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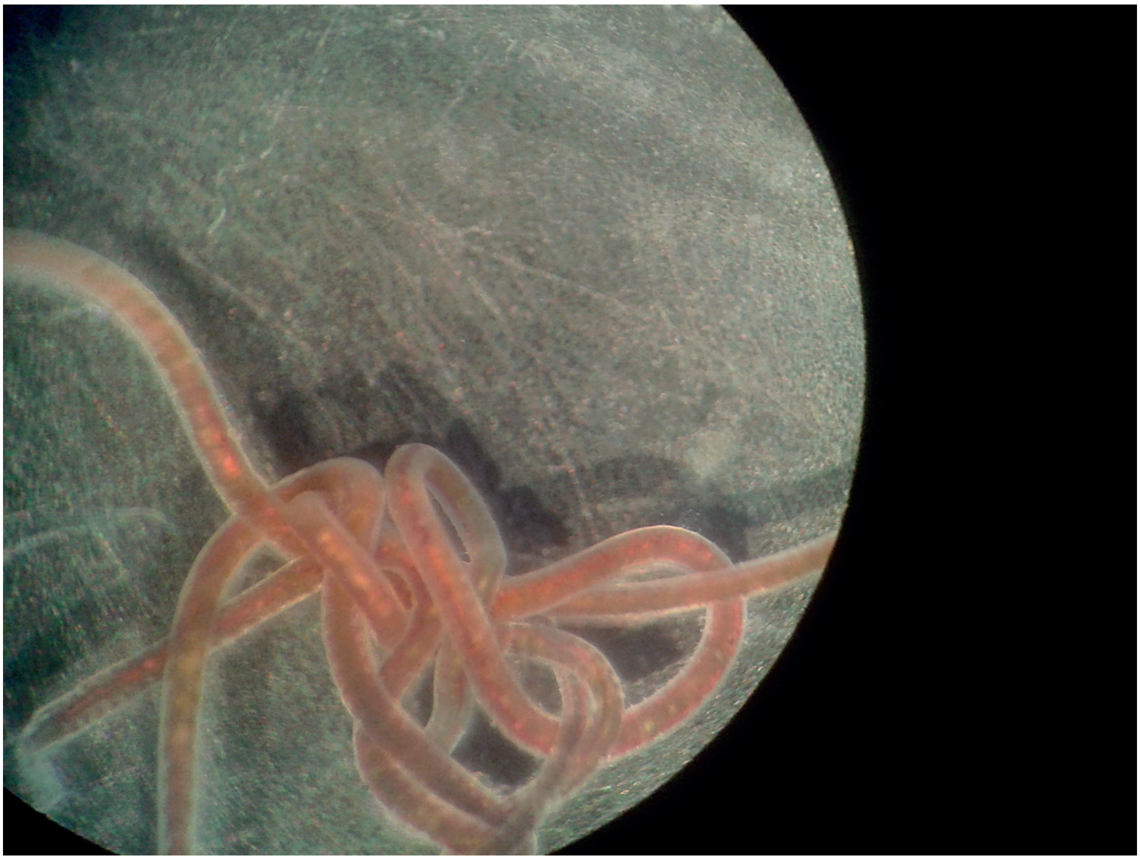
Jaana Wallin

Aquatic Effects and Risk Assessment of Multi-metal Leachates from Metal Mining and Acid Sulphate Soils



UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS AND SCIENCE

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Aquatic Effects and Risk Assessment of Multi-metal Leachates from Metal Mining and Acid Sulphate Soils

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
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in building Ambiotica, hall YAA303, on August 24, 2018 at 12 o'clock noon.

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ABSTRACT

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Diss.

Leachates and effluents from acid sulphate soils and metal mining compose a significant part of metal and sulphate loads into aquatic environments in Finland. Acid sulphate soils have deteriorated the water systems of the Ostrobothnian region for several decades. Discharges from metal mining have similar characteristics as leachates from acid sulphate soils. The extent of metal contamination and its effects were surveyed altogether in 10 estuaries in western Finland and in 9 lakes affected by biomining and conventional mining activities. Assessment also included monitoring data and toxicity tests with benthic macroinvertebrates. Metal concentrations in the estuary sediments were elevated and acidity was transported to the estuaries. The most evident acid sulphate soil induced effect was deterioration of the benthic communities in the estuaries. The risk of having negative effects due to acid sulphate soils was elevated in 9 out of 10 estuaries. Effects observed in the mining-affected lakes were comparable to effects induced by acid sulphate soil leachates. In both mining sites, concentrations of the ore metals were elevated especially in the lake sediments close to the mine. Sequential extractions of the sediments suggested that the partitioning of the ore metals Ni and Cu in the studied sediments were of anthropogenic origin. The laboratory toxicity tests with the standard test species *Lumbriculus variegatus* indicated reduced growth and reproduction and altered behavioural responses, with growth and reproduction being more sensitive endpoints. However, behavioural responses seemed to be sensitive enough for quick screening-level assessments. The field-collected lake sediments did not sustain their original properties, such as pH, which made their ecotoxicity assessment challenging. Thus, there remains a need to develop methods for comprehensive risk assessment of challenging sediments.

Keywords: Acid sulphate soils; benthic macroinvertebrates; ecotoxicity; metals; mining; risk assessment; sediment.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I–III.

The original idea in I was by K-MV, and by AKK in II and III. I took part in the planning of I–III with other co-authors. AV was responsible for elemental analyses in II and III. I was responsible for the majority of laboratory work of I–III. Minor contributions are mentioned in the acknowledgements of each paper. I analysed the data with help from co-authors and wrote the first drafts of I–III. All the co-authors participated in the finalization of the papers.

I Wallin J., Karjalainen A.K., Schultz E., Järvistö J., Leppänen M.T. & Vuori K.-M. 2015. Weight-of-evidence approach in assessment of ecotoxicological risks of acid sulphate soils in the Baltic Sea river estuaries. *Science of The Total Environment* 508: 452–461.

II Wallin J., Karjalainen J.S., Väisänen A., Kukkonen J.V.K. & Karjalainen A.K. 2018. Ecotoxicity assessment of mining-affected lake sediments by *Lumbriculus variegatus* biotest. Manuscript.

III Wallin J., Vuori K.-M., Väisänen A., Salmelin J. & Karjalainen A.K. 2018. *Lumbriculus variegatus* (Annelida) biological responses and sediment sequential extractions indicate ecotoxicity of lake sediments contaminated by biomining. *Science of The Total Environment* 645: 1253–1263.

ABBREVIATIONS

AA	annual average
AFW	artificial freshwater
AMD	acid mine drainage
AS	acid sulphate
BBI	brackish water benthic index
DI	deformity index
DOC	dissolved organic carbon
dw	dry weight
EC_{50}	effective concentration that yields 50% response in the test population
EQS	Environmental Quality Standard
EPA	United States Environmental Protection Agency
EC	European Council
EU	European Union
HLW	hypolimnetic water
HQ	hazard quotient
Hz	hertz
ISO	International Organization for Standardization
JYU	University of Jyväskylä
LC_{50}	concentration lethal to 50% of the test population
LOI	loss on ignition
MAC	Maximum Acceptable Concentration
MFB	multispecies freshwater biomonitor
OECD	Organisation for Economic Co-operation and Development
SEs	sequential extractions
SFS	Finnish Standards Association
SYKE	Finnish Environment Institute
SQC	sediment quality criteria
SQG	sediment quality guideline
t	temperature, °C
TIC	total inorganic carbon
TOC	total organic carbon
WFD	Water Framework Directive
ww	wet weight

1 INTRODUCTION

Aquatic environments are an essential part of the national identity and heritage of Finland, a country often described by the internationally advertised catch phrase 'land of a thousand lakes' (Alahuhta *et al.* 2013). Clean waters are valued and their recreational value is significant for the country (Vesterinen *et al.* 2010, Artell 2014). Historically, water systems have been essential for the development of permanent population and nowadays society is dependent on water bodies in various ways (Horppila and Muotka 2011, Alahuhta *et al.* 2013). Yet industrial development has been extensive and many water bodies have been impaired from the vast use of natural resources (Alahuhta *et al.* 2013). In the EU member countries, a trigger for harmonisation of protection, enhancement and restoration of aquatic environments has been the WFD (2000/60/EC). At present, the ecological and chemical status of most of the inland water bodies in Finland is classified as good (Mäenpää 2011). Although many water systems are recovered or in recovery, there are several unresolved questions regarding the protection of aquatic environments. There are both nonpoint source and point source environmental issues which need assessment as the environment changes. In this thesis, the focus is on sediments, which can reflect the past and the future of Finnish water bodies.

1.1 Significant sources of metals and sulphate

1.1.1 Nonpoint source: acid sulphate soils

The area of acid sulphate (AS) soils is larger in Finland than in any other European country, and although notable areas are also situated in Sweden, the most abundant areas of AS soils are in tropical areas (Roos and Åström 2005, Andriesse and van Mensvoort 2006). Depending on the classification criteria, the estimated area of AS soils varies from 43,000 to 340,000 ha in Finland and approximately 5000 ha in Sweden (Palko 1994, Yli-Halla *et al.* 1999, Andriesse and van Mensvoort 2006). Finnish AS soils were formed during the Litorina Sea period of Baltic Sea approximately 8000–4000 years BP (Palko 1994). Acid sulphate soils were developed when microbial activity reduced sulphide rich sediments under anoxic conditions and they now lie above sea level because of isostatic land uplift (e.g. Dent and Pons 1995, Nystrand and Österholm 2013). The area of former Litorina Sea and potential AS soils is nowadays on the western coast of Finland (Yli-Halla *et al.* 2017, Anon. 2018a). The special characteristic of Finnish AS soils is their relatively low sulphur content in comparison with tropical AS soils (Åström and Björklund 1997, Yli-Halla *et al.* 1999). However, the buffering capacity of AS soils is low in Finland, which makes them as potentially hazardous as the global AS soils (Dent and Pons 1995, Åström and Björklund 1997, Yli-Halla *et al.* 1999).

As undisturbed and below groundwater level, AS soils remain reduced (Dent and Pons 1995). The environmental issues arise when the AS soils are exposed to atmospheric oxygen. Land use activities, such as agricultural use with ditching or draining, lower the groundwater level. Sulphides are oxidized to sulphates and sulphuric acid is formed (Dent and Pons 1995). Acidity and declining pH result in the dissolution of metals. When the water level rises especially during high discharge period, the metals and acidity are transported together to receiving water bodies. Therefore, high amounts of metals are leached from AS soils in spring and autumn, when snow thaws and precipitation is high. Metals that are most easily leached from Finnish AS soils are Al, Cd, Co, Cu, Mn, Ni and Zn (e.g. Åström and Björklund 1997, Åström and Corin 2000, Boman *et al.* 2010). It has been estimated that, between 1972 and 2002, metal loads from ASs exceeded that of industrial effluents in Finland (Sundström *et al.* 2002).

Stream research concerning sites affected by AS soils has been quite abundant in comparison to estuary locations. Moreover, knowledge of the effects of AS soils on the estuary sites is scarce. The most apparent reported effects from river sites include mass deaths of adult fish, which were most recently observed in autumn and winter 2006–2007 (Nordmyr *et al.* 2008a, Toivonen and Österholm 2011). Besides adult fish, leachates from AS soils can have adverse effects on, for example, fish larvae and benthic invertebrate communities (Hudd and Kjellman 2002, Fältmarsch *et al.* 2008).

1.1.2 Point source: metal mining

Modern technology demands vast amounts of metals and many old mines are running out of ore. Mining activities are growing worldwide and emissions from mining are expected to grow as well. There is also a pressure to mine in remote or sensitive areas (Anon. 2008). Mining activities in boreal regions often occur close to freshwater reservoirs, hence exploiting these areas can be a significant threat to aquatic ecosystem health (Kreutzweiser *et al.* 2013, Thienpont *et al.* 2016, Väänänen *et al.* 2016).

Mining operations in Finland have a long history, and in recent years the metal mining industry has grown (Anon. 2017). Metal production has focused on Cu, Ni, Zn, Co, Cr, Fe and V. Advances in mining technology have led to a situation where deposits with low-grade ore can be utilized with non-conventional techniques (Rawlings and Johnson 2007). Biomining, in particular, has been in the headlines in Finland during the last decade, and analysed from every possible perspective, from environmental to social (Sairinen *et al.* 2017). Biomining processes enable the recovery of valuable metals from low-grade ore with bacteria. Yet even though biomining is commonly referred to as being a more environmentally friendly option than conventional mining, environmental impacts cannot be excluded (Jerez 2017).

Emissions from metal mining have similar properties as those from AS soils. Both are of anthropogenic origin generally but mining effluents come more commonly from point source. Metals are often released to the environment from mining sites because of accidents, such as leakages from wastewater ponds or the breaking of tailings dams, and effluents that comply with environmental permission procedures. Effluents and leachates from mining sites are often heterogeneous and their characteristics such as pH, metal composition and conductivity can fluctuate considerably over time (Dudka and Adriano 1997, Mäkinen and Lerssi 2007, Nieto *et al.* 2013). In addition to metals, mining effluents may contain other chemicals used in the mining processes. For example, the pH of the effluents may need adjustment so that their pH is in the range defined in the environmental permit (Heikkinen and Väisänen 2007). This can be a significant source of major ions over time. Thus, the effluents are complex mixtures of contaminants.

