	2.45	1.11	2.27	1.13	0.41	1.78	0.69	0.47
Lagenodifflugia vas	0.00	4.41	0.30	0.97	1.72	1.56	0.87	0.84	0.00	0.92	0.89	1.81	1.13	0.41	0.22	2.06	0.93
Lesquereusia spiralis	1.04	1.10	1.82	1.29	1.29	1.56	2.17	0.84	1.22	1.53	0.67	2.49	0.00	0.00	1.34	0.92	2.34
Pontigulasia compressa	0.35	0.74	1.21	2.27	2.16	1.17	1.30	1.39	1.22	0.31	1.77	2.49	0.75	2.90	1.34	1.14	1.87
Difflugia strain A	7.27	6.25	4.55	6.47	6.90	6.23	5.22	8.08	3.66	6.42	8.65	9.52	3.01	11.62	6.68	3.20	7.94
Difflugia strain B	5.19	3.68	4.55	5.18	5.17	4.67	2.17	4.46	4.07	4.89	4.88	3.85	0.38	4.56	2.23	2.75	6.54
TEST A	8.30	5.88	8.18	9.06	9.91	7.39	5.22	4.74	4.88	7.34	4.88	5.67	1.88	0.83	7.57	6.86	4.21
Difflugia protaeiformis 'species A'	0.35	0.37	0.61	0.00	0.00	0.00	0.00	0.00	0.41	0.00	0.00	0.00	0.38	0.41	0.00	0.00	0.00

Appendix 2. cont.

Luikonlahti. the mine impacted core RET-99 (% data)																			
SampleID (Depth cm)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Sample weight (g)	1.870	2.170	1.752	1.632	1.573	1.570	1.498	1.499	1.495	1.442	1.390	1.390	1.390	1.423	1.389	1.351	1.380	1.358	1.356
Water content (%)	95.1	94.3	93.6	93.1	91.7	89.0	96.7	87.5	84.2	70.7	75.1	88.8	89.1	89.4	86.8	86.3	87.8	87.9	81.0
Total counts (specs)	257	216	259	304	327	265	234	285	253	219	319	225	274	330	284	278	251	332	287
Counted subsamples (X/8)	6	3	3	3	2	2	1	1	1	2	2	1	1	2	2	2	1	1	2
Arcella vulgaris	1.17	1.39	0.39	0.66	0.00	0.38	0.85	0.35	0.00	0.91	0.63	0.89	0.36	0.00	0.00	0.36	0.80	1.20	1.05
Centropyxis aculeata	2.72	3.70	3.86	1.32	2.45	2.26	3.85	3.51	3.16	7.76	17.24	16.89	18.61	20.30	14.08	17.63	13.55	12.65	14.98
Centropyxis aculeata 'discoides'	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	0.79	1.37	0.63	0.00	0.73	0.00	0.70	1.08	0.80	0.00	0.70
Centropyxis constricta	1.17	0.00	0.39	1.97	0.92	1.51	1.28	0.70	1.19	4.57	7.21	8.00	7.66	7.88	8.45	6.47	9.16	7.83	3.14
Difflugia bidens	0.00	0.46	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.89	0.73	0.61	0.35	0.00	0.40	0.90	1.05
Difflugia corona	0.00	0.00	0.00	0.00	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.60	0.00
Difflugia globulus	3.50	5.09	3.47	6.91	13.76	9.81	16.24	12.63	4.35	12.79	10.03	5.33	6.57	3.94	3.52	3.96	2.79	3.61	3.14
Difflugia oblonga 'oblonga'	5.84	6.48	2.32	7.24	5.50	7.92	10.26	13.33	15.02	13.70	9.40	9.78	9.49	10.91	11.27	12.23	16.73	13.86	13.24
Difflugia oblonga 'linearis'	0.00	0.00	0.00	0.00	0.31	0.75	0.00	3.16	1.58	2.28	2.51	0.89	1.09	1.82	1.76	0.36	0.80	0.90	2.79
Difflugia oblonga 'glans'	0.00	0.00	0.39	0.66	0.00	0.00	0.00	0.00	0.40	0.00	0.63	0.44	0.00	0.91	0.00	0.00	0.80	0.00	0.00
Difflugia oblonga (small.short neck)	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia oblonga 'lanceolata'	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia oblonga 'tenuis'	5.84	5.56	5.02	3.95	13.46	10.57	10.26	6.32	8.70	4.11	5.33	9.78	6.57	7.58	9.51	7.19	8.37	7.53	9.41
Difflugia oblonga 'bryophila'	7.39	2.31	4.25	4.93	16.82	13.21	14.53	14.39	9.49	5.02	16.93	16.44	16.79	12.73	12.68	15.11	12.75	9.94	8.36
Difflugia protaeiformis 'protaeiformis'	17.12	22.69	13.90	15.79	14.07	16.98	11.54	15.79	19.76	9.13	11.91	11.11	12.41	9.39	9.51	7.55	9.96	16.57	13.24
Difflugia protaeiformis 'crassa'	1.17	4.63	1.54	0.66	0.92	1.13	0.43	0.70	1.58	0.46	1.25	1.33	2.19	1.82	2.11	0.36	1.20	1.20	2.79
Difflugia protaeiformis 'rapa'	5.06	6.02	6.18	6.25	3.98	4.53	3.85	4.21	3.56	7.76	3.45	7.11	6.20	6.67	5.63	7.19	7.97	6.63	5.92
Cucurbitella tricuspis	42.80	38.89	50.97	36.84	19.57	18.87	20.51	18.25	22.13	25.11	5.02	4.89	4.01	5.76	6.69	7.19	6.77	7.23	12.89
Difflugia urceolata	0.78	0.00	0.00	0.33	0.92	1.51	0.00	1.40	1.58	0.00	0.63	0.00	1.09	0.61	1.06	1.80	0.00	0.00	0.35
Lagenodifflugia vas	1.95	0.46	3.09	7.24	4.28	5.66	3.42	3.16	2.77	0.91	0.63	0.00	0.73	0.61	0.35	1.44	0.40	0.60	1.39
Lesquereusia spiralis	0.00	0.46	0.39	0.00	0.00	0.00	0.43	0.70	0.40	3.65	5.96	2.22	2.92	6.06	8.45	7.55	5.58	7.23	3.83
Pontigulasia compressa	0.00	0.00	0.39	0.00	0.00	0.00	1.28	0.70	1.98	0.46	0.31	1.33	0.36	0.91	1.41	1.44	0.40	0.90	0.00
TEST A	0.00	0.46	0.00	0.00	0.00	1.51	0.00	0.00	1.58	0.00	0.31	1.33	1.46	1.21	2.46	1.08	0.40	0.30	1.74
Difflugia protaeiformis 'multi-spined'	3.50	0.93	3.09	5.26	3.06	3.02	1.28	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.30	0.00

Appendix 2. cont.