Metal mining has substantial environmental effects and emissions related to mining are known to alter ecosystem processes (Dudka and Adriano 1997, Chapman *et al.* 1998, Cadmus *et al.* 2016). For example, declines in benthic macroinvertebrate abundances and alterations in zooplankton and phytoplankton community compositions together with reduced species richness have been reported (Cadmus *et al.* 2016, Leppänen *et al.* 2017). Ecotoxicity of stream waters affected by mining effluents are quite well reported (e.g. Gerhardt *et al.* 2004, Sardo and Soares 2010, Cadmus *et al.* 2016) but less information is available on the ecotoxicity of mining-affected lake sediments (Väänänen 2017). Especially knowledge of the lake sites affected by biomining is fairly restricted.

1.2 Sediments as sink and source of metals

Boreal lakes are globally important freshwater reservoirs. In the boreal region of northern Europe the winter is long, average temperature is low and recovery from pollution can be long (Anon. 2008). Thus, they are sensitive to excess ion loading. Metals are stable and are not broken down in the environment. In the freshwater catchments, metals are transported from effluent sources onwards into the receiving streams and lakes. Substances that are discharged into the aquatic systems can sink to sediments (Chapman *et al.* 1999b, Burton 2010, Tessier *et al.* 2011). Environmental conditions such as pH, temperature, water hardness and association with organic matter will determine the metal speciation in aquatic systems and where the metals will eventually be precipitated (Chapman *et al.* 1998, Cadmus *et al.* 2016). In the estuaries, precipitation is also controlled by dilution with saline brackish water (Nystrand *et al.* 2016).

Although sediments are often referred to as sinks of elements, their dynamic nature is recognized and sediments can act as a source of metals (Chapman *et al.* 1999b). As in the water phase, bioavailability and toxicity are determined by sediment's physical and chemical properties, and metal speciation is metal-, site- and time-specific (Chapman *et al.* 1998, Vink 2009). Sediment-bound non-bioavailable metals can become bioavailable when environmental conditions change in the sediment–water interface and when they are taken up by the exposed biota (Chapman *et al.* 1998, Vink 2009, Burton 2010, Camusso *et al.* 2012).

Sediment-dwelling organisms can also have an effect on the mobility and toxicity of sediment-deposited metals (Burton 2010, Blankson and Klerks 2016, Blankson *et al.* 2017). For example, deposit-feeding oligochaetes burrow in sediments and are able to modify the sediment structure (e.g. Leppänen and Kukkonen 1998, Thibodeaux and Bierman 2003). Bioturbation has been shown to release metals from sediments into the water phase (Peterson *et al.* 1996, Chandler *et al.* 2014, Blankson and Klerks 2016). Sediment characteristics are not solely responsible for the bioavailability of contaminants and bioturbation can have a locally significant effect on metal mobilization from sediments (Remaili *et al.* 2016).

1.3 Assessment of contaminated sediments

1.3.1 Risk assessment

Ecological risk is the combined outcome of exposure and effects (Suter 2007). There are several risk assessment methods and frameworks. The most common risk assessment framework consists of problem formulation, analysis and risk characterization (Fig. 1). The problem formulation phase includes gathering and integrating the available information for a risk assessment plan. The analysis portion consists of characterizing exposure and effects, and in the risk characterization phase the final risk is evaluated and described (Suter 2007). The aim of risk characterization is to produce reliable information for risk management, and regarding sediments the emphasis should be on the biological aspect rather than chemistry (Chapman and Smith 2012).

Sediments are complex and heterogeneous in nature, and their detailed physicochemical characterization is often impossible. Contaminant distribution in sediments is also often patchy (Burton and Johnston 2010). Concentrations vary horizontally and vertically, but also temporally. Risks associated with metals can fluctuate when bioavailability under dynamic environmental conditions due to seasons is considered (Väänänen *et al.* 2016). Furthermore, sediments are commonly contaminated with mixtures of metals rather than with a single element (Väänänen *et al.* 2018), which complicates their risk assessment (Chapman 2001, Norwood *et al.* 2003, Kreutzweiser *et al.* 2013).

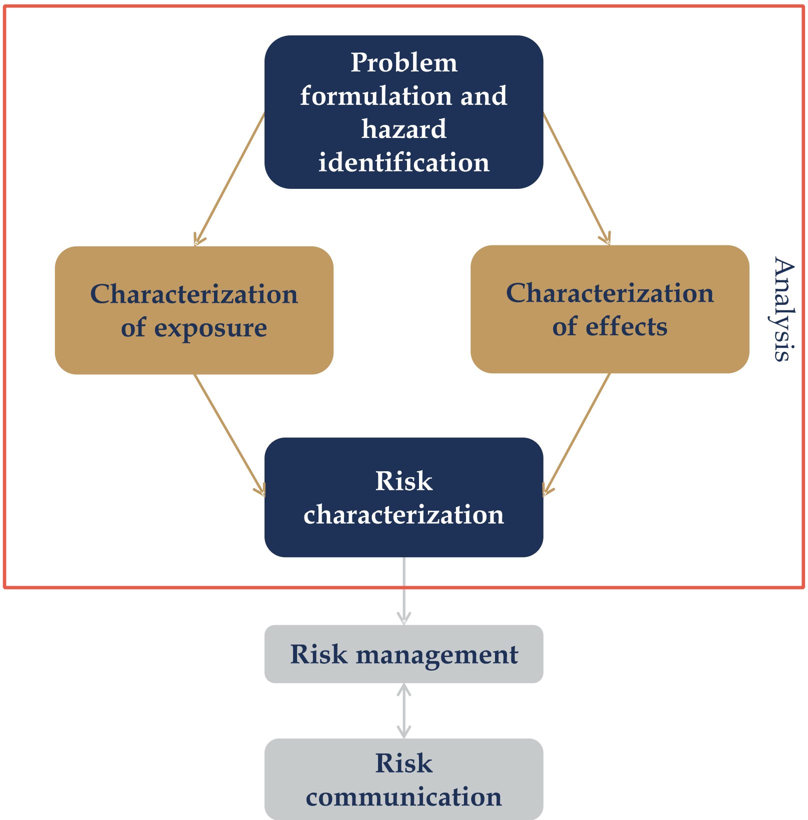

FIGURE 1 Simplified ecological risk assessment framework of EPA. Redesigned from Anon. (1992a).

Sediment risk assessment has focused on development of different guidelines, and they are convenient for regulatory purposes (Chapman and Smith 2012, Väänänen *et al.* 2016). However, single guidelines are usually not sufficient for protecting aquatic environments because contamination occurs as mixtures as noted. Toxic effects to metal mixtures vary and responses can be antagonistic, additive or synergistic (e.g. Norwood *et al.* 2003, Burton 2010, Väänänen *et al.* 2018), and these interactions are excluded with the SQG for single metals (Chapman *et al.* 1999b). Sediment quality values can be applied in screening-level risk assessment to identify the main contaminants in the studied area (Chapman and Smith 2012). Amendments to WFD include also obligations to monitor sediment contamination (2008/105/EC, 2013/39/EU), and the EU member countries can establish SQG at the national level. In Finland, SQGs are not applied and the only guidelines involving sediments are defined for depositing of dredged sediments (Anon. 2015). In addition to measuring sediment contamination, potential contaminant-induced effects need assessment. The most traditional methods include toxicity tests in the laboratory and monitoring of the contaminated site (Chapman 1990).

1.3.2 Use of benthic invertebrates in sediment risk assessment

Benthic invertebrates are an essential part of aquatic food webs (Luoma 1989). Sediment-burrowing species rework surface sediments and are an essential food source to many predators (Chapman 2001, Rainbow 2002, Suter and Cormier 2015). Therefore, they have an important role in contaminant and sediment geochemical behaviour and properties: benthic invertebrates are a vital part of functioning aquatic ecosystems (Luoma 1989, Suter and Cormier 2015). Assessment of benthic macroinvertebrate communities is used to classify the sediment quality, and they have been connected to sediment metal contamination (Chapman 2001, Rainbow 2002). Responses of benthic macroinvertebrates vary according to contaminant, species, exposure route, and properties of sediment and water (Phipps *et al.* 1995, Rainbow 2002). Metal exposure has been linked, for example, to alterations of the benthic communities or to increased body burdens of metals (Rainbow 2002, Cain *et al.* 2004, Custer *et al.* 2016). Metals can be transferred in aquatic environments and consumers in higher positions in the aquatic food webs may be exposed to sediment metals via benthic macroinvertebrates (Rainbow 2002, Xie *et al.* 2008).

There is no single organism that would fulfil all requirements of an ideal sediment toxicity assessment (Giesy and Hoke 1989). Yet there are several advantages when using aquatic oligochaetes in ecotoxicity assessments, because they are ecologically representative species, exposed to sediment contaminants via several routes, relatively easy to handle in the laboratory conditions and can be used in several types of ecotoxicity studies (Chapman 2001). Chironomids also share a majority of these favourable properties mentioned for oligochaetes (Phipps *et al.* 1993). One advantage is that when using species that ingest sediment in sediment bioassays, exposure from the diet is also considered (Phipps *et al.* 1995, Camusso *et al.* 2012).

Interest in biological indicators of toxicity has been noteworthy, which has enabled the development of methods employing benthic macroinvertebrates. The number of ecotoxicological studies employing behavioural endpoints has grown during the last decade and they are regarded as encouraging tools in environmental risk assessment (Pyle and Ford 2017). Behaviour is mentioned as a sensitive response for stress because an organism represents the final integrated results of a diversity of biochemical and physiological processes in the short term as well as in the long term (Gerhardt *et al.* 1994, Melvin and Wilson 2013). Behavioural responses are thus considered as an early warning signal because they arise before adverse effects, such as mortality and population regression. Sediment burrowing behaviour has high energetic demands (Jumars and Wheatcroft 1989), and altered behaviour may have detrimental effects on benthic invertebrates if their acquisition of food or ability to react is impaired.

2 OBJECTIVES

The aim of this thesis was to estimate the effects and assess the risk of multi-metal exposure to boreal aquatic environments, with an emphasis on assessment of contaminated sediments. Metal, nutrient and acidity loading from acid sulphate soils and metal mining were investigated. It was hypothesized that leachates from these 2 nationally significant contamination sources with similar properties impair sediment quality in the recipient water bodies. One of the main tasks was to assess the ecotoxicological test methods of field-collected sediments under laboratory conditions and evaluate the sensitivity of different toxicity test endpoints (growth, reproduction and behaviour). Hypothesis was that growth of benthic invertebrates is reduced and reproduction is impaired in the mining-affected sediments. Furthermore, it was expected that behaviour of the test species *Lumbriculus variegatus* is altered in the contaminated sediments. The importance of environmental conditions was also addressed. An overall objective was to produce information for the development of risk assessment methods suitable for boreal environments. The following specific questions were addressed:

Are the contaminant concentrations deviating from a good ecological status from a regulatory perspective (I–III)?

Is sediment a sink or a source of metals, and what is the accumulation potential of metals in benthic invertebrates (II, III)?

Do the estuary sediments have negative effects on the benthic invertebrate community (I)?

Can standard sediment toxicity tests be applied in the risk assessment of boreal metal-contaminated sediments (I–III)?

3 MATERIALS AND METHODS

3.1 Study sites

The study sites were situated on the coast of Ostrobothnia (I), Central Finland (II) and north-eastern Finland (II, III) (Fig. 2). The sites consisted of 10 different estuaries and 11 lakes. The catchment areas of the lakes and rivers upstream from the estuaries varied from 13 to 7479 km^2. The proportion of arable land area was higher in the catchment areas of rivers upstream from the estuaries (range 10–27 km^2) in comparison to mining-influenced lakes (range 0–5.6 km^2) (Table 1).

The studied estuaries are low salinity estuaries and the effect of the tide is negligible in the Baltic Sea (Cieslinski and Drwal 2005). In the Bothnian Bay, surface salinity varies from 3 to 5 ‰ (Omstedt and Axell 2003) and in the estuaries salinity can be lower (Nordmyr *et al.* 2008a). Freshwater inflow has a substantial effect on the estuary hydrology during peak discharge (Nystrand *et al.* 2016). The upstream river catchments of the estuary sites were in the AS soil area but the precise area of AS soils is not known. In general, arable land area correlates roughly with the high likelihood for AS soils (Anon. 2018a). The estuaries of the rivers Lestijoki, Perhonjoki and Lapväärtinjoki were probably the least affected by AS soils, while Vöyrinjoki, Kyrönjoki and Laihianjoki were the most affected.