Luikonlahti. the mine impacted core RET-99 (% data)																		
SampleID (Depth cm)	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Sample weight (g)	1.441	1.433	1.435	1.296	1.263	1.260	1.298	1.211	1.217	1.216	1.463	1.289	1.159	1.124	1.180	1.219	1.217	1.138
Water content (%)	82.1	79.2	78.8	78.3	75.0	71.5	66.5	70.6	70.1	77.8	77.2	77.1	76.8	75.9	79.4	79.2	79.3	77.8
Total counts (specs)	321	313	262	242	319	282	294	242	290	313	395	245	298	285	328	295	306	297
Counted subsamples (X/8)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Arcella vulgaris	0.00	0.32	2.67	0.41	0.63	0.35	0.68	0.41	0.34	0.96	0.00	0.82	1.01	0.70	0.00	0.00	0.00	0.34
Centropyxis aculeata	11.53	15.65	19.08	10.74	16.30	17.38	20.41	11.16	17.93	14.06	21.01	18.37	16.11	16.14	15.24	15.25	16.67	16.50
Centropyxis aculeata 'discoides'	2.49	1.28	2.67	3.31	2.51	0.71	4.08	4.96	1.03	1.60	1.77	1.63	1.68	1.40	3.05	1.02	0.00	0.67
Centropyxis constricta	4.05	6.07	5.34	4.13	3.76	3.55	3.40	2.07	2.76	1.60	1.77	2.86	1.34	1.75	0.00	0.00	0.00	0.34
Difflugia bidens	0.62	0.32	0.76	1.24	0.63	0.00	0.00	1.24	0.34	1.28	0.51	0.41	0.67	0.35	0.00	0.00	0.33	0.00
Difflugia corona	0.00	0.00	0.00	0.41	0.00	0.00	0.34	0.83	0.34	0.96	0.76	0.41	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia globulus	4.05	2.88	3.05	4.13	2.51	3.55	4.42	5.79	4.83	3.19	4.05	3.27	3.69	5.96	2.74	3.39	2.61	3.37
Difflugia oblonga 'oblonga'	13.71	13.74	10.69	11.98	10.97	12.41	12.59	14.88	11.72	8.31	13.42	11.84	17.45	15.44	20.12	17.29	17.32	14.81
Difflugia oblonga 'linearis'	2.80	3.19	0.76	1.65	3.13	5.32	2.38	4.96	3.10	3.19	1.52	3.67	4.36	2.46	4.27	4.75	3.92	5.39
Difflugia oblonga 'glans'	0.62	0.32	0.00	0.83	0.31	0.00	0.00	0.41	0.69	0.00	0.25	0.00	0.00	1.40	0.91	0.00	0.00	0.00
Difflugia oblonga (small.short neck)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.51	1.22	1.68	0.70	0.00	0.34	1.31	0.67
Difflugia oblonga 'lanceolata'	0.00	0.00	0.00	0.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00	0.00	0.00
Difflugia oblonga 'tenuis'	8.72	10.86	9.54	11.57	10.03	10.99	7.48	10.74	9.66	11.82	10.89	9.80	10.74	12.28	9.45	12.88	9.80	11.78
Difflugia oblonga 'bryophila'	13.08	11.82	11.45	14.88	14.73	15.60	13.61	15.70	12.41	15.02	10.89	10.61	14.09	14.04	10.06	16.61	15.69	15.49
Difflugia protaeiformis 'protaeiformis'	9.03	9.58	5.73	9.50	10.97	8.16	8.50	5.37	7.93	9.27	6.84	8.57	6.04	5.96	8.84	8.81	6.86	7.74
Difflugia protaeiformis 'crassa'	1.25	1.28	2.29	1.65	2.51	3.55	2.04	1.65	2.07	1.60	1.27	2.04	2.01	2.46	2.13	0.68	2.29	1.35
Difflugia protaeiformis 'rapa'	6.54	4.15	6.11	4.55	5.02	4.61	5.44	5.79	3.10	3.83	3.54	4.90	3.36	2.81	4.88	1.69	3.59	2.36
Cucurbitella tricuspis	13.08	9.27	5.73	7.02	5.96	2.48	4.42	4.96	7.24	7.03	7.09	6.94	6.71	6.32	4.88	4.07	9.48	6.40
Difflugia urceolata	0.62	0.00	1.15	0.83	0.31	0.71	0.34	0.41	2.07	2.24	1.52	1.63	0.67	0.70	1.22	2.71	1.63	4.71
Lagenodifflugia vas	0.62	0.96	2.29	2.48	0.94	2.13	1.36	2.07	3.10	3.19	2.53	3.27	1.01	1.05	2.74	0.68	0.65	1.35
Lesquereusia spiralis	0.62	0.96	3.44	1.24	2.82	3.19	1.02	0.83	0.69	2.24	3.29	1.63	1.34	1.75	1.52	0.68	0.65	1.68
Pontigulasia compressa	0.31	1.28	1.15	1.65	1.57	0.00	2.38	1.65	1.38	1.28	1.27	0.82	1.01	0.70	2.13	1.69	0.98	1.35
TEST A	5.92	6.07	6.11	4.96	3.76	5.32	4.76	3.72	6.90	7.03	5.32	5.31	4.70	5.61	5.79	7.46	6.21	3.70
Difflugia protaeiformis 'multi-spined'	0.31	0.00	0.00	0.00	0.63	0.00	0.34	0.41	0.34	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix 2. cont.