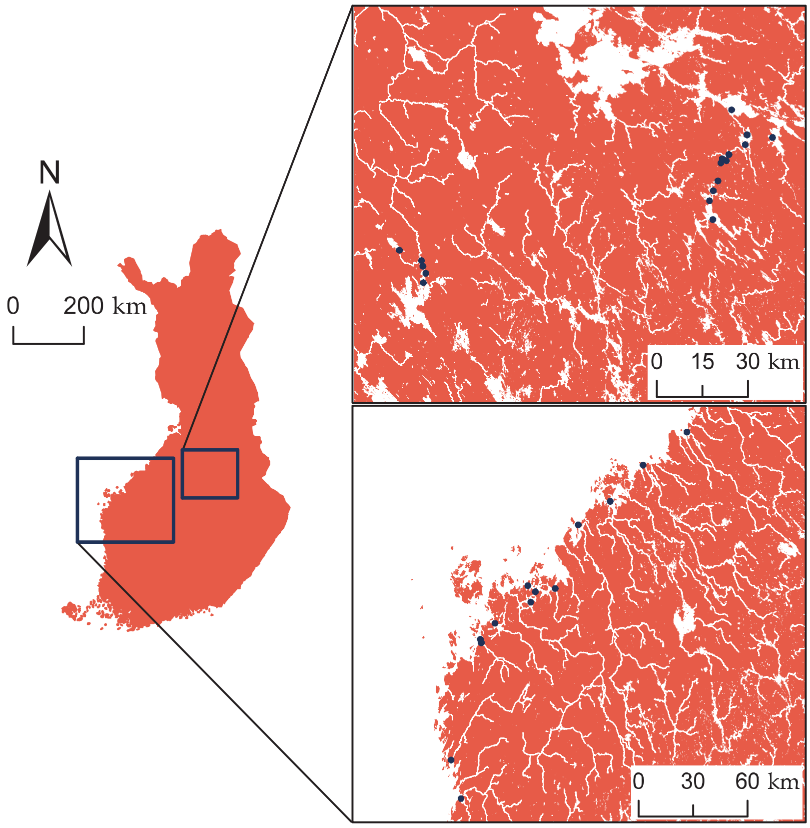

FIGURE 2 Map of sampling sites in Central and north-eastern Finland (upper box) and on the coast of Ostrobothnia (lower box). Map data: General map of Finland ©National Land Survey of Finland 2/2018; Lakes, rivers ©Finnish Environment Institute (SYKE), 6/2017; Maps were constructed with ArcGIS® v. 10.3.1 (ESRI Inc., Redlands, CA).

The long operating history of the Pyhäsalmi mine began in 1962 (Anon. 2014). It started as an open pit mine, but nowadays it is an underground mine with one of the deepest points in Europe. The main products have been Zn, Co and pyrite. Metals are recovered by conventional flotation process. The mine's effluents loaded the receiving waterbody the most in the early 1970s (Mäkinen 2011). Treated effluents are run north into Junttiselkä, which is a separate basin of the larger Lake Pyhäjärvi. There is also backflow towards the south into the Kirkkoselkä basin of Lake Pyhäjärvi occasionally during high discharge. Currently, Pyhäsalmi mine is preparing to shut down its operations due to the depletion of ore. It is expected that 2019 will be the mine's last year of operation (Anon. 2014).

The Terrafame mine (formerly known as the Talvivaara mine) is an open pit mine, and its main products are Ni, Zn, Cu and Co. It employs heap bioleaching technique to recover metals from low-grade ore with bacteria under acidic conditions (pH 1.5–3) (Saari and Riekkola-Vanhanen 2012, Riekkola-Vanhanen 2013). Treated wastewater is discharged into the Oulujoki and Vuoksi watersheds. In November 2015 Terrafame began to run treated effluents north through a bank outfall pipe (Mäkinen 2017). The mine has been in operation since 2008 and during its period of operation, mining effluents have been discharged to receiving water bodies by accident many times (Kauppi *et al.* 2013, Leppänen *et al.* 2017, Salmelin *et al.* 2017).

TABLE 1 Studied lakes and estuaries, their catchment areas and proportions of arable land. Catchment areas and Corine land cover data 2012 were derived from the VALUE tool of the Finnish Environment Institute (SYKE) (Anon. 2018b). The column on the right indicates in which paper the location was studied.

Lakes	Catchment area km^2	Arable land %	Paper
Salminen	13	0.1	III
Kalliojärvi	18	0	II, III
Kolmisoppi	111	0.2	II, III
Jormasjärvi	299	1.2	II, III
Nuasjärvi	7479	1.5	II
Kiantajärvi (reference)	325	2.2	II, III
Ylä-Lumijärvi	16	0	II
Kivijärvi	53	0	II
Laakajärvi	465	0.3	II
Pyhäjärvi	674	5.3	II
Parkkimanjärvi (reference)	38	5.6	II
River estuaries			
Lestijoki	1309	10.2	I
Perhonjoki	2410	10.2	I
Ähtävänjoki	2021	13.6	I
Lapuanjoki	4089	21.4	I
Vöyrinjoki	228	27.8	I
Kyrönjoki	4783	24.3	I
Laihianjoki	507	27.3	I
Maalahdenjoki	429	13.8	I
Närpiönjoki	953	20.0	I
Lapväärtinjoki	1051	12.3	I

Major ions, such as Ca^{2+} or SO_4^{2-}, are released from mines in addition to metals. Wastewater and leachates from mining are commonly acidic and metal-rich, thus their refinement is needed to neutralize and precipitate metals and to alleviate AMD. The Pyhäsalmi and Terrafame mines have both utilized liming and gypsum (Mäkinen and Lerssi 2007, Riekkola-Vanhanen 2013, Marttila 2017), which elevate the Ca^{2+} and SO_4^{2-} concentrations in mining effluents.

3.2 Sampling and sample handling

Field sampling was carried out in summer and autumn 2010 (I) and in early spring during ice season 2015 (II) and 2016 (III). Epilimnion and hypolimnion specific conductivity, oxygen concentration and saturation, pH, and temperature were measured with a multi-parameter water quality sonde (YSI V2 6600) in the mining-affected sites (II, III). Water samples were taken with a Limnos water sampler for the toxicity test (II) and chemical analyses (II, III). Additional water samples were taken for alkalinity, DOC, TIC, Cl^-, SO_4^{2-} and elemental analyses (II). Water samples were filtered *in situ* through 0.45 µm syringe filter (Whatman® GD/X or GD/XP) and acidified with 65 % HNO_3 (suprapur, Sigma-Aldrich®) (II) for elemental analyses.

Sediment samples were taken with Ekman grab sampler (I) or Sandman gravity corer (I–III). Surface sediments were collected to depths of 3 cm (I) or 5 cm (II, III) from the sediment-water interface. In the Pyhäsalmi area, added samples were collected from 5–10 cm and approximately 10–20 cm because of the long mining history of the area's mine (II). The replicate sediment samples were combined to 1 bulk sample in the mining-affected sites (II, III). Sediment pH was measured in field from a separate subsample of the bulk sediment sample within 12 h from the sampling with a portable pH sonde (VWR pH100) (II, III).

All samples were stored in the field in a cooler box at approximately +4–7 °C. In the laboratory, all water samples (II, III) and the sediment samples for *L. variegatus* toxicity tests (I, III) were stored in a refrigerating room at +4 °C in the dark. Sediment samples for kinetic the *A. fischeri* toxicity test (I), *C. riparius* toxicity test (I) and elemental analyses (II, III) were stored in a freezer at –20 °C. Sediments samples for kinetic *A. fischeri* toxicity test (I) and element analyses (I–III) were freeze-dried at the accredited laboratory of SYKE (I) or at JYU (Christ Alpha) (II, III).

3.3 Ecotoxicological effect assessment

3.3.1 Study species and their use in toxicity tests

3.3.1.1 Lumbriculus variegatus (II, III)

Lumbriculus variegatus (Annelida: Clitellata: Lumbriculidae; Müller 1774) is an aquatic oligochaete worm, which spends its whole life cycle in the sediments. In normal conditions, *L. variegatus* live in colonies and the worms burrow into the sediment and rest their tail part in the overlying water (Penttinen *et al.* 1996). They burrow into the sediment and feed on sediment particles, which can be observed from the egestion of faecal pellets on the sediment surface. In laboratory conditions, *L. variegatus* reproduces asexually and the doubling rate fluctuates between 10 and 14 days in standard laboratory conditions

(Brinkhurst 1987, Phipps *et al.* 1993, Rodriguez and Reynoldson 2011). After fragmentation, it takes longer for *L. variegatus* to start feeding after growing a new posterior part rather than an anterior part (Leppänen and Kukkonen 1998b).

Since the *L. variegatus* can be found from various environments and lives its whole lifecycle in sediment it is a good model organism for toxicity tests representing benthic community. As an aquatic oligochaete, it is relatively easily cultured for use in toxicity tests in laboratory conditions (Phipps *et al.* 1993, Leppänen and Kukkonen 1998b). *L. variegatus* is one of the most used species in toxicity testing of freshwater sediments (Rodriguez and Reynoldson 2011). It has been used in acute, chronic and behavioural studies of metal contamination both with field-collected and laboratory-spiked sediments (Chapman *et al.* 1999a, Sardo and Soares 2010, 2011, Blankson and Klerks 2016). Growth and reproduction are the most common test endpoints. Although *L. variegatus* is not the species most sensitive to metals, it is usually more sensitive than chironomids are (Phipps *et al.* 1995).

3.3.1.2 Chironomus riparius (I)

Chironomus riparius (Diptera: Chironomidae; Meigen 1804) belongs to the non-biting midges. They are also commonly found from various environments and distributed worldwide (Armitage *et al.* 1995). The life cycle of *C. riparius* comprises 4 phases, from which the larval phase is sediment-dwelling. Larvae of *C. riparius* exhibit tube-building behaviour, which aids in gas exchange and provides shelter (Armitage *et al.* 1995). The larval phase has 4 instars, and the duration of each is 4–8 d (Anon. 2004a). They are collector-gatherers feeding on fine particulate organic matter (Armitage *et al.* 1995). Chironomids are rather insensitive to metal contamination, and their relative abundancies are generally high in polluted sites (Armitage *et al.* 1995, Phipps *et al.* 1995).

Guidelines for toxicity tests with *C. riparius* range from an acute immobilization test to a whole life-cycle test. Responses to be recorded in the chronic test include emergence and the sex of the emerged adults, but additional larval growth and survival can also be used. Depending on the testing conditions, the test duration of OECD standard 218 (Anon. 2004a) sediment toxicity test is commonly around 28 d. Providing additional food to the larvae is essential during the chronic exposure. *Chironomus riparius* also has potential for use in behavioural tests (Salmelin *et al.* 2016). Chironomid mentum deformities have also been used as indicators of stress but care should be taken when defining and analysing deformities (Salmelin *et al.* 2015).

3.3.1.3 Aliivibrio fischeri (I)

Aliivibrio fischeri (formerly *Vibrio fischeri*; Beijerinck 1889) is a light-emitting bacterium present in marine environments (Anon. 1992b). It is commonly used in screening-level toxicity tests. Bioluminescence inhibition assays of *A. fischeri* have been reported as most sensitive in comparison with other bacterial assays (Girotti *et al.* 2008). The kinetic method, in which change in luminescence is measured, has enabled the testing of solid and coloured samples (Lappalainen *et al.* 1999) and the ISO standard method 21338 (Anon. 2010) has been developed. Sample toxicity is determined by inhibition in the ability of *A. fischeri* to produce light (Girotti *et al.* 2008).

3.3.2 Toxicity tests

The acute toxicity of estuary sediments was evaluated with a kinetic *A. fischeri* test. A sediment sample was extracted with 2 % NaCl solution. The test was conducted according to ISO standard 21338 (Anon. 2010), but the pH of the sediment extracts were buffered and adjusted to 7 with $NaHCO_3$. The luminescent bacteria were exposed to a dilution series of sediment extracts ranging from 50–0.10 %.

Chronic toxicity tests with *C. riparius* and *L. variegatus* were carried out mainly as stated in standards OECD 218 (Anon. 2004a) and 225 (Anon. 2007). Field-collected sediments were used as unsieved in all toxicity tests (I–III). The toxicity test duration was 28 d (I, II) or 21 d (III). Elendt M7 medium without RbCl prepared according to the standard OECD 218 (Anon. 2004a) was used in the *C. riparius* toxicity test (I). Hypolimnetic water or diluted AFW (hardness 1 mmol l^{-1}) prepared according to standard ISO 6341 (Anon. 2012) was used in the *L. variegatus* toxicity test (II, III). Ultrapure water (conductivity < 0.056 µS cm^{-1}) was used throughout all tests (I–III). Toxicity tests were conducted at 20 ± 2 °C under a 16:9 light:dark cycle while oxygen concentration, water/sediment pH, *t* and NH_3/NH_4^+ were monitored (I–III). Overlying water was aerated throughout the tests, and *C. riparius* were fed during the exposures (I) but the *L. variegatus* were not (II, III). After the exposure period the organic matter of estuary sediments were eliminated with 10 % KOH, sediments were sieved through 250 µm sieve and *C. riparius* head capsules were collected (I). Sediments from mining-affected locations were also sieved and *L. variegatus* were collected, rinsed in AFW, blotted dry, weighed (*ww*), vacuum freeze-dried and weighed again (*dw*) (II, III).

The mentum deformities of *C. riparius* were evaluated conservatively, which included only apparent deformities (Salmelin *et al.* 2015) under a light microscope (I). The proportion (%) of deformed menta (i.e. the deformity index) was calculated according to Hämäläinen (1999), and deformity indices observed in *C. riparius* exposed in the estuary sediments were compared with the artificial control sediment (I). The proportions of emerged *C. riparius*, their sex ratios, mentum deformities and median emergence times were compared with control (I). The growth and reproduction of *L. variegatus* were compared with the reference (III) or with the reference in regard to sediment pH at 28 d (II).

3.3.3 Multispecies freshwater biomonitor (III)

The multispecies freshwater biomonitor® (MFB) is an applicable tool for measuring light-sensitive sediment-dwelling organisms, and it enables the quantification of behavioural responses. The biomonitoring system is based on quadrupole impedance conversion technology (Gerhardt *et al.* 1994, Sardo *et al.*

2007), in which the movements of the test organism in an electrical field of a test chamber are recorded (Figure 3). Movements generate alterations in the electrical field of the chamber that are measured as impedance changes (Gerhardt *et al.* 1994, Gerhardt 2007). Different behavioural patterns produce their specific electrical signals (Gerhardt 2007, Sardo *et al.* 2007). These electrical signals are converted with a discrete fast Fourier transformation, and as an outcome the MFB software yields a percentage of time that the test organism spends on a certain predefined frequency range (Sardo *et al.* 2007, Sardo and Soares 2011).

The behaviour of *L. variegatus* was measured at the outset and at the end of the 21 d exposure period. Necessary adjustments to the system settings and operation of the MFB chambers were tested in a preliminary test setup. Sardo and Soares (2010, 2011) reported that *L. variegatus* peristaltic movements could be observed at frequency range 0.1–1.0 Hz and overall locomotory activity at 1.0–3.0 Hz, thus these frequencies were recorded during the behavioural measurements. The bottom of the chamber covered with parafilm under the cap to prevent worms from escaping the chamber. A mesh was placed under the cap of top part. The MFB chambers were half-filled with the test sediment and AFW was used above the sediment. The MFB chamber was put vertically into a 1 l beaker filled with AFW (Fig. 3). Each lake sediment was measured as 6 replicates except for Lake Kalliojärvi, because 1 chamber was unsuccessful in recording the signal at 21 d. Additionally, 1 empty chamber with sediment and 1 only with AFW were used as control for background noise.

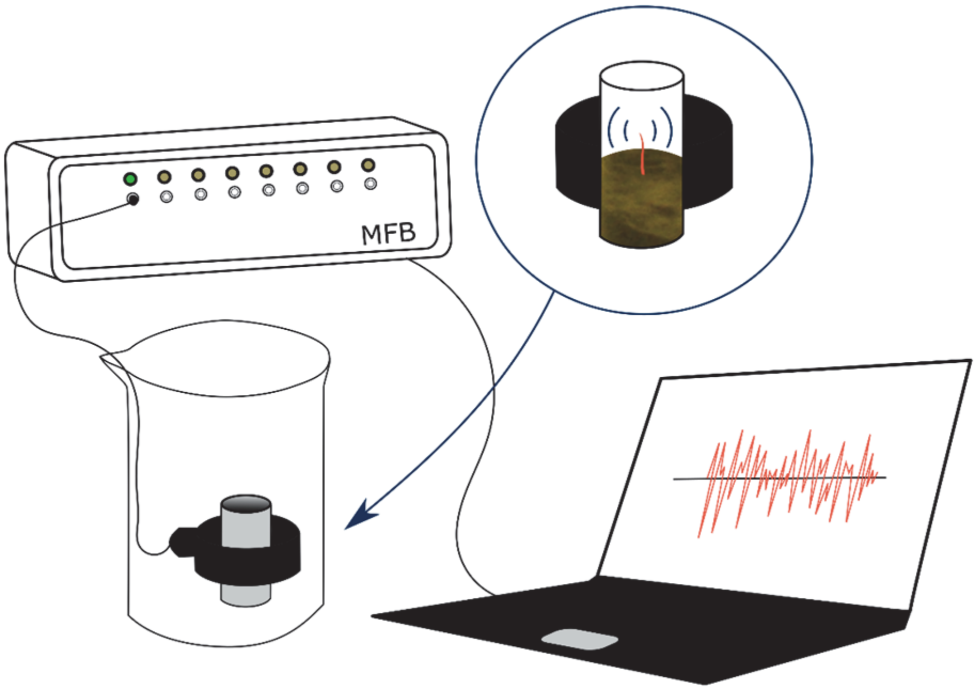

FIGURE 3 Schematic figure of the MFB device and behavioural measurement of *L. variegatus*. In the circle, *L. variegatus* locomotion in a measuring chamber half-filled with the test sediment is represented. The chamber was in a beaker filled with AFW. Locomotion was recorded by the MFB-device, which was connected to a computer.

The behavioural measurements were conducted with 1 randomly selected worm in each chamber. After introducing the worm into the test chamber, the chamber was settled for 20–30 min so that the worm could burrow into the sediment properly. The behaviour was automatically recorded for 3 h at 10 min intervals, in which the behaviour was registered for 4 min. Oxygen saturation was monitored from 1 replicate beaker before and after the recording period. Average times of *L. variegatus* in peristaltic or overall locomotory frequency range in mining-affected sediments were compared with the local reference sediment.

3.4 Analyses

3.4.1 Water analyses

Sulphate, alkalinity, TIC and Cl^- were determined in the accredited laboratory of SYKE from separate field water sample (n = 1) (II). In addition, DOC was measured from each field-collected water sample at JYU (II). The samples were filtered in the laboratory prior to DOC measurements. Dissolved concentrations of Na, P, S, Ca, Mg, Al, Fe, K, As, Cd, Co, Cr, Cu, Ni, Sr, U, Zn and Mn were analysed with inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer Optima 8300) from the field-collected water samples (II), from the overlying water samples in the toxicity tests (II, III). The total concentrations of the same elements were measured from the samples of the overlying water in the MFB setup (III). Metal concentration in the field-collected samples were compared with MAC-EQSs for Cd, Ni and Pb (Government Decree 1090/2016) (II).

3.4.2 Sediment and *L. variegatus* analyses

Dry weight and LOI were determined at 105 °C for 24 h and at 800 °C (I) for 1 h (I) or 550 °C for 2.5 h (II,III), respectively. The sediment organic carbon was analysed once from the field-collected sediments from mining sites at JYU from acid-treated (1 M HCl) samples with elemental analyser (Elemental Vario EL III) (II). Organic carbon content in sediments was otherwise approximated from the LOI according to Pajunen (2004).

The elemental analysis of AS-soil-affected estuary sediments was conducted in the accredited laboratory of SYKE with inductively coupled plasma mass spectrometry (ICP-MS) (Perkin-Elmer Elan DRC II) after microwave digestion with HNO_3, and Al, Fe, As, Cd, Co, Cr, Cu, Ni, Pb, Zn and Mn were included in the analysis (I). Total elemental concentration analysis of sediments and *L. variegatus* from mining-affected locations and toxicity tests was conducted at JYU and the analyses included Na, P, S, Ca, Mg, Al, Fe, K, As, Cd, Cr, Cu, Ni, Pb, Sr, U, Zn and Mn (II,III). Vacuum freeze-dried samples were digested with *aqua regia* (HNO_3:HCl, 1:3, *v/v*) and sonicated (ELMA Transonic

820/H or Bandelin Sonorex RK 512/H) for 3–4 min in a 50 °C bath. Digested sediment samples were filtered (Whatman no. 41). The sediment extracts or digested *L. variegatus* were diluted to a final volume of 50 ml or 10 ml with ultrapure water, respectively. Elemental concentrations of the samples were analysed as the water samples (II, III).

The same elements without Na were analysed in SEs as in the total concentration analysis of mining-affected sediments at JYU (III). Partitioning of elements in the field-collected sediments was evaluated with ultrasound-assisted SEs (Tessier *et al.* 1979, Väisänen and Kiljunen 2005). Extracted fractions were exchangeable (F1), carbonate bound (F2), Fe and Mn oxides bound (F3), organic matter sulphide bound (F4) and residual (F5) fraction (Tessier *et al.* 1979). Extraction method was conducted according to Väisänen and Kiljunen (2005), but $MgCl_2$ was replaced with 0.5 M NH_4Cl in exchangeable fraction extraction. The exchangeable and carbonate-bound fractions were considered to represent the most mobile, that is, the potentially most bioavailable. The residual fraction consisted of primary and secondary minerals and was regarded as non-bioavailable to the biota in any environmental conditions (Tessier *et al.* 1979, Torres and Auleda 2013).

3.5 Background and monitoring data

Background metal concentrations were approximated from the literature and contemporary guidelines for water and sediments (I, II). Metal concentrations of estuary sediments were compared with Swedish SQC and national dredging and banking guidelines (Anon. 1999, 2004b).

Water quality data for the estuaries was derived from the HERTTA database of SYKE (version 5.6) (I). Monitoring data for alkalinity, conductivity, salinity, TOC, pH and metals (Al, As, Cd, Co, Cr, Cu, Pb, Ni, Zn) were gathered from a 19-month period from March 2009 to September 2010 representing exposure conditions during and before the sediment field sampling. If there was not a monitoring site in the estuary, the nearest monitoring site to the river mouth was selected. Average concentrations were compared with AA-EQSs for Cd, Ni and Pb (Government Decree 868/2010).

The ecological status of benthic macroinvertebrate communities in the studied estuaries was assessed from BBI (Perus *et al.* 2007), which was also obtained from the HERTTA database. The indices were derived from monitoring data collected in the second national river basin management plan from 2010 to 2012, which was implemented according to WFD. In case BBI was not defined for the estuary, the nearest river benthic invertebrate quality index was used. For the Kyrönjoki and Maalahdenjoki estuaries, only 1 BBI was available although sediment sampling was conducted at 3 different distances (I).

Acute toxicity data (EC/LC_{50}) for HQ were derived from the US EPA Ecotox database for 3 selected metals (Al, Cd and Zn). Only toxicity test data corresponding to Finnish water chemistry for alkalinity, DOC and pH during high-discharge period in the estuaries affected by AS soils were selected (I). Final HQ was calculated from the maximum concentration in the estuary water derived from the HERTTA database during the observation period, which was divided by the sum of the 5th percentile of selected metal derived from the Ecotox database and background concentration (I).

3.6 Classification according to risk or contamination gradient

All assessment endpoints are summarized in Table 2. For the estuary sites (I), final risk was evaluated using the weight of evidence method (Suter 2007). Each assessment endpoint corresponded to an individual line of evidence, and each line of evidence had equal weight. Different lines of evidence were summarized and a final risk class of negligible, low, moderate or high was assigned to every estuary site (I).

At the Terrafame mining site, sampling locations were classified into 3 groups in relation to mining effect in 2015 (II). Mining effect was defined as low, medium or high according to hypolimnion sulphate concentration and specific conductivity together with sediment Na, S, Ni and Mn concentrations (II). Each mining effect group included 3 lakes, and 1 lake (Kiantajärvi) was classified as a reference lake.

TABLE 2 Assessment endpoints and papers where they were used.

Assessment method	Endpoint	Paper
AA-EQS	chronic	I
MAC-EQS	acute	I, II
pH minimum	acute	I
HQ	acute	I
SQC	concentration based	I
BBI	chronic	I
A. fischeri bioassay	acute	I
C. riparius bioassay	chronic	I
L. variegatus bioassay	chronic	II, III
Behaviour of *L. variegatus*	acute and chronic	III

4 RESULTS AND DISCUSSION

4.1 Concentrations in biota (II, III)

Body residues of measured elements in *L. variegatus* exposed to mining-affected sediments were generally highest in the AFW setup and lowest in the MFB setup. However, body residues were within the same order of magnitude for most elements, and the largest variations were observed in Al, Fe and Mn body residues. The smaller concentrations in the MFB setup may be explained with 7 d shorter exposure period than in the OECD 225 standard (Anon. 2007). Performance of *L. variegatus* was weakest in the AFW setup, which might explain the higher body residues due to larger biomass and growth dilution in other test setups.

The body residues of the ore metals of TF and PS mines increased in a concentration-dependent matter similarly as in the sediments. The phenomenon was most evident in the HLW setup. The accumulation of Mn was not as straightforward, but Mn concentrations in *L. variegatus* were also correlated with sediment total or organic matter and sulphide-bound fraction.

Ni was detected from *L. variegatus* only from the setup with HLW above sediment or exposed to sediments of the high mining effect group. Pore water has been reported to be the main source of Ni uptake for *L. variegatus* and *Tubifex tubifex* and uptake from ingested sediment particles is not significant (Camusso *et al.* 2012, Méndez-Fernández *et al.* 2014). Our results supported the observations that Ni is not extensively accumulated in *L. variegatus* from sediments unless the sediment is heavily polluted. It might not be relevant to monitor Ni from benthic oligochaete worms unless the source of Ni is the water or the pore water.

As opposed to Ni, sediment was a more significant source of Cu and Zn uptake. Increasing concentrations of Cu and Zn in *L. variegatus* along with sediment concentration gradient were more evident in the longer 28 d setups with AFW and HLW in comparison with MFB setup. With chironomids, body burdens of As, Cd, Cr, Cu, Ni, Pb and Zn have been connected to ecological responses (Bervoets *et al.* 2016). Tissue concentrations of freshwater oligochaeta have also shown potential in predicting the ecological effects and concentrations of Hg, As and Zn have been demonstrated as distinguishing toxic and non-toxic river sites (Bervoets *et al.* 2016, Méndez-Fernández *et al.* 2017).