Luikonlahti. the reference core RET-23b (% data)												
SampleID (Depth cm)	0	1	2	3	4	9	14	19	24	29	34	37
Sample weight (g)	0.912	1.056	1.005	1.000	0.998	1.000	1.024	0.997	0.971	0.968	1.015	0.983
Water content (%)	93.5	92.5	91.5	91.1	90.4	86.1	84.6	87.0	87.6	86.8	86.3	86.1
Total counts (specs)	224	336	360	371	375	302	385	403	282	257	297	221
Counted subsamples (X/8)	1	1	1	1	1	1	1	1	1	1	1	1
Arcella vulgaris	0.00	0.00	0.00	0.27	0.00	0.00	0.00	0.25	0.35	0.39	0.67	0.45
Centropyxis aculeata	5.80	4.46	4.44	4.58	5.07	6.62	8.05	7.69	7.09	7.78	5.05	6.33
Centropyxis aculeata 'discoides'	0.00	0.60	0.00	0.81	0.00	0.00	0.52	0.74	1.06	0.78	0.67	2.26
Centropyxis constricta	1.79	1.79	1.11	2.16	1.60	1.32	2.86	2.73	2.13	1.95	2.69	0.90
Difflugia bidens	1.79	0.30	0.00	0.54	0.80	0.33	0.78	1.49	1.42	1.56	0.34	1.36
Difflugia corona	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia globulus	3.13	1.79	1.67	1.08	3.73	3.97	2.86	4.96	4.26	2.33	3.03	1.36
Difflugia oblonga 'oblonga'	8.04	7.14	8.06	7.28	8.53	9.60	10.91	11.17	13.48	12.45	11.45	11.31
Difflugia oblonga 'linearis'	4.91	3.27	2.78	3.50	5.33	6.95	3.64	4.22	2.48	1.95	2.36	2.26
Difflugia oblonga 'glans'	0.45	0.30	0.56	0.00	0.27	0.00	0.52	0.00	0.00	0.00	0.00	0.45
Difflugia oblonga (small.short neck)	0.00	0.00	0.00	0.00	0.00	0.00	1.30	0.99	0.00	0.00	0.00	0.00
Difflugia oblonga 'lanceolata'	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia oblonga 'tenuis'	4.46	5.06	7.50	5.12	4.00	6.95	7.53	9.43	10.64	13.23	10.77	9.50
Difflugia oblonga 'bryophila'	0.45	0.89	0.56	0.81	0.27	0.00	1.04	0.74	1.06	0.78	0.67	2.71
Difflugia protaeiformis 'protaeiformis'	29.02	30.95	28.61	29.38	27.47	25.50	21.82	21.59	23.05	22.96	27.95	26.24
Difflugia protaeiformis 'crassa'	1.79	1.49	1.11	1.89	2.67	1.66	0.78	0.99	0.35	0.78	0.67	0.45
Difflugia protaeiformis 'rapa'	2.68	6.25	4.44	5.39	3.47	4.30	2.86	1.49	2.13	3.50	1.01	1.81
Cucurbitella tricuspis	22.77	23.21	24.44	24.53	21.60	11.59	10.13	12.66	10.28	10.89	12.46	13.12
Difflugia urceolata	1.34	0.60	0.28	1.35	1.33	0.66	0.26	0.00	0.00	0.00	0.34	0.45
Lagenodifflugia vas	0.00	1.49	0.83	0.81	1.33	3.64	1.82	2.98	3.55	2.33	1.68	2.71
Lesquereusia spiralis	1.34	0.60	1.67	0.00	1.07	2.32	1.56	0.50	0.71	0.00	0.34	0.90
Pontigulasia compressa	1.34	0.30	1.39	1.08	1.07	0.66	1.30	0.74	0.00	0.39	1.35	0.90
TEST A	0.89	0.00	0.83	0.54	0.53	0.99	2.60	0.74	0.35	0.78	0.34	0.90
Difflugia protaeiformis 'multi-spined'	0.00	0.30	0.28	0.27	0.27	0.33	0.26	0.00	0.00	0.00	0.00	0.00

Appendix 2. cont.

Haveri. the mine impacted core HAV2 (% data)																		
SampleID (Depth cm)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Sample weight (g)	1.325	1.302	1.271	1.057	1.027	1.089	1.097	1.027	1.037	1.063	1.057	1.031	1.047	1.075	1.067	1.068	1.067	1.043
Water content (%)	80.4	75.6	74.3	65.1	63.2	70.6	66.7	66.1	65.7	70.6	71.3	71.1	70.6	66.5	63.6	57.4	59.0	59.2
Total counts (specs)	242	288	322	234	270	249	385	268	261	269	372	359	364	213	276	265	269	256
Counted subsamples (X/8)	2	2	2	2	2	2	2	2	2	2	2	2	2	3	4	4	3	2
Arcella vulgaris	2.07	0.35	1.55	0.85	0.37	0.40	1.04	0.00	1.15	1.12	0.81	1.11	0.27	1.88	1.09	1.13	0.74	0.00
Centropyxis aculeata	4.96	5.21	4.04	2.99	6.30	4.02	4.94	2.61	3.45	5.20	3.23	5.57	3.57	4.69	8.33	4.53	5.58	7.42
Centropyxis aculeata 'species 1'	4.96	1.74	3.42	3.42	1.11	2.41	4.94	2.99	6.90	4.46	1.88	2.79	3.02	2.35	1.45	3.77	1.86	2.34
Centropyxis aculeata 'discoides'	1.65	0.69	0.93	0.00	1.11	0.80	0.52	1.12	1.53	0.00	0.54	1.67	0.55	0.94	0.00	0.38	1.12	1.56
Centropyxis constricta 'aerophila'	2.48	0.69	1.86	2.14	0.37	2.81	2.08	1.49	2.68	1.86	2.69	1.67	4.12	7.98	3.62	1.89	1.49	0.00
Centropyxis constricta 'spinosa'	0.00	0.00	0.93	0.43	4.07	0.40	0.78	1.12	2.30	1.49	3.76	1.39	1.92	4.69	0.36	1.89	1.49	2.34
Centropyxis constricta 'constricta'	0.83	2.43	1.24	0.43	0.00	0.00	0.00	0.00	0.00	0.37	0.27	0.00	1.10	0.94	0.72	0.38	0.00	0.00
Difflugia bidens	0.41	1.04	0.31	0.85	0.74	0.00	0.00	0.00	0.77	1.12	0.27	0.00	0.27	0.00	0.36	0.75	1.49	0.39
Difflugia corona	0.41	0.69	0.31	1.28	0.00	0.40	0.00	0.75	0.00	0.00	0.81	0.00	0.00	0.00	0.00	1.13	0.37	0.00
Difflugia globula	3.72	4.17	2.80	1.71	3.70	3.21	3.38	3.36	3.07	2.60	2.69	1.67	3.57	2.82	3.62	3.40	4.09	3.91
Difflugia oblonga 'oblonga'	10.74	6.60	9.01	8.55	7.04	8.03	8.05	10.07	8.81	13.01	12.90	14.76	10.44	11.27	10.87	7.92	12.27	12.50
Difflugia oblonga 'linearis'	3.31	3.82	2.48	3.42	2.22	1.61	1.30	3.36	1.92	1.86	1.61	1.39	2.20	3.29	2.54	6.79	8.92	7.81
Difflugia oblonga 'glans'	0.00	0.00	0.00	0.43	0.74	0.40	1.30	0.37	0.38	1.12	0.81	0.00	0.27	0.00	0.36	0.38	0.00	0.78
Difflugia oblonga 'spinosa'	0.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.37	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia oblonga 'lanceolata'	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia oblonga 'tenuis'	4.55	5.21	5.59	2.99	5.19	4.02	5.19	4.10	4.21	2.60	4.03	4.46	3.30	5.63	6.88	5.66	5.58	7.81
Difflugia oblonga 'bryophila'	6.61	4.51	6.21	5.13	6.67	5.62	9.09	9.33	8.05	8.18	6.99	5.01	4.40	7.51	8.33	10.57	14.50	14.06
Difflugia protaeiformis 'claviformis'	8.68	9.03	5.28	5.98	6.67	7.23	4.94	4.48	4.98	7.43	6.72	6.41	6.87	4.69	7.25	7.92	9.29	7.42
Difflugia protaeiformis 'acuminata'	0.00	5.21	1.55	1.28	2.96	2.81	2.08	1.87	3.45	2.60	2.69	2.79	3.85	1.41	1.81	4.15	2.97	3.13
Difflugia protaeiformis 'amphoralis'	0.00	5.56	6.21	7.69	10.74	8.43	6.49	7.46	9.20	8.18	9.95	13.37	10.44	11.27	6.88	10.57	5.58	4.69
D.protaeiformis 'species 1'	2.07	1.04	0.62	0.85	0.74	2.01	1.04	0.37	0.77	2.60	1.34	1.11	3.57	1.88	1.45	0.38	1.12	0.39
Difflugia protaeiformis 'multicornis'	2.89	1.74	1.55	2.56	2.59	3.61	1.04	2.99	3.83	2.60	2.42	2.23	2.75	1.88	1.81	1.51	1.86	1.95
Cucurbitella tricuspis	23.97	30.21	27.64	28.63	21.11	28.11	23.64	24.63	21.07	19.70	20.16	17.27	23.08	17.37	18.84	11.32	7.06	8.59
Cucurbitella tricuspis 'spec1'	7.85	4.51	8.70	11.97	8.52	6.43	10.91	12.31	5.36	4.83	9.14	8.64	3.85	3.29	5.43	4.91	1.12	3.91
Difflugia urceolata	0.83	0.35	0.62	0.85	0.74	0.00	0.52	0.00	0.00	1.12	1.08	0.56	0.82	0.00	1.45	0.75	2.60	0.78
Lagenodifflugia vas	2.48	3.47	3.73	1.28	4.07	2.81	3.90	1.87	2.68	1.86	2.42	2.79	2.47	0.47	3.62	3.77	2.23	2.34
Lesquereusia spiralis	3.31	1.04	2.80	4.27	1.48	4.02	2.60	1.87	1.53	2.60	0.00	2.23	1.92	3.76	1.81	1.89	3.35	5.08
Pontigulasia compressa	0.41	0.69	0.62	0.00	0.74	0.40	0.26	1.49	1.53	1.12	0.54	1.11	1.37	0.00	1.09	2.26	3.35	0.78

Appendix 2. cont.

Haveri. the mine impacted core HAV2 (% data)										
SampleID (Depth cm)	18	19	20	21	22	23	24	25	26	27
Sample weight (g)	1.022	0.996	1.012	0.968	1.02	1.015	1.036	1.015	1.007	1.06
Water content (%)	57.6	59.4	61.2	57.1	56.1	52.3	53.1	58.6	63.4	65.2
Total counts (specs)	283	355	323	313	330	290	360	240	308	277
Counted subsamples (X/8)	2	2	2	2	2	2	2	1	1	1
Arcella vulgaris	0.35	2.54	0.31	0.64	0.61	0.34	0.56	0.00	0.32	0.36
Centropyxis aculeata	7.07	8.45	5.88	3.83	6.36	7.24	3.61	3.33	5.84	2.89
Centropyxis aculeata 'species 1'	5.30	3.10	4.64	2.88	0.61	1.03	0.00	1.67	0.00	0.72
Centropyxis aculeata 'discoides'	0.71	0.85	0.93	2.24	0.61	0.34	2.78	1.67	0.65	1.08
Centropyxis constricta 'aerophila'	0.35	0.00	0.62	0.96	0.00	0.00	0.56	0.42	0.00	0.36
Centropyxis constricta 'spinosa'	1.06	0.28	1.24	0.00	1.21	0.00	0.56	0.00	1.62	0.00
Centropyxis constricta 'constricta'	0.00	1.41	0.93	0.00	0.00	0.69	0.00	0.00	0.00	0.00
Difflugia bidens	0.35	0.56	1.24	0.64	0.00	0.00	0.00	0.00	0.65	0.36
Difflugia corona	0.71	0.00	0.31	0.00	0.00	1.03	0.00	0.42	0.00	0.00
Difflugia globula	7.42	5.07	4.64	5.43	5.76	3.79	4.72	3.75	3.90	1.44
Difflugia oblonga 'oblonga'	8.83	13.52	12.69	16.61	15.76	17.93	11.67	13.75	15.26	14.80
Difflugia oblonga 'linearis'	8.48	5.92	8.36	6.39	5.15	10.00	13.06	14.58	6.17	4.69
Difflugia oblonga 'glans'	0.35	0.28	0.93	1.60	0.30	0.00	0.00	0.00	0.65	0.00
Difflugia oblonga 'spinosa'	0.35	0.00	0.00	1.28	0.00	0.00	0.00	0.00	0.32	0.36
Difflugia oblonga 'lanceolata'	0.35	0.85	0.00	0.32	0.30	0.69	0.00	0.00	0.00	0.00
Difflugia oblonga 'tenuis'	7.42	9.58	10.22	8.95	11.21	7.59	9.17	10.83	11.36	7.22
Difflugia oblonga 'bryophila'	11.66	10.14	10.22	8.95	12.12	8.62	11.39	12.50	11.69	17.33
Difflugia protaeiformis 'claviformis'	7.77	8.45	8.36	7.03	8.79	9.31	11.39	9.17	12.01	13.00
Difflugia protaeiformis 'acuminata'	3.18	1.41	2.48	2.56	2.42	4.14	3.06	3.75	4.55	5.05
Difflugia protaeiformis 'amphoralis'	1.41	5.07	7.74	7.67	5.45	7.24	7.22	6.25	5.52	5.42
D.protaeiformis 'species 1'	1.41	0.85	1.86	0.00	2.12	2.07	2.22	1.25	2.27	1.44
Difflugia protaeiformis 'multicornis'	0.71	1.97	0.93	0.64	0.30	0.00	0.00	0.42	0.32	1.08
Cucurbitella tricuspis	12.72	5.63	6.50	3.83	3.33	4.48	2.50	3.75	4.55	3.61
Cucurbitella tricuspis 'spec1'	1.41	2.82	2.17	1.28	1.82	1.03	1.39	0.42	0.97	1.44
Difflugia urceolata	1.77	0.56	1.86	4.47	2.42	1.03	2.22	2.08	0.97	4.69
Lagenodifflugia vas	3.18	5.07	1.55	5.11	6.06	6.21	4.17	5.00	3.90	6.86
Lesquereusia spiralis	4.59	3.94	1.55	2.24	1.82	1.38	1.39	0.42	0.32	1.08
Pontigulasia compressa	1.06	1.69	1.86	4.47	5.45	3.79	6.39	4.58	6.17	4.69

Appendix 2. cont.