Lumbriculus variegatus was a representative species selection to study body residues of metals because it is a relatively resistant species and suitable organism for studying accumulation (Bervoets *et al.* 2016). Incorporating body burdens of metals in sediment risk assessment may appear to be possible at least for Zn in the studied mining sites. It has been observed that oligochaetes exposed in the laboratory accumulated more metals than field-collected oligochaetes but the concentrations of As, Hg, Cu and Zn were within the same order of magnitude (Méndez-Fernández *et al.* 2017). Thus, it should be noted that the body residues of *L. variegatus* that were measured can overestimate the concentrations that could be recovered from field-collected individuals.

4.2 Metal concentrations and acidity in the estuaries (I)

Elevated concentrations of metals known to be extensively leached from AS soils (Al, Cd, Co, Cu, Mn, Ni, Zn) were observed in the sediments. Furthermore, metals not abundantly leached from AS soils were present on background concentrations in the estuary sediments. Comparisons of metal concentrations with contemporary Swedish SQCs (Anon. 1999) suggested that Cd, Co, Cu, Ni and Zn were the main contaminants (Table 2 in I) whereas comparisons with national dredging guideline (Anon. 2004b) suggested contamination due to Cd, Ni and Zn concentrations (Fig. 3 in I). Multi-metal contamination was noticed especially in the estuaries of Vöyrinjoki, Kyrönjoki 2 and 3, Laihianjoki, Maalahdenjoki 3 and Närpiönjoki, where at least 50 % of metals that had a reference/classification limit exceeded their concentration (Fig. 4). Comparisons with total concentration based Swedish SQC implied contamination to a greater extent than comparisons with the national dredging guideline. Total metal concentrations in the estuaries were on a level similar to the one reported earlier by Nordmyr *et al.* (2008a). In the estuaries of Kyrönjoki and Maalahdenjoki, most metals were enriched in the surface sediments of the furthest sampling site. It might be that metals are in dissolved form near the river mouth and are deposited further in the estuary where the influence of brackish water increases. In the Vöyrinjoki estuary, it has been reported that Al, Cu, La and U are deposited first with organic matter when water pH is neutralized whereas Co, Mn, Ni and Zn precipitate further down the estuary (Nordmyr *et al.* 2008a, Nystrand and Österholm 2013). Precipitation of Al, Cu, Cr, Fe, Pb and U has been detected to need less saline water than Cd, Co Mn, Ni and Zn (Nystrand *et al.* 2016). Similar behaviour of sediment metals was observed in the estuaries of Kyrönjoki and Maalahdenjoki, where Mn was most notably enriched in the furthest sampling site.

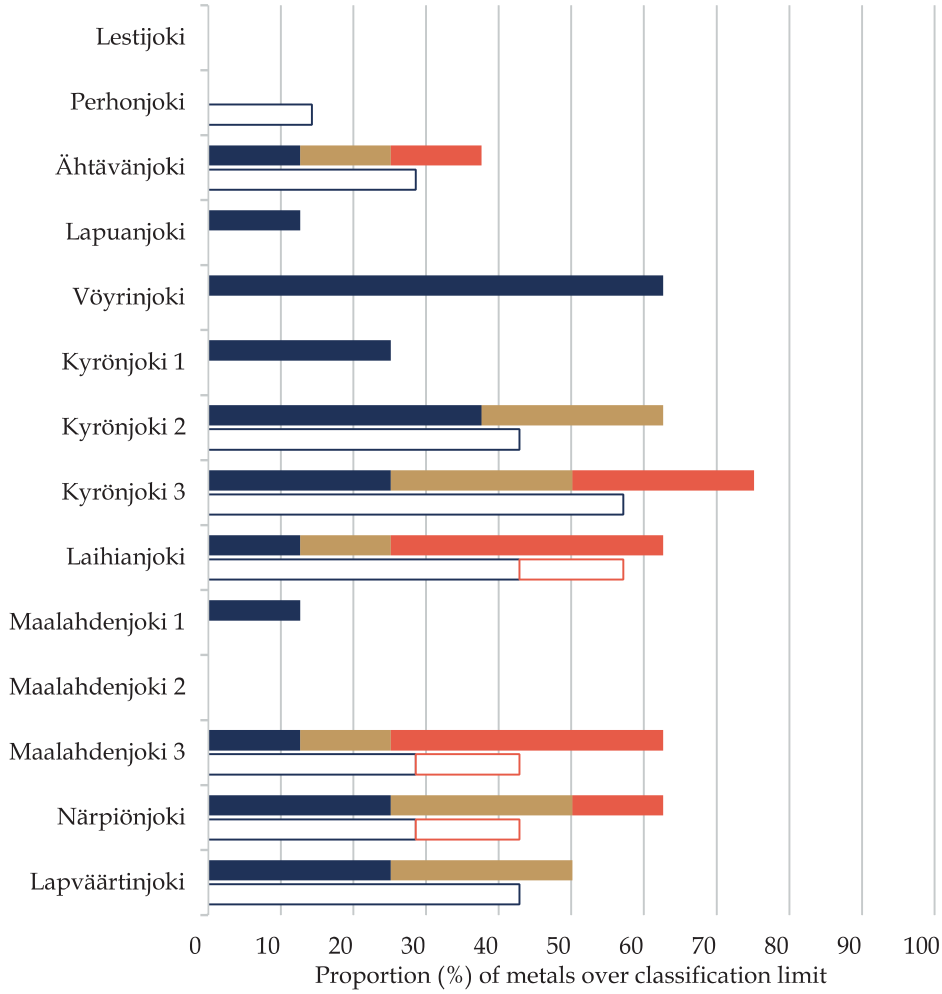

FIGURE 4 Proportion (%) of metal concentrations in the estuary sediments exceeding their classification limit when compared with Swedish SQC (solid beams) or national dredging guideline (open beams). Swedish SQC included 8 metals and dredging guideline 7. Solid beams indicate clear (blue), large (ochre) or very large (red) deviation from reference concentrations. Open beams indicate potentially contaminated (blue outline) or contaminated (red outline) sediment metal concentrations.

Mainly the same metal concentrations were elevated in the water as in the sediment, and they indicated contamination of the estuary water. Instead, metals that are meagrely leached from AS soils (As, Fe, Cr, Pb) were present in background concentration levels (Verta *et al.* 2010) in water. Cadmium was the only metal of the EUs WFD priority substances in which the concentration exceeded the national AA-EQSs in approximately 35 % of the study sites, representing 3 out of 10 estuaries. The risk of harmful effects due to chronic Cd exposure was therefore elevated in the estuaries of Kyrönjoki, Maalahdenjoki and Närpiönjoki. The estuary sites were usually monitored during the overrun periods in spring and autumn, so the average concentrations may overestimate actual annual average concentrations. In contrast to AA-EQS, HQ for Cd was below 1 in all estuaries.

Aluminium is easily mobilized from the AS soils (Österholm and Åström 2004), and it was also transported to the estuary sites. There are no guidelines for Al, but high HQs for Al in all estuary sites implied that Al concentrations can pose a risk during peak discharge. During peak discharge, the metal concentrations are typically the highest and the pH is low, and Al concentrations up to 7.3 mg l^{-1} were noted in the monitoring data. Although probably less than 30 % of Al occurs as free ion in the river systems affected by AS soil, lower than the Al concentrations in the monitoring data have been observed to be detrimental to aquatic animals (e.g. Vuorinen *et al.* 1993, Keinänen *et al.* 2000, Nystrand and Österholm 2013). In addition to Al, the HQ for Zn were over 1 in the estuaries of Vöyrinjoki, Maalahdenjoki and Laihianjoki. Aquatic insects are usually quite tolerant under acute exposure of divalent metals although negative effects are observed in chronic exposures (Brix *et al.* 2011).

The estuary of Vöyrinjoki is the only estuary affected by AS soil along the western coast of Finland, in which behaviour of metals is well reported (e.g. Nordmyr *et al.* 2008a, b, Nystrand *et al.* 2016). Vöyrinjoki is adversely affected by AS soils (Roos and Åström 2005, Nordmyr *et al.* 2008a) and similar behaviour of metals might be expected in other estuaries that receive leachates from AS soil hotspot areas.

Acidity was evident in the estuary sites in addition to metals, and pH values even lower than 4.5 were observed in the estuaries of Vöyrinjoki and Laihianjoki. A minimum pH from 4.5 to 5.5 was observed additionally at 5 study sites. This showed that acidity at least occasionally deteriorated the water quality at 50 % of the study sites. Acidic conditions were diluted with the Baltic Sea brackish water in the estuaries of Kyrönjoki and Maalahdenjoki, in which the monitoring data were collected from 3 different distances. Water was neutralized earlier in the more open estuary of Maalahdenjoki.

4.3 Element concentrations at mining sites (II, III)

Metals that were the known components of effluents (Kauppi *et al.* 2013) were also present in the sediment samples. The Terrafame mine is located in a black shale region and concentrations of Ni and Zn are typically high in the parent bedrock (Loukola-Ruskeeniemi *et al.* 1998). One of the lakes studied (Kolmisoppi; medium mining effect group) was located in the black shale area and, for example, Ni concentrations were 30 % higher in the Kolmisoppi sediment than in the black shale parent material. The ore metals Ni and Zn were identified as main contaminants near the Terrafame mining site and Cu and Zn near the Pyhäsalmi mining site. Total concentrations were high especially in the lakes nearest to the mining sites.

Sediment contamination extended through all the sampling sites downstream of both the Terrafame and Pyhäsalmi mines. There was a decreasing concentration gradient away from the mine for several metals at the

Terrafame mining site (Fig. 5), and the gradient was more apparent in the direction of the Vuoksi watershed than the Oulujoki watershed. However, the opposite situation was observed in Pyhäsalmi. In the surface sediments, the highest Cu and Zn concentrations were detected in the surface sediment (0–5 cm) of Pyhäjärvi KS, although the effluent flow is towards Junttiselkä. This was most likely explained by the long operating history of the Pyhäsalmi mine and different sedimentation rates at separate sampling sites. The sedimentation rate is lowest in Pyhäjärvi KS and highest in Junttiselkä N (Mäkinen and Lerssi 2007, Mäkinen 2011); hence, sediment slices from different sampling sites represented unequal time spans. Consequently, Cu and Zn concentrations peaked in deeper cores (5–10 and > 10 cm) at the Junttiselkä S and N sampling sites (Table 5 in II), impeding comparisons between equally sliced samples and their retrospective risk assessment.

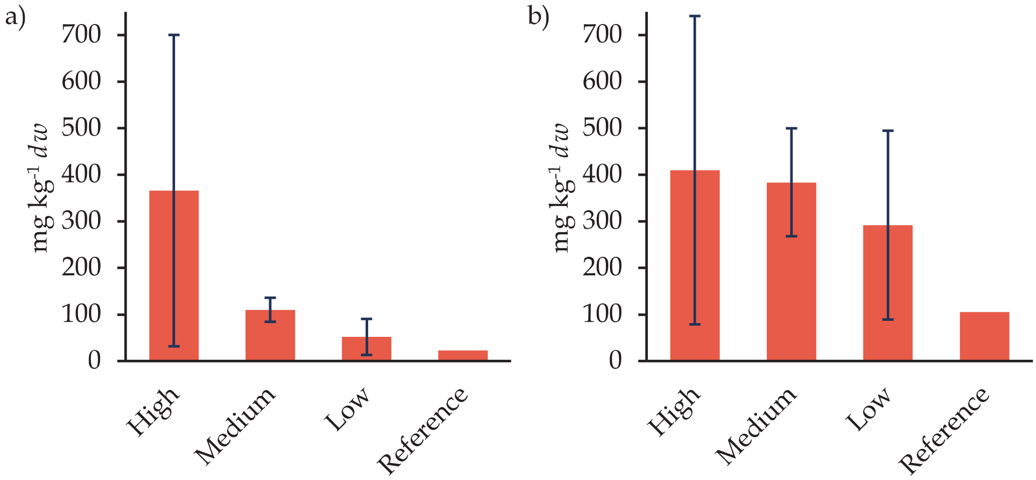

FIGURE 5 Mean (± SD) sediment a) Ni and b) Zn concentrations in the field-collected samples in different mining effect groups at the Terrafame (Talvivaara) mining site (n = 3) and the reference lake Kiantajärvi (n = 1) in spring 2015.