Haveri. the reference core HAV4 (% data)																		
SampleID (Depth cm)	0	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	22	24
Sample weight (g)	3.087	3.227	2.368	2.309	2.416	2.405	2.437	1.089	2.482	2.432	2.365	2.08	2.462	2.492	2.46	2.457	2.482	2.462
Water content (%)	91.6	83.2	77.1	54.8	72.1	75.7	73.7	73.2	75.3	68.7	69.7	58.7	56.4	60.3	53.1	46.9	45.7	45.6
Total counts (specs)	138	244	217	228	248	264	219	173	251	230	133	230	266	238	260	202	159	230
Counted subsamples (X/8)	8	5	3	3	4	5	4	8	5	5	8	8	4	5	5	5	4	4
Arcella vulgaris	0.72	0.82	0.00	0.44	0.00	0.76	0.46	1.16	1.20	0.00	2.26	0.43	0.00	0.42	1.92	0.00	0.00	0.00
Centropyxis aculeata	2.17	2.87	1.38	3.51	2.82	3.41	3.65	3.47	2.79	1.74	4.51	2.61	6.77	3.36	4.62	3.47	4.40	4.78
Centropyxis aculeata 'species 1'	0.72	1.23	2.30	1.75	0.40	2.65	2.74	2.31	2.39	3.04	6.77	2.61	1.50	1.68	1.54	0.50	0.63	2.61
Centropyxis aculeata 'discoides'	0.00	0.41	0.92	0.44	2.42	0.76	0.46	1.16	1.99	0.87	2.26	0.43	0.00	2.10	1.15	0.99	1.26	1.74
Centropyxis constricta 'aerophila'	0.72	0.00	0.00	0.44	2.42	0.38	0.00	0.58	1.59	3.91	8.27	0.43	1.13	0.00	1.15	0.50	1.26	1.74
Centropyxis constricta 'spinosa'	0.00	2.05	0.00	2.19	3.63	1.52	1.37	2.31	2.79	1.30	3.01	2.61	4.14	1.68	1.15	0.50	0.00	1.74
Centropyxis constricta 'constricta'	3.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.43	0.75	0.43	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia bidens	1.45	0.00	0.46	0.88	0.81	0.00	0.46	0.00	0.80	0.00	1.50	0.00	0.38	0.00	0.77	0.00	0.00	0.00
Difflugia corona	0.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia globula	0.72	0.00	0.46	0.44	0.00	3.03	3.20	2.89	1.59	0.87	1.50	0.87	1.50	0.42	0.00	0.99	0.00	0.43
Difflugia oblonga 'oblonga'	0.00	0.82	2.76	1.75	1.61	1.14	2.74	2.89	1.59	1.74	1.50	1.74	2.63	3.36	5.38	3.96	5.03	5.22
Difflugia oblonga 'linearis'	7.25	4.92	5.07	5.26	7.26	5.68	3.65	5.78	7.57	6.96	14.29	12.17	9.40	11.34	17.69	19.31	18.24	16.96
Difflugia oblonga 'glans'	3.62	1.64	2.76	0.88	2.02	1.52	2.28	2.89	1.99	3.48	4.51	9.13	6.39	5.04	5.00	10.40	9.43	13.48
Difflugia oblonga 'spinosa'	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.00	0.00	0.00	0.00	0.00	0.84	0.77	1.49	3.14	0.00
Difflugia oblonga 'lanceolata'	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia oblonga 'tenuis'	0.00	0.00	0.00	0.44	0.81	0.00	0.00	0.00	1.99	1.30	0.00	0.00	0.38	0.84	0.77	4.46	4.40	1.30
Difflugia oblonga 'bryophila'	2.90	0.82	1.84	2.19	2.82	1.89	0.46	4.05	3.19	2.17	3.76	8.70	6.77	18.91	21.54	17.82	22.01	8.26
Difflugia protaeiformis 'claviformis'	4.35	0.41	3.23	2.63	2.02	5.30	3.65	4.62	3.59	3.04	12.03	8.70	13.16	14.71	17.69	22.77	16.35	14.35
Difflugia protaeiformis 'acuminata'	2.17	3.28	3.23	4.82	4.03	2.65	2.74	3.47	1.99	3.48	3.76	3.91	2.26	3.36	0.38	0.99	5.03	4.35
Difflugia protaeiformis 'amphoralis'	2.90	1.64	0.00	1.32	0.00	1.52	0.91	2.31	7.57	0.87	0.00	2.17	1.50	2.10	0.38	0.00	1.89	2.61
D.protaeiformis 'species 1'	7.97	3.28	4.15	4.82	7.26	4.55	7.31	8.09	7.57	5.65	6.02	3.48	4.14	7.14	3.46	5.45	1.26	9.57
Difflugia protaeiformis 'multicornis'	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cucurbitella tricuspis	47.83	54.10	49.31	46.05	40.32	38.64	47.49	40.46	35.86	38.26	6.77	23.04	18.05	5.04	5.38	3.47	1.26	4.35
Cucurbitella tricuspis 'spec1'	6.52	18.85	19.35	13.60	13.71	19.32	13.70	7.51	7.97	19.57	10.53	9.13	10.90	10.08	3.46	0.99	0.63	2.61
Difflugia urceolata	0.72	0.00	0.00	0.00	0.00	0.38	0.00	0.00	1.20	0.00	0.75	0.00	1.88	2.10	1.92	0.99	0.00	0.43
Lagenodifflugia vas	2.17	0.82	2.76	4.39	4.84	4.17	1.83	2.89	0.40	0.43	1.50	6.52	6.39	5.04	1.15	0.50	3.77	3.04
Lesquereusia spiralis	0.72	1.23	0.00	1.32	0.81	0.00	0.46	0.58	1.59	0.43	1.50	0.00	0.75	0.42	1.92	0.50	0.00	0.43
Pontigulasia compressa	0.72	0.41	0.00	0.44	0.00	0.76	0.46	0.00	0.00	0.43	1.50	0.87	0.00	0.00	0.77	0.00	0.00	0.00

Appendix 2. cont.

Pyhäsalmi post disturbance samples (% data)																									
SampleID (Site number)	10	9	8	7	6	5	4	3	2	1	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Sample weight (g)	2.606	2.614	3.594	2.701	2.618	2.007	2.035	2.076	1.163	2.172	3.072	3.064	3.092	3.249	2.096	2.047	2.971	2.061	2.537	2.065	2.607	2.600	1.555	2.030	1.454
Water content (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Total counts (specs)	298	257	346	230	242	241	336	421	292	439	380	340	255	298	432	313	271	377	236	213	274	328	299	323	279
Counted subsamples (X/8)	1	1	1	1	1	1	2	2	2	1	1	1	1	1	2	2	1	2	1	1	1	1	1	2	1
Arcella species	0.00	0.34	0.48	0.60	0.00	0.41	0.00	1.16	0.39	0.00	0.79	0.00	0.00	0.34	0.23	0.32	0.37	0.27	0.00	0.47	0.00	0.61	0.00	0.00	0.36
Centropyxis aculeata	9.57	15.07	5.70	7.14	2.49	4.55	3.04	5.20	8.56	9.06	4.47	4.41	2.35	1.01	3.47	1.60	1.48	3.71	0.85	4.23	1.09	2.44	6.02	8.05	5.73
Centropyxis constricta 'aerophila'	3.87	1.37	0.48	0.89	0.00	0.83	0.00	0.00	0.00	0.34	1.05	1.76	0.78	0.00	1.16	0.32	2.58	0.27	0.85	0.00	0.00	0.30	1.00	0.00	0.00
Centropyxis constricta	2.73	1.37	2.14	1.19	1.24	0.83	2.17	2.89	1.17	1.34	1.32	0.29	1.18	2.01	0.69	0.64	1.48	1.06	1.27	0.47	1.09	1.22	2.01	0.93	0.72
Difflugia bidens	2.73	1.03	0.71	2.68	0.83	0.00	0.43	0.00	0.00	0.34	0.00	0.00	0.00	0.34	1.16	0.00	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.08
Difflugia corona	0.00	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.26	0.00	0.00	0.34	0.00	0.32	0.37	0.00	0.00	0.00	1.46	0.30	0.00	0.00	0.00
Difflugia protaeiformis 'multicornis'	4.10	1.71	3.09	1.19	1.24	2.07	3.04	4.34	2.72	3.36	3.95	4.12	1.96	1.68	1.16	2.56	1.85	2.92	1.27	1.88	0.36	2.44	1.34	2.79	1.79
Difflugia globula	1.59	1.37	0.00	0.89	1.24	0.83	1.30	4.62	0.78	2.01	2.37	2.65	1.18	1.01	1.39	1.60	0.37	1.59	0.85	3.29	2.19	1.22	1.34	2.17	0.72
Difflugia oblonga 'oblonga'	6.61	7.19	6.41	2.68	5.81	8.68	7.83	5.78	7.39	8.72	9.21	9.12	12.55	15.77	10.88	8.31	9.23	11.94	10.17	7.04	12.41	10.37	13.38	12.07	5.73
Difflugia oblonga 'linearis'	4.56	2.05	3.33	2.68	3.32	4.13	6.52	4.62	4.67	2.68	6.84	8.53	9.41	7.05	10.65	6.39	9.59	11.14	11.44	9.86	14.96	12.80	12.71	14.24	16.13
Difflugia oblonga 'glans'	2.05	1.03	0.71	0.00	0.00	1.65	0.87	2.31	0.78	0.00	0.79	2.94	1.96	0.34	0.46	0.00	0.37	1.33	2.54	2.35	0.36	1.22	2.34	0.00	0.72
Difflugia oblonga 'spinosa'	0.91	2.74	5.46	2.08	2.49	1.24	0.00	0.29	2.33	1.68	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.36
Difflugia oblonga 'lanceolata'	0.68	0.00	2.61	1.79	0.83	1.24	0.87	1.16	0.00	0.00	0.53	0.00	1.96	1.34	1.39	0.32	1.48	0.27	0.42	3.76	0.73	1.22	0.00	0.00	0.00
Difflugia oblonga 'tenuis'	5.24	4.11	4.04	4.46	2.90	7.02	6.96	4.05	4.28	5.70	4.21	5.59	9.41	5.70	5.56	6.71	3.69	8.22	5.08	7.04	6.57	3.96	4.35	5.57	5.02
Difflugia oblonga 'bryophila'	7.97	4.79	5.23	4.76	6.22	8.26	8.70	8.96	3.11	6.71	10.26	9.12	14.51	10.07	10.88	13.10	13.28	9.02	13.14	9.39	10.22	11.28	9.03	7.74	8.96
Difflugia protaeiformis (acuminata)	5.47	3.42	5.70	8.93	6.64	12.40	9.13	4.62	9.34	7.72	13.42	11.76	8.63	15.44	10.65	14.06	15.87	12.20	8.05	7.04	10.95	13.11	11.71	14.24	17.20
Difflugia protaeiformis 'claviformis'	5.01	3.42	4.28	2.68	2.07	2.07	2.61	2.31	1.95	3.36	2.11	3.53	0.78	2.01	2.55	1.60	3.69	2.65	2.54	3.76	1.09	0.91	0.33	0.00	0.36
Difflugia protaeiformis 'acuminata'	4.33	2.74	3.80	1.49	2.90	2.07	3.91	4.91	1.56	1.34	3.68	1.76	4.31	4.36	5.09	7.67	3.32	2.92	3.39	6.57	4.01	4.88	3.01	4.33	5.02
Difflugia protaeiformis 'amphoralis'	4.78	8.22	8.31	6.85	11.62	6.20	6.09	6.36	8.17	5.70	11.05	10.29	9.41	8.05	8.56	11.18	5.17	6.63	9.32	6.57	9.12	8.54	8.36	7.43	7.17
Cucurbitella tricuspis	8.20	23.97	27.32	34.23	35.27	23.97	20.00	20.23	34.24	29.53	10.26	12.94	10.20	13.76	13.43	11.18	13.28	12.20	14.41	12.21	15.33	10.37	13.04	12.38	12.54
Difflugia urceolata 'urceolata'	0.46	0.00	0.00	0.00	0.00	0.41	0.87	0.87	0.00	0.34	0.26	0.00	0.78	0.67	0.23	0.64	0.74	0.53	2.12	0.94	0.36	1.22	0.33	0.00	0.72
Difflugia urceolata 'ebes'	2.51	1.37	0.48	1.19	0.00	0.41	0.87	1.45	0.78	0.00	1.32	1.18	1.18	2.01	1.62	3.51	1.11	3.18	0.42	3.29	0.73	1.22	0.00	1.86	0.72
Lagenodifflugia vas	5.92	2.05	3.33	2.38	1.24	2.48	5.65	8.38	0.78	1.34	3.42	4.12	2.75	1.34	4.40	2.88	7.75	3.18	8.05	4.69	3.28	5.18	3.68	3.10	3.58
Lesquereusia spiralis	7.97	8.22	4.51	7.14	9.13	4.96	4.35	3.76	5.84	6.38	3.68	3.24	1.57	1.68	1.16	2.88	0.74	1.59	3.39	1.88	1.09	1.83	2.01	0.62	3.58
Pontigulasia compressa	2.05	0.68	0.71	1.19	2.07	2.48	3.48	1.45	0.78	1.01	3.16	2.06	2.35	2.01	2.31	1.92	1.11	2.65	0.42	2.82	2.19	3.35	3.34	1.86	1.43

Appendix 2. cont.