Sequential fractions indicated that the behaviour of elements was site- and element-specific. The decreasing gradient of S, Cu, Ni and U with declining apparent anthropogenic effect of Cu and Ni away from the Terrafame mining location was observed. A minority of the elements were present in residual fractions, suggesting potential changes in metal speciation are possible if the environmental conditions change, because only the residual fraction is stable (Tessier *et al.* 1979, Torres and Auleda 2013). The non-residual part of SEs is commonly considered to be of anthropogenic origin (Pagnanelli *et al.* 2004). Therefore, increasing gradient of the residual fraction away from the mine implied that metals in sediments, such as Cu and Ni, were of human origin. It was estimated that the mobilization potential was the highest for Sr and Mn because they were recovered in the exchangeable fraction by 50–60 % (Fig. 2 in III). The exchangeable fraction, suggesting the highest bioavailability, of S, Ni and U peaked in the sediments of the lakes Kalliojärvi and Salminen. Only Cd and Zn concentrations were related considerably (> 20 %) with Fe and Mn oxides bound fraction. On average, 30–85 % of S, Cd, Cr Cu, Ni, Pb and U were associated with organic matter and sulphide bound fraction in the mining-affected sediments. Organic matter and sulphide bound fraction were not distinguished because they were recovered in the same fraction. It has been reported that the relationship between Cu complexed with organic compounds and sulphide bound fraction is approximately 1:3 in mining-affected river sediments (Pagnanelli *et al.* 2004) but for mining-affected lake sediments there is no similar information available. Cu partitioning was similar in the residual and non-residual fraction as in the river sediments downstream from abandoned pyrite mine in Gómez-Álvarez *et al.* (2007). Residual concentrations of Fe and Al dominated nearly all sediments, and a similar phenomenon with Fe has been observed in AMD contaminated river sediments (Pagnanelli *et al.* 2004, Gómez-Álvarez *et al.* 2007).

The partitioning of S in sediments was analogous to metals. The exchangeable fraction was highest in the lakes most affected by mining. Association with the most easily extracted fraction suggested that S was largely as sulphates in the sediments of the lakes Kalliojärvi and Salminen.

Partitioning of metals in mining-affected lake sediments has not been extensively studied and because extraction methods vary between studies there is a lack of confidence when comparisons are made, for example, against river sediments from different regions. Pre-treatments have an influence on the distribution of metals in the sediments, but freeze-drying is considered the least disturbing drying method (Rapin *et al.* 1986, Bordas and Bourg 1998). Although ultrasound-assisted SEs are faster than the traditional method (Tessier *et al.* 1979, Väisänen and Kiljunen 2005), SEs remain laborious. Thus, SEs cannot be recommended to be added to regular monitoring programmes.

Water chemistry indicated an increase of some major ions (Na^{+}, SO_4^{2-}, Ca^{2+}, Mg^{2+}) particularly in the hypolimnion of the mining-affected lakes. The hypolimnions of the lakes Kalliojärvi and Kivijärvi near the Terrafame mine were saline. Nickel and Cd concentrations exceeded MAC-EQS in the hypolimnion of Kalliojärvi.

4.4 Assessment of effects

4.4.1 Estuary sites (I)

The most evident effect was deterioration of the benthic invertebrate community in the estuary locations. The quality of the benthic community was estimated to be the poorest in the estuaries of Vöyrinjoki and Kyrönjoki. Moderate BBI was designated to estuaries of Lapuanjoki and Laihianjoki. The deterioration of the benthic community was most likely affected by the acid multi-metal leachates from AS soils. However, estuaries received run-offs from the whole upstream catchment area. Effluents from other sources, such as industry or residential areas, may also have loaded the estuaries.

Inconsistent effects were observed in the laboratory tests. Although the estuary sediments showed toxicity in the acute *A. fischeri* test, no explicit reason for the toxicity was discovered. Photoluminescent bacteria have been used in the assessment of soils and sediments contaminated with metals but the ability to distinguish polluted sediments from unpolluted with luminescent organisms varies (Girotti *et al.* 2008). In addition, responses were equivocal in the chronic *C. riparius* toxicity test and there were no differences in adult emergence ratios or times. Chironomids are not the most sensitive taxa to metals (Phipps *et al.* 1995). They were also fed during the exposure, which may have reduced their exposure to sediment metals. Indeed, it has been noted that responses of *C. riparius* to sediment contaminants are less evident than responses to food quality and quantity (De Haas *et al.* 2002). The conditioning period and 1 renewal of overlying water may have leached the most soluble metals, and metal concentrations during the exposure may have been lower than the original concentrations. Mentum deformities were low (≤ 11 %) and consistent through all exposures and with survival data. The sediment quality triad provides a classic example in cases where toxicity is not observed but contamination on site and biota alteration are observed: the measured contaminants are either in a non-bioavailable form or another contaminant is the reason for toxic effects (Chapman 1990). The stabilization period and providing food to *C. riparius* during the toxicity test period are the most likely causes that have diminished the exposure of *C. riparius* to sediment contaminants.

4.4.2 Mining sites (II, III)

The results were more consistent in the toxicity tests with the sediments contaminated with mining effluents in comparison to the estuary sites. Although responses in the toxicity test with *C. riparius* were not observed with metal-contaminated estuary sediments, decrease in growth and reproduction of *L. variegatus* exposed to mining-affected sediments was observed. Responses were observed in both conventional and biomining-affected lake sediments. Growth was more sensitive test endpoint than reproduction. Similar results have been observed with *L. variegatus* exposed to sediments spiked with retene, where differences were not observed in reproduction between exposures (Nikkilä *et al.* 2001). Because the reproduction of *L. variegatus* is asexual, sublethal and lethal endpoints cannot always be distinguished definitely (Rodriguez and Reynoldson 2011). Therefore, the reproduction of *L. variegatus* is not expected to be the most sensitive endpoint.

Responses in *L. variegatus* growth and reproduction were most apparent in the HLW setup (Fig. 2 in II). The least sensitive was the AFW setup. In the HLW setup, pairwise comparisons showed that growth and reproduction were lowered in all mining effect groups but differences were not significant in the AFW setup (Table 3). In the MFB setup, the number of worms and *dw* were decreased in the sediments from the lakes Jormasjärvi, Kalliojärvi and Salminen (Fig. 3 in III). However, responses were not observed in the *L. variegatus* exposed to Lake Kolmisoppi sediment, which belong to medium mining effect group in the HLW and AFW setups.

TABLE 3 Results of ANCOVA and combined Fisher's probability statistics from *L. variegatus* toxicity tests exposed to Terrafame (TF) mining-affected sediments. HLW = test setup with hypolimnetic water, AFW = test setup with artificial freshwater, MFB = 21 d test setup with behavioural measurements, RF = reproduction factor, *ww* = wet weight, *dw* = dry weight.

Site/covariate		HLW		AFW		Combined		MFB		Combined	
		F	p	F	p	X^2_4	p	F	p	X^2_6	p
TF mining effect	RF	6.6	0.002	2.111	0.124	16.6	0.002	46.4	< 0.001	57.5	< 0.001
pH	RF	1.9	0.178	1.074	0.310	5.8	0.215				
TF mining effect	*ww*	35.8	< 0.001	2.327	0.099	42.8	< 0.001	46.9	< 0.001	94.5	< 0.001
pH	*ww*	3.4	0.076	0.611	0.611	6.1	0.096				
TV mining effect	*dw*	37.9	< 0.001	3.915	0.020	47.1	< 0.001	46.4	< 0.001	89.9	< 0.001
pH	*dw*	5.1	0.033	0.303	0.587	7.9	0.189				

At the Pyhäsalmi sampling site, responses were most apparent in the Junttiselkä basin. As a whole, sampling depth was more important than sample site (Table 4). This might be associated with lower food quality in deeper sediment slices. Differences in both *ww* and *dw* of *L. variegatus* were observed between sediments near the Pyhäsalmi mining site when both sampling site and depth were incorporated in the analysis (Table 4).

A major issue during the toxicity tests was the decline in the sediment and overlying water pH. In the reference sediments, pH decreased similarly during all toxicity tests as in the mining-affected sediments (Fig. 4 in II and Fig. 4 in III). The sediment from Lake Parkkimanjärvi may not be a representative reference lake in toxicity testing due to large shift in pH below 5.0. It is relatively challenging to find natural sediment that would fully meet the requirements for reference sediment (Anon. 2007) and support the sufficient growth of the toxicity test species (Burton 2010, Wolfram *et al.* 2012).

It was not possible to separate the effects induced by metals from the effects potentially induced by acidity. Although oligochaetes are tolerant to acidic conditions (Chapman *et al.* 1982, Rodriguez and Reynoldson 2011), chronic exposure to low pH values (pH = 5) can, for example, result in chaetal changes and loss of hairs in *T. tubifex* (Chapman and Brinkhurst 1987). Effect of sediment pH on the *dw* of *L. variegatus* or on its reproduction were significant only in the HLW setup of Terrafame sediments or the AFW setup of Pyhäsalmi sediments (Table 3, 4). The overlying water was not buffered, but it has been observed that even the buffering may not be enough to sustain the original pH during chronic exposure setup (Väänänen 2017). Metals and sulphide rich sediments are easily acidified when the environmental conditions change. Decrease in water pH has been reported near the Pyhäsalmi mining site in Junttiselkä, where water pH dropped under 5 during the spring turnover period (Heikkinen and Väisänen 2007). A similar effect was observed in the toxicity test setups and they may resemble the scenario in the spring when the anoxic/hypoxic hypolimnion and surface sediments are oxidized together with sulphides, acidic conditions are formed and metals are solubilized as in the AS soils. Physical and chemical changes in the sediments after sampling complicated their risk assessment and toxicity tests in the laboratory.

TABLE 4 Results of ANCOVA and combined Fisher's probability statistics from *L. variegatus* toxicity tests exposed to Pyhäsalmi (PS) mining-affected sediments. HLW = test setup with hypolimnetic water, AFW = test setup with artificial freshwater, RF = reproduction factor, *ww* = wet weight, *dw* = dry weight.

Site/covariate		HLW		AFW		Combined	
		F	p	F	p	X^2_4	p
PS 0–5 cm	RF	5.3	0.032	2.611	0.133	10.9	0.027
pH	RF	2.4	0.167	0.417	0.539	4.8	0.307
PS 0–5 cm	*ww*	12.0	0.004	6.495	0.020	19.0	< 0.001
pH	*ww*	6.5	0.262	0.224	0.650	3.5	0.472
PS 0–5 cm	*dw*	7.9	0.012	2.429	0.150	12.6	0.013
pH	*dw*	1.5	0.260	0.072	0.796	3.2	0.533
PS site	RF	4.4	0.029	2.84	0.086	12.0	0.017
PS depth	RF	1.9	0.179	2.15	0.147	7.3	0.122
pH	RF	3.0	0.103	4.86	0.042	10.9	0.028
PSsite × PSdepth	RF	0.6	0.701	1.368	0.286	3.2	0.523
PS site	*ww*	5.5	0.014	1.53	0.246	11.3	0.024
PS depth	*ww*	22.5	< 0.001	29.09	< 0.001	47.3	< 0.001
pH	*ww*	1.4	0.261	1.63	0.218	5.7	0.220
PSsite × PSdepth	*ww*	5.1	0.007	2.473	0.084	14.9	0.005
PS site	*dw*	4.7	0.024	0.08	0.924	7.6	0.107
PS depth	*dw*	16.8	< 0.001	13.73	< 0.001	34.9	< 0.001
pH	*dw*	1.1	0.307	0.23	0.636	3.3	0.514
PSsite × PSdepth	*dw*	3.5	0.029	3.883	0.020	14.9	0.005

Comparisons of responses between the HLW and AFW setup included uncertainties due to longer sediment storage time between the 2 setups. It is known that storing sediments can have an influence on the toxicity of sediment (Becker and Ginn 1995, Moore *et al.* 1995, De Lange *et al.* 2008). On the contrary, it has been reported that sediments retain their toxicity when responses are compared in relation to concurrent control (DeFoe and Ankley 1998). The responses were evident in the HLW setup, in which field-collected hypolimnetic water was used as overlying water. AFW is commonly used as overlying water in standard toxicity tests also when employing field-collected sediments. The significance of using AFW above sediment in toxicity tests might merit further assessment.

Chronic toxicity test species *L. variegatus* and *C. riparius* were relevant in risk assessment because both species are present in boreal lakes and estuaries (e.g. Phipps *et al.* 1993, Armitage *et al.* 1995) whereas applicability of *A. fischeri* is restricted to laboratory conditions. Both *L. variegatus* and *C. riparius* are quite easily maintained in the laboratory, *L. variegatus* being easier when constructing test setup. However, laboratory cultures may be more sensitive to metals than the natural populations, which makes extrapolation to the natural conditions difficult (Chapman *et al.* 1999b).

Although *ww*, *dw* and the reproduction of *L. variegatus* were the most evident toxicity endpoints, the most mining-affected lake was differentiated from the reference lake by behavioural measurements. Both peristaltic and overall locomotory activity remained similar at 0 d and 21 d in most of the tested lake sediments so MFB was a rather quick instrument for the detection of effects. When chronic responses were studied, *dw* was the most sensitive response. There is usually more variation in *ww* than *dw* and measuring *ww* is not required in the standard. Growth has also earlier been reported to be a more easily detectable response to metal exposure than behaviour in general (Melvin and Wilson 2013). However, behavioural responses of *L. variegatus* have been related to metal and acid mine drainage exposure (O'Gara *et al.* 2004, Gerhardt 2009, Sardo and Soares 2010). Reduced peristaltic and the overall activity of *L. variegatus* can indicate reduced foraging behaviour and ability to hide or escape predators. Effects of contaminants on a species may have broader indirect consequences in the aquatic environment if, for example, the vulnerability to predation is altered (Fleeger *et al.* 2003, Blankson *et al.* 2017). It is also common that there is variation in behavioural responses between species and individuals (Gerhardt 2007, Salmelin *et al.* 2016). For example, simulated pulse to acid mine drainage has induced behavioural responses in *Echinogammarus meridionalis* and to a lesser extent in *Choroterpes picteti* but not in *Hydropsyche pellucidula* (Macedo-Sousa *et al.* 2008).