Pyhäsalmi peak disturbance samples (% data)																									
SampleID (Site number)	10	9	8	7	6	5	4	3	2	1	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Sample weight (g)	1.043	1.075	1.035	1.097	1.156	1.196	1.143	1.112	1.054	1.095	1.180	1.110	1.223	1.106	1.132	1.174	1.071	1.095	1.073	1.092	1.120	1.191	1.052	1.148	1.195
Water content (%)	79.8	78.0	76.3	81.2	80.9	77.9	78.8	77.3	73.5	67.4	80.9	80.1	85.2	86.2	85.4	85.0	83.7	82.3	84.1	87.2	86.3	85.2	79.4	83.4	81.5
Total counts (specs)	191	355	266	288	229	272	203	153	368	352	274	160	242	202	238	218	238	246	217	177	232	245	238	250	312
Counted subsamples (X/8)	1	2	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Arcella species	0.28	0.00	0.65	0.49	0.37	0.00	0.00	0.00	0.28	0.00	0.36	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.22	0.00	0.40	0.00
Centropyxis aculeata	11.93	8.97	7.19	4.43	6.25	3.49	2.78	2.63	12.11	8.38	8.39	16.25	5.79	2.97	4.74	3.67	4.20	4.07	3.23	2.82	6.90	2.45	10.50	6.40	8.65
Centropyxis constricta 'aerophila'	0.85	1.09	0.00	0.49	0.00	0.00	0.35	0.00	0.85	0.52	2.19	1.88	1.24	0.99	0.00	0.00	0.00	0.00	0.46	0.56	1.29	0.00	0.00	0.00	0.32
Centropyxis constricta	2.56	1.09	0.65	1.48	1.10	0.87	0.00	2.63	2.54	3.66	1.82	2.50	1.65	0.99	1.90	2.75	0.84	1.63	0.00	0.00	1.72	2.45	2.10	1.20	1.92
Difflugia bidens	0.85	0.54	0.00	0.00	0.74	0.00	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32
Difflugia corona	0.28	0.00	0.00	0.49	0.00	0.44	1.39	1.50	0.28	0.00	2.19	1.25	0.41	0.99	1.42	1.38	0.00	0.41	0.92	0.56	2.16	1.63	2.52	1.60	0.96
Difflugia protaeiformis 'multicornis'	1.70	2.99	1.31	3.45	2.94	0.44	4.17	3.01	2.54	2.09	1.82	1.25	3.72	1.98	0.47	0.46	2.10	1.63	1.84	0.56	0.86	1.22	0.00	1.60	0.96
Difflugia globula	0.00	1.09	0.00	0.00	0.37	0.44	0.35	0.00	0.28	0.00	2.92	0.63	1.24	0.00	1.42	0.92	1.68	2.44	1.38	0.00	0.43	1.22	0.42	1.60	2.24
Difflugia oblonga 'oblonga'	3.41	6.52	3.27	7.39	4.04	5.24	4.86	6.02	5.63	9.42	6.20	11.88	10.33	8.91	10.43	6.42	11.76	5.28	9.68	12.43	6.90	11.02	11.76	11.60	8.97
Difflugia oblonga 'linearis'	5.97	4.62	5.88	6.40	6.25	6.99	5.21	4.14	1.97	5.24	8.76	7.50	9.92	6.93	13.27	11.47	13.45	11.79	15.21	7.91	14.22	12.24	8.82	12.80	13.46
Difflugia oblonga 'glans'	1.14	0.54	0.00	0.49	0.00	0.00	0.69	1.13	2.25	1.05	0.36	0.63	0.41	0.50	0.00	0.46	0.00	0.00	1.38	0.00	0.43	0.82	0.00	0.40	0.64
Difflugia oblonga 'spinosa'	1.70	0.54	3.27	1.97	1.84	3.06	3.13	1.50	1.69	1.05	0.36	0.00	0.00	0.50	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia oblonga 'lanceolata'	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38	1.13	0.00	0.36	0.63	1.24	0.00	0.00	0.00	2.52	1.22	1.38	1.13	0.86	1.22	0.84	0.80	0.96
Difflugia oblonga 'tenuis'	5.68	7.61	5.23	5.42	5.15	6.55	7.64	7.14	5.35	3.14	4.74	6.25	4.55	6.93	4.74	7.80	9.24	6.91	7.37	10.73	8.62	4.49	6.72	6.00	6.09
Difflugia oblonga 'bryophila'	10.23	8.97	7.84	10.34	7.35	11.35	14.93	7.89	9.30	8.90	12.04	4.38	7.44	14.36	17.06	12.84	9.24	12.20	11.06	9.04	11.21	11.43	12.18	14.80	12.82
Difflugia protaeiformis (acuminata)	3.41	6.79	4.58	9.85	5.88	7.86	5.90	7.89	8.45	4.71	5.84	6.88	6.61	9.41	4.27	11.93	9.66	10.57	7.37	11.30	7.33	8.98	8.82	6.40	8.65
Difflugia protaeiformis 'claviformis'	4.26	2.72	1.96	0.49	0.74	5.24	2.78	2.63	3.66	2.62	3.28	3.13	0.83	2.97	1.42	1.38	2.94	1.63	0.92	1.69	1.72	0.41	0.00	0.80	2.56
Difflugia protaeiformis 'acuminata'	1.70	2.17	1.31	2.46	4.41	1.75	4.17	4.89	3.38	1.05	3.65	1.88	4.96	2.97	1.90	3.21	2.52	4.47	2.30	3.95	2.59	4.08	2.94	2.40	2.56
Difflugia protaeiformis 'amphoralis'	7.95	11.96	7.19	10.84	7.35	8.73	10.76	9.02	7.89	12.04	9.49	13.75	13.64	7.92	9.48	9.17	6.72	6.50	6.91	11.86	9.91	9.80	8.82	8.40	5.45
Cucurbitella tricuspis	17.05	19.29	37.91	26.60	34.93	24.45	18.06	25.94	16.90	16.75	8.76	10.63	15.29	21.29	14.69	15.14	13.03	15.04	13.82	11.86	9.91	16.33	9.66	11.60	11.54
Difflugia urceolata 'urceolata'	0.57	0.00	0.00	0.00	0.00	0.00	1.04	0.00	1.13	2.62	0.36	0.00	0.41	0.50	0.00	0.00	1.26	0.00	0.92	0.56	0.00	0.00	0.00	0.00	0.00
Difflugia urceolata 'lebes'	1.70	0.27	0.65	0.00	1.10	0.87	0.69	1.50	1.13	0.00	1.82	0.63	0.41	0.50	6.16	1.38	0.42	1.63	2.30	0.56	1.72	0.00	2.10	0.40	0.96
Lagenodifflugia vas	8.24	4.89	1.31	2.96	1.47	4.37	4.51	3.76	3.94	8.38	6.93	3.13	4.13	2.97	1.42	4.13	4.20	5.28	6.45	7.91	5.17	3.27	5.88	4.80	3.21
Lesquereusia spiralis	5.68	2.72	8.50	3.94	7.72	5.68	5.56	3.38	4.51	5.24	2.92	1.88	3.31	2.48	1.90	1.83	2.10	2.85	3.23	1.69	3.45	3.27	2.94	2.40	1.60
Pontigulasia compressa	2.84	4.35	1.31	0.00	0.00	2.18	1.04	2.63	2.54	3.14	3.65	3.13	1.24	1.98	1.90	2.75	1.26	2.03	1.84	2.26	1.29	1.63	2.52	2.80	4.17

Appendix 2. cont.