The interpretation of behavioural responses needs special expertise, which can constrict the applicability of MFB in risk assessment. On the other hand, there are currently not many methods to estimate behavioural responses of sediment-dwelling animals, which are sensitive to light. With MFB, it was possible to detect the peristaltic and overall locomotory activity in the frequency ranges found in the literature (Sardo and Soares 2010, 2011) and inactivity was also observed (Fig. 6). Thus, MFB is a noteworthy piece in the risk assessment toolbox because it is readily applicable. An advantage of using MFB is that it can be used to monitor these light-sensitive and sediment-burrowing species. Yet there were challenges in behavioural measurements of

L. variegatus. Peristaltic and overall locomotory activities overlapped partly. Forward crawling frequency has been reported to occur at 1.21 Hz (Ding *et al.* 2001), which corresponds with the peristaltic movements recorded with MFB. Validating specific activities is somewhat intractable because simultaneous video monitoring is impossible. Preliminary tests indicated that behaviour was different in water and occurred in a higher frequency range similarly as in Ding *et al.* (2001), in which swimming frequency was at 10.7 Hz. At least the overall activity could require additional validation.

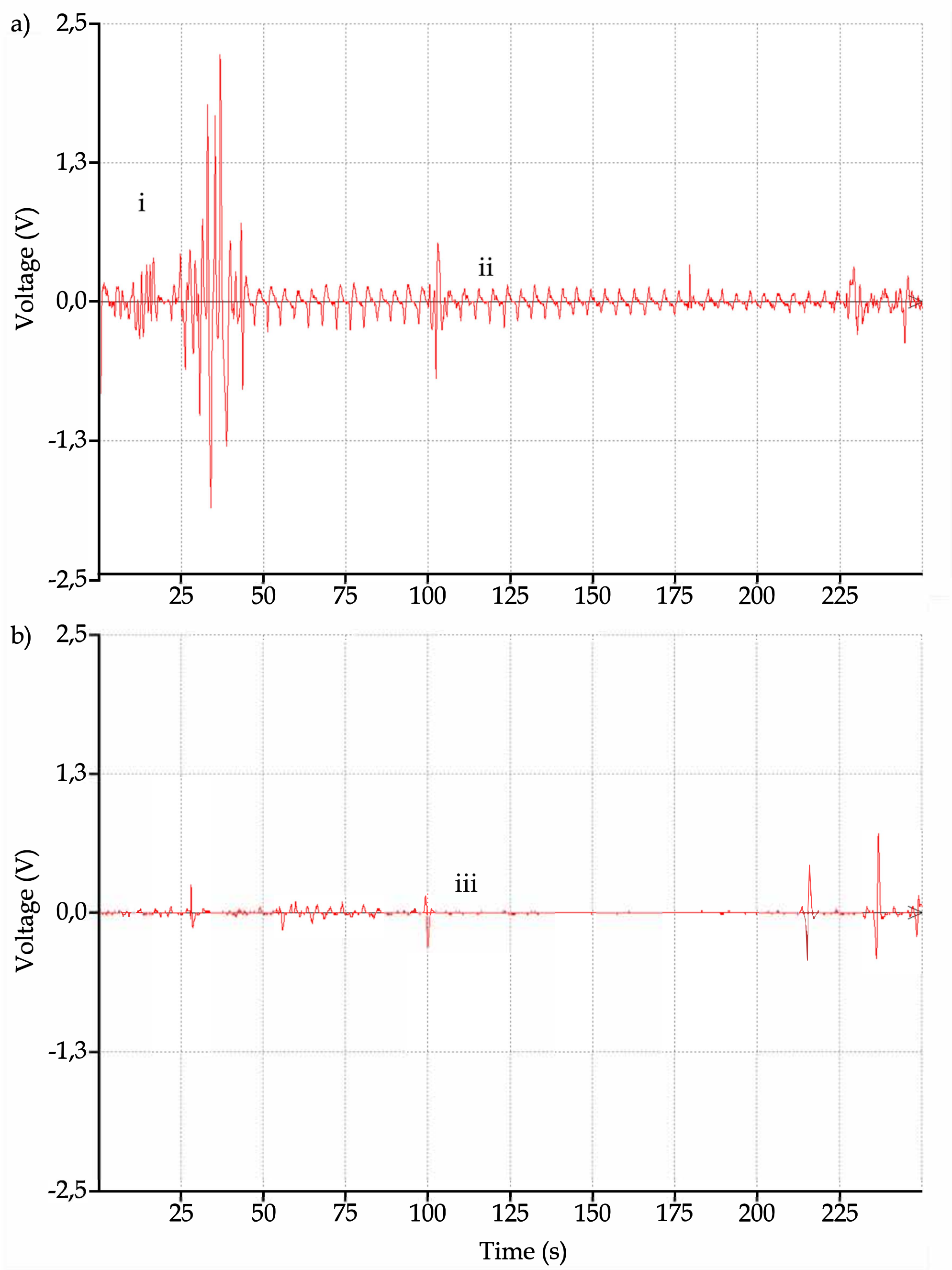

FIGURE 6 Characterized signals of a) the overall activity (i) and peristaltic movements (ii) of *L. variegatus* in the Lake Kiantajärvi sediment at 0 d, and b) immobility (iii) of *L. variegatus* in the Lake Salminen sediment at 21 d.

Six chambers with 1 worm per chamber were used in behavioural measurements so the number of individual worms was low. The maximum number of chambers was 8 in the system used in this thesis, and there is an option to add up to 96 parallel chambers. Even though measuring several animals would produce a more representative concept of the behavioural patterns of the species, the increased workload is a drawback in relation to easy applicability of MFB in risk assessment. One benefit of using biological monitoring is that the work related to traditional toxicity tests is reduced, so this advantage could be lost with numerous measured individuals.

In addition, operating with MFB should be considered when measuring sediment-dwelling organisms. One chamber filled with sediment interfered with the recording of behaviour, and it gave only weak signals during measurement at 21 d (III). The signal was probably suppressed due to the sediment because a normal signal returned when the chamber was cleaned. Vertical positioning is also needed for behavioural measurements with sediments. This could diminish the oxygen flow through mesh from the upper part with the surrounding water in comparison to horizontal positioning of a measurement chamber. Therefore, measurement time could be more limited with sediments than with water-only tests. Although oxygen concentration was over 96 % in all test beakers (III), oxygen was not monitored directly from the chamber and oxygen concentration may have decreased during the 3-hour measurement period in the sediment-water interface. Nevertheless, MFB proved to be at its best in the screening level risk assessment of metal-contaminated sediments. It could be applied when quick effect evaluations are needed.

5 CONCLUSIONS

It has been known for decades that AS soils load the water bodies in the Ostrobothnian region, but the assessment of AS-soil-induced effects has been, and remains, scarce in the estuary sites. It was observed that metals that are easily leached from AS soils were transported together with acidity to the estuaries. Metal contamination of estuary sediments was evident. It appeared that the ecological quality was deteriorated in several estuary sites receiving leachates from the AS soil hotspot areas.

Effluents from biomining and conventional mining shared similar properties as leachates from AS soils. Sediment metal concentrations showed a contamination gradient in the mining-affected lakes. Sequential extractions provided insight about site- and metal-specific partitioning of studied elements in mining-affected sediments. An anthropogenic effect on the element concentrations was estimated to be highest in the lake sediments nearest to the mining site. When additional information is needed, SEs could be useful and attention should be given to sample handling in order to achieve representative sediment samples for SEs. In addition to metals, clear salinization was observed especially in the hypolimnion of lakes contaminated by biomining. Environmental quality standards were exceeded in some AS soil and mining-affected sites but the EQSs for water were not efficient in the separation of sites of a different grade of contamination. Traditional monitoring of contaminants from the water is laborious, and frequent sampling is needed if the aim is to detect the concentration peak. Thus, additional approaches should be used more frequently, which would also detect long-term loading. It could be beneficial to develop site-specific sediment quality guidelines for the classification of contaminated sediments although quality guidelines for single metals are not solely sufficient when assessing toxicity of metal mixtures.

Toxicity tests with *L. variegatus* suggested that exposure to mining-affected lake sediments had an effect on growth and reproduction. Toxicity tests were estimated to express the worst-case scenario in nature. Sensitivity to these traditional toxicity test endpoints was higher than behavioural responses were. However, behavioural responses were observed already in acute exposure with the most polluted sediment. Thus, MFB was a suitable screening level risk assessment tool and it could be employed when fast evaluations of effects or additional toxicity test endpoints are needed.

Sediment metals were bioavailable to *L. variegatus* in the laboratory exposures. *Lumbriculus variegatus* showed accumulation potential for metals according to concentration gradient. Monitoring body residues of metals from benthic macroinvertebrates might be advantageous, but it needs further assessment and validation.

Risk assessment of lake sediments experimentally was challenging, because under laboratory conditions they did not sustain their original properties. The major issue in the sediment risk assessment of inorganic contaminants appeared to be reliable assessment and quantification of effects under conditions that prevailed at the field sites. Accumulation basins are commonly loaded by various contaminants from various sources. Therefore, identifying a single source which is responsible for sediment ecotoxicity is not simple or feasible. It must also be acknowledged that several of the risk assessment methods used in this study have their limitations, and there is no single package of risk assessment tools that could be applied in every case. When whole-sediment toxicity tests are applied, the results should be expressed in relation to reference conditions so that risk is not overestimated. Care should be taken when selecting representative reference sites and it is advantageous to include several reference sediments. There is a demand for environmentally realistic whole-sediment toxicity tests which could be applied in the risk assessment of metal-contaminated sediments on a national level.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Kaivosteollisuudesta ja happamilta sulfaattimailta tulevan metallikuormituksen vesistövaikutukset ja riskien arviointi

Merkittävä osa Suomen vesistöjä kuormittavista epäorgaanisista päästöistä on peräisin paikallisesta maa- ja kallioperästä. Happamat sulfaattimaat ovat merkittävin vesistöjen metallikuormituksen lähde, ja metallikuormituksen lisäksi ne nimensä mukaisesti myös happamoittavat vesistöjä. Suomessa on Euroopan laajimmat happamien sulfaattimaiden esiintymisalueet, jotka sijaitsevat suurimmaksi osaksi Pohjanmaan rannikkoalueilla. Happamat sulfaattimaat ovat muodostuneet useita vuosituhansia sitten Itämeren Litorina-vaiheen aikana. Ne eivät itsessään aiheuta vesistöhaittoja, mutta ongelmia syntyy maankäytön seurauksena. Happamia sulfaattimaita on otettu laajalti viljelyskäyttöön. Kun pohjaveden pinta laskee esimerkiksi salaojituksen vuoksi, sulfidisavi hapettuu ja metallit vapautuvat liukoiseen muotoon. Metallit kulkeutuvat vastaanottaviin vesistöihin happamien valumavesien mukana korkean virtaaman aikana erityisesti keväisin ja syksyisin. Vaikka jokivesien metallipitoisuudet ja happamien sulfaattimaiden ympäristövaikutukset jokivesissä ovat varsin hyvin tunnettuja, jokien suistoalueiden kuormituksesta ja sen vaikutuksista eliöstöön ei ole juuri tietoa saatavilla. Tämän vuoksi tutkimuksessa selvitettiin happamilta sulfaattimailta tulevien valumavesien vaikutuksia kymmenessä eri länsirannikon jokisuistossa.

Metallikaivannaisteollisuuden jätevedet muistuttavat ominaisuuksiltaan happamien sulfaattimaiden valumavesiä, mutta kuormitus on pistemäisempää happamiin sulfaattimaihin verrattuna. Kaivostoiminnan jätevedet voivat myös olla koostumukseltaan vaihtelevampia ja sisältää liuenneiden metallien lisäksi esimerkiksi prosessikemikaalien jäämiä. Kaivosalueilta johdetaan ylijäämävesiä luontoon ympäristölupien puitteissa, mutta lisäksi jätevettä voi päätyä vesistöihin esimerkiksi altaiden tai patojen vuotojen tai rikkoutumisen vuoksi. Tässä tutkimuksessa arvioitiin metallikaivostoiminnan ekotoksikologisia vaikutuksia yhteensä yhdessätoista eri järvikohteessa, jotka sijaitsivat kahden eri kaivoksen vaikutuspiirissä kolmella eri vesistöalueella.

Väitöskirjatyön tavoitteena oli arvioida monimetallikuormituksen riskejä suomalaisissa vesistöissä ja selvittää, eroavatko tutkittujen vesistöjen metallipitoisuudet säädöksissä määritellystä hyvästä kemiallisesta tilasta. Riskinarvioinnissa keskityttiin erityisesti sedimentteihin, jotka ovat vesistöihin kulkeutuneiden ja niiden pohjalle laskeutuneiden maa-ainesten muodostamia kerrostumia. Sedimenttien tilan arviointi on tärkeää, sillä ne voivat toimia yhtä lailla yhdisteiden nieluina tai lähteinä riippuen ympäröivän ympäristön tilasta. Sedimentit ovat aliedustettuina nykyisessä ympäristön laatua määrittelevässä lainsäädännössä. Esimerkiksi tietyille vedessä esiintyville haitta-aineille on asetettu kansallisia laatunormeja, mutta vastaavia ei ole sedimenteille. Lainsäädännössä käsitellään vain ruopattavia sedimenttimassoja ja niiden loppusijoitusta. Tässä työssä tutkittiin, voivatko sedimentteihin päätyneet alkuaineet olla biologisesti saatavilla ja päätyä pohjaeläimiin. Lisäksi arvioitiin ekotoksikologisten testimenetelmien soveltuvuutta sedimentissä esiintyvien useiden haitta-aineiden yhteisvaikutuksen arviointiin.