Pyhäsalmi pre disturbance samples (% data)																									
SampleID (Site number)	10	9	8	7	6	5	4	3	2	1	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Sample weight (g)	1.014	1.008	1.066	1.047	1.063	1.059	1.109	1.070	1.003	1.076	1.029	1.131	1.088	1.150	1.141	1.064	1.055	1.086	1.121	1.128	1.173	1.215	1.177	1.177	1.224
Water content (%)	77.0	76.8	79.2	81.6	82.3	80.6	77.0	76.1	70.6	72.0	82.1	84.0	85.4	87.5	85.2	85.1	86.0	83.6	83.0	79.4	87.6	84.5	84.8	85.0	83.3
Total counts (specs)	354	279	382	236	356	204	270	259	293	260	381	240	266	248	262	211	198	212	207	165	181	223	227	221	372
Counted subsamples (X/8)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	4	3	3	1	1	1
Arcella species	0.00	0.34	0.39	1.11	0.00	0.00	0.00	0.26	0.00	0.28	0.00	0.00	0.38	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.00	0.00	0.27
Centropyxis aculeata	15.00	8.87	7.34	5.56	1.47	0.56	0.42	3.14	7.89	15.54	6.30	1.67	4.51	4.03	4.58	5.21	3.03	4.72	2.90	3.03	2.76	6.73	6.61	6.79	11.56
Centropyxis constricta 'aerophila'	1.92	2.05	1.93	1.11	1.47	0.56	0.00	1.31	1.79	1.13	0.00	1.25	1.13	0.81	0.38	0.00	0.00	0.00	0.00	1.21	1.10	0.00	0.88	0.90	0.81
Centropyxis constricta	3.85	5.46	3.09	4.81	2.94	2.25	2.54	4.19	5.02	4.24	6.56	2.08	1.88	2.42	0.76	4.27	1.01	1.42	0.00	0.00	1.66	0.90	3.52	1.36	3.76
Difflugia bidens	0.00	0.00	0.00	0.74	0.00	0.00	0.00	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.54
Difflugia corona	0.38	0.00	0.39	0.37	1.47	0.56	1.27	1.31	0.00	0.28	6.56	0.00	1.50	1.61	2.29	0.95	2.02	0.00	2.42	2.42	2.21	2.24	2.20	2.71	1.08
Difflugia protaeiformis 'multicornis'	0.38	1.37	1.93	0.37	1.47	0.00	3.39	1.31	1.08	1.98	0.00	0.83	2.26	0.40	0.38	0.47	0.00	0.94	0.00	0.00	0.00	0.00	0.00	0.00	0.81
Difflugia globula	3.46	1.37	1.93	0.74	0.49	4.49	3.81	3.40	2.51	1.98	2.62	2.92	0.38	0.81	1.91	0.95	1.01	2.83	4.83	1.21	1.10	1.35	3.08	0.00	3.49
Difflugia oblonga 'oblonga'	10.00	8.19	10.04	7.78	12.25	6.18	5.08	7.85	10.04	7.34	8.40	12.08	7.89	8.06	11.07	7.11	6.57	12.74	16.43	14.55	8.84	11.21	10.57	11.31	8.87
Difflugia oblonga 'linearis'	10.77	9.90	7.34	7.78	10.29	12.36	13.14	7.85	2.51	5.08	7.35	15.83	9.77	10.48	8.78	13.74	15.66	11.32	25.12	23.64	23.76	17.94	16.30	16.29	15.86
Difflugia oblonga 'glans'	1.15	0.68	0.39	0.00	0.00	0.00	0.00	0.26	0.00	0.85	0.00	0.83	0.38	0.00	0.76	0.00	0.51	0.00	1.93	3.03	6.08	1.35	0.44	0.90	0.81
Difflugia oblonga 'spinosa'	0.00	0.68	0.00	0.00	0.49	0.00	0.00	0.26	0.36	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Difflugia oblonga 'lanceolata'	0.00	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.10	0.83	1.88	2.02	3.44	1.90	1.52	2.36	0.00	0.00	0.00	0.00	1.76	2.26	1.08
Difflugia oblonga 'tenuis'	4.23	6.83	5.02	5.19	6.37	8.99	4.24	4.97	6.09	5.93	7.87	7.92	9.40	10.48	7.25	6.16	10.10	10.38	3.86	8.48	3.87	5.38	5.29	6.33	4.57
Difflugia oblonga 'bryophila'	15.00	13.99	11.97	13.33	18.63	18.82	19.07	15.71	14.70	9.60	17.32	21.25	15.04	11.69	14.12	19.91	15.15	14.62	18.36	21.21	14.92	28.25	14.54	14.48	14.52
Difflugia protaeiformis (acuminata)	3.85	8.53	10.42	14.44	11.27	8.99	10.17	9.16	8.24	14.69	4.20	5.83	7.52	7.66	6.49	6.16	5.56	4.25	1.45	1.21	2.21	2.24	5.29	5.43	7.53
Difflugia protaeiformis 'claviformis'	1.54	0.34	3.47	1.11	0.49	1.40	1.27	1.83	2.87	1.98	0.26	1.25	1.13	1.21	1.91	1.42	0.51	0.00	0.00	0.00	0.00	0.45	0.00	0.45	1.08
Difflugia protaeiformis 'acuminata'	2.69	1.37	2.32	3.70	3.92	3.65	2.97	2.36	1.79	1.69	3.67	2.92	2.63	4.84	3.05	3.79	3.54	3.77	1.45	0.61	1.10	0.45	2.20	2.26	2.15
Difflugia protaeiformis 'amphoralis'	8.46	6.83	10.81	8.52	6.37	8.71	10.59	8.38	11.47	9.32	10.50	12.08	11.65	13.71	14.89	13.74	14.14	12.26	7.73	6.67	14.92	6.28	6.61	10.41	7.26
Cucurbitella tricuspis	7.31	11.60	14.29	13.70	9.31	9.83	12.71	9.69	12.19	9.60	6.30	6.67	11.65	11.69	8.78	8.53	11.62	11.79	1.93	3.64	6.08	3.59	11.89	6.33	7.80
Difflugia urceolata 'urceolata'	0.77	0.34	0.00	0.74	0.49	0.28	0.42	0.26	0.00	0.28	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.55	0.45	0.44	0.00	0.00
Difflugia urceolata 'lebes'	0.77	1.02	1.54	1.11	0.00	3.65	1.27	4.97	2.15	0.85	1.31	0.42	1.50	1.61	0.76	3.32	3.03	2.83	3.38	0.00	2.21	0.00	3.08	1.81	1.88
Lagenodifflugia vas	4.62	5.12	3.47	4.44	4.41	3.65	2.54	6.81	3.94	2.54	4.20	1.25	3.01	1.21	4.20	0.95	1.52	1.89	4.83	4.85	3.87	3.59	3.52	1.36	1.61
Lesquereusia spiralis	3.85	2.39	1.54	2.59	5.39	0.84	0.42	2.36	4.66	2.26	2.10	0.83	1.13	1.61	1.53	0.47	0.51	0.47	0.00	0.00	0.55	0.45	0.44	2.71	2.15
Pontigulasia compressa	0,00	2,39	0,39	0,37	0,98	3,65	3,81	1,83	0,00	1,69	1,84	0,83	1,88	1,61	1,53	0,00	1,52	1,42	2,90	3,64	1,10	5,38	0,88	4,98	0,54