Sedimenttien haitallisuutta arvioitiin laboratoriokokein kahdella eri pohjaeläinlajilla, *Chironomus riparius* -surviaissääskentoukalla ja *Lumbriculus variegatus* -harvasukamadolla. Vaikka happamien sulfaattimaiden vaikutuspiirissä olevat suistosedimentit eivät olleet myrkyllisiä surviaissääskentoukalle, suistokohteiden pohjaeläinyhteisöjen laatua mittaavat pohjaeläinindeksit olivat hyvää huonompia suistoissa, joiden valuma-alueella oli paljon happamia sulfaattimaita. Heikentynyt pohjaeläimistön laatu oli selkein yksittäinen vaikutusmittari, kun arvioitiin metallipitoisuuksien ja happamuuden yhteisvaikutuksia suistoalueilla.

Tutkimuksessa arvioitiin, että lähellä kaivoksia sijaitsevien järvisedimenttien laatu oli heikentynyt, sillä harvasukamatojen kasvu ja lisääntyminen oli heikompaa erityisesti kuormitetuimpien järvien sedimenteissä verrattuna vertailujärven sedimenttiin. Erot olivat selvimpiä, kun sedimentin päällä käytettiin kultakin järveltä kerättyä alusvettä standardin mukaisen keinotekoisen makean veden asemesta. Lisäksi käyttäytymismittauksissa harvasukamadot olivat aktiivisempia vertailujärven sedimentissä kuin lähimpänä kaivoskohdetta sijaitsevan järven sedimentissä. Kaivosvaikutteisille sedimenteille altistettujen harvasukamatojen aktiivisuus oli samaa tasoa sekä lyhytaikaisen että pitkäaikaisen altistuksen jälkeen. Perinteiset ekotoksikologiset testivasteet, kasvu ja lisääntyminen, olivat näissä kokeissa selvempiä vaikutusmittareita käyttäytymismuutoksiin verrattuna. Harvasukamatojen käyttäytymismittauksia voidaan käyttää perinteisten vasteiden ohella, erityisesti kun vaikutuksia tulee arvioida nopeasti tai muuttuvissa tilanteissa.

Happamilta sulfaattimailta helposti liukenevien metallien (Al, Cd, Co, Cu, Mn, Ni ja Zn) pitoisuudet olivat kohonneet suistovesissä verrattuna taustapitoisuuksiin, ja metallit kertyivät suistosedimentteihin. Kertyminen suistoalueen sedimentteihin vaihteli eri metallien välillä. Joista valuvat happamat vedet lisäsivät myös suistovesien happamuutta. Eräässä tutkimuskohteessa veden pH oli pienimmillään 4,3, ja 19 kuukauden tarkastelujaksolla noin puolella suistokohteista pH-arvot olivat pienempiä kuin 5,0.

Metalleja tuottavien kaivoskohteiden vesistöissä havaittiin vastaavasti malmiometallien korkeita pitoisuuksia sekä vedessä että sedimentissä. Metallipitoisuuksien vertailu vesiympäristölle asetettuihin laatunormeihin ei antanut riittävää kokonaiskuvaa metallien vesistövaikutuksista. Metallipitoisuuksien lisäksi myös muiden epäorgaanisten yhdisteiden, kuten sulfaatin, pitoisuudet olivat korkeita alusvedessä ja sedimentissä. Sedimenttien alkuainepitoisuudet olivat pääsääntöisesti suurimmat lähimpänä kaivosaluetta. Kun metallien jakaantumista tarkasteltiin vaiheittaisten uuttojen avulla, havaittiin metallien sitoutumisen sedimentissä olevan metalli-, sedimentti- ja paikkakohtaista. Esimerkiksi metallien helppoliukoisin osuus oli pääsääntöisesti suurin lähimpänä kaivosaluetta sijaitsevien järvien sedimenteissä.

Useat harvasukamadoista mitatut alkuainepitoisuudet suurenivat, kun niitä vastaavat alkuainepitoisuudet sedimenteissä suurenivat. Metalleilla saastuneiden paikkojen luokittelu onnistui harvasukamatojen avulla. Harvasukamadot sietävät metallikuormitusta melko hyvin, jolloin myös matojen kudosten metallipitoisuuksia voidaan määrittää. Metallien kertyminen matoon oli kuitenkin osin metalli- ja sedimenttikohtaista. Esimerkiksi madoista oli oleellista mitata nikkelipitoisuuksia, jos altistus tuli alusveden kautta. Sen sijaan sinkin määrää tarkasteltaessa altistus myös sedimentin kautta oli tärkeää.
Happamilta sulfaattimailta ja metallikaivostoiminnasta tuleva vesistökuormitus oli samankaltaista. Sedimentit olivat harvasukamatojen merkittävä epäorgaanisten aineiden, kuten metallien, lähde. Sedimentit tulisikin sisällyttää osaksi säädeltyä vesistövaikutusten arviointia. Metalleilla saastuneiden rikkipitoisten sedimenttien riskinarvioinnin haasteena oli niiden fysikaalisten ja kemiallisten ominaisuuksien, kuten pH:n, muuttuminen kuljetuksen ja varastoinnin aikana ennen testausta. Myös edustavan vertailupaikan valinta on tehtävä huolellisesti, vaikka ominaisuuksiltaan esimerkiksi orgaanisen aineksen määrän suhteen tutkimuskohteita vastaavan sedimentin löytäminen voi olla hankalaa. Sedimenttien haitallisuuden arvioinnissa käytettäviä ekotoksikologisia testimenetelmiä tulee edelleen kehittää, jotta niiden vertailtavuus todellisiin ympäristöoloihin nähden tulee luotettavammaksi. Vaikutusten sekä niiden määrän ja laadun arviointiin tarvitaankin edelleen useita riskinarviointimenetelmiä.

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ORIGINAL PAPERS

I

WEIGHT-OF-EVIDENCE APPROACH IN ASSESSMENT OF ECOTOXICOLOGICAL RISKS OF ACID SULPHATE SOILS IN THE BALTIC SEA RIVER ESTUARIES

by

Jaana Wallin, Anna K. Karjalainen, Eija Schultz, Johanna Järvistö, Matti T. Leppänen & Kari-Matti Vuori 2015

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Weight-of-evidence approach in assessment of ecotoxicological risks of acid sulphate soils in the Baltic Sea river estuaries

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HIGHLIGHTS

• Acid sulphate soils release high amounts of metals and acidity.
• Metals and acidity are transported to estuary sites.
• Acid sulphate soils impair the ecological status of several Baltic Sea estuaries.
• More information is needed on low salinity estuaries.

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ABSTRACT

Acidity and leaching of metals from acid sulphate soils (ASSs) impair the water quality of receiving surface waters. The largest ASS areas in Europe are found in the coasts of the northern Baltic Sea. We used weight-of-evidence (WoE) approach to assess potential risks in 14 estuary sites affected by ASS in the Gulf of Finland, northern Baltic Sea. The assessment was based on exposure and effect profiles utilizing sediment and water metal concentrations and concurrent pH variation, sediment toxicity tests using the luminescent bacterium *Vibrio fischeri* and the midge *Chironomus riparius*, and the ecological status of benthic macroinvertebrate communities. Sediment metal concentrations were compared to national sediment quality criteria/guidelines, and water metal concentrations to environmental quality standards (EQSs). Hazard quotients (HQs) were established for maximum aluminium, cadmium and zinc concentrations at low pH based on applicable US EPA toxicity database. Sediment metal concentrations were clearly elevated in most of the studied estuaries. The EQS of cadmium (0.1 μg/l) was exceeded in 3 estuaries out of 14. The pH-minima were below the national threshold value (5.5) between good and satisfactory water quality in 10 estuaries. *V. fischeri* bioluminescence indicated toxicity of the sediments but toxic response was not observed in the *C. riparius* emergence test. Benthic invertebrate communities were deteriorated in 6 out of 14 sites based on the benthic invertebrate quality index. The overall ecotoxicological risk was assessed as low in five, moderate in three and high in five of the estuary sites. The risk assessment utilizing the WoE approach indicated that harmful effects of ASSs are likely to occur in the Baltic Sea river estuaries located at the ASS hotspot area.

1. Introduction

Acid sulphate soils (ASSs) are described as the nastiest soils in the world (Dent and Pons, 1995). This is due to their ability to generate sulphuric acid and extremely low pH to water phase. ASSs have developed in coastal areas mainly during Holocene as a result of microbial activity which reduced sulphide rich sediments under anoxic conditions. Global area of ASSs is approximately 12 to 13 million hectares, mainly in tropical areas (Andriesse and van Mensvoort, 2006).

Finland has the largest ASS area in Europe (Roos and Åström, 2005). ASSs in Finland have been formed during Litorina period of Baltic Sea, approximately 4000–8000 bp (Palko, 1994). Area of ASSs has been estimated to cover 1600–3400 km^2 in Finland (Andriesse and van Mensvoort, 2006; Palko, 1994). The particular feature of Finnish ASSs is generally low sulphur content and buffering capacity in comparison to the tropical ASSs (Dent and Pons, 1995; Yli-Halla et al., 1999; Åström and Björklund, 1997).

When ASSs are exposed to oxygen, sulphides are rapidly oxidized and sulphuric acid is formed (Dent and Pons, 1995). Draining of ASSs

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for agriculture and other land use activities enhances oxidation of sulphide layers and consequent formation of sulphuric acid and leaching of metals. Thus acid runoff and high concentrations of dissolved metals have deteriorated ecological and chemical status of water bodies for centuries along the western coast of Finland. Massive drainage of ASSs for agricultural purposes occurred during 1960s and 1970s in Finland (Saarinen et al., 2010; Åström et al., 2005), and it has been estimated that metal loads from ASSs exceed the metal discharges of industrial effluents (Sundström et al., 2002). It is expected that climate change will increase acid runoff from ASSs (Saarinen et al., 2010).

Metals known to be abundantly leached from the Baltic ASSs include aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn) (Boman et al., 2010; Nordmyr et al., 2008a; Åström and Björklund, 1997; Åström and Corin, 2000; Åström and Spiro, 2000). Acidity and high metal concentrations may have detrimental effects on biodiversity and community structure of fish, benthic invertebrates and aquatic plants (Fältmarsch et al., 2008). Occasional large fish kills are distinct effects of acidity and metal exposure. Acidity and high metal concentrations also impair reproduction, development and metabolism of fish (Hudd, 2000).

Concentrations, behaviour and speciation of metals in water bodies, especially estuaries, affected by runoff from ASSs are yet largely unknown (Fältmarsch et al., 2008; Nystrand et al., 2012; Åström and Corin, 2000). Therefore assessing hazardous effects of ASSs is challenging. Estuaries are considered as ecologically sensitive environments since their water chemistry, *e.g.* pH and salinity levels, varies according to rainfall, river discharge, tides and the overall climate conditions. In Finland, estuaries are unique environments since they locate in the coastal areas of Baltic Sea where tide is negligible and salinity is low (1–2‰) compared to tidal estuaries, and where salinity level fluctuations are mostly affected by the river discharge.

Weight-of-evidence (WoE) approach consists of multiple lines of evidence (LoE) (Chapman, 2007). Different LoE include screening of contaminant levels, evaluating their bioavailability and effects (Chapman, 2007; EPA, 1998). Laboratory and field toxicity tests are often included also. Different lines of evidence are weighed and based on the evidence, the risk of studied contaminants in the environment is determined. It is a flexible method, and WoE approach can be assembled individually to each studied subject area. WoE approach is suitable in comprehensive risk assessments, because evaluation of all the available data is by definition required (EPA, 1998), and it has been applied successfully in various subject areas (Chapman, 2007; Wolfram et al., 2012).

The objective of this study was to characterize ecotoxicological risks in 10 Baltic Sea river estuaries affected by ASS hotspot area based on their exposure and effect profiles using a WoE approach. Environmental quality objectives set by EU Water Framework Directive (WFD, Directive 2000/60/EC) call out for detailed information on the chemistry and biology of estuaries; hence, we addressed the following questions: 1) do the metal concentrations in water and sediment deviate from Environmental Quality Standards (EQSs) set by WFD and other applicable guidelines regulating metal pollution, 2) do the estuary sediments have toxic effects on biota, and 3) does the ecological status of estuaries reach the WFD quality objectives?

2. Material and methods

2.1. Study area

The study area is located in the Western coast of Finland and is comprised of 10 river estuaries in the Gulf of Bothnia (Fig. 1). All estuaries are affected by ASS runoff, but the exact area of ASSs in river catchments is unknown. River catchments ranged from 500 km^2 to 4923 km^2 and cultivated area upstream estuaries from 63 km^2 to 1160 km^2.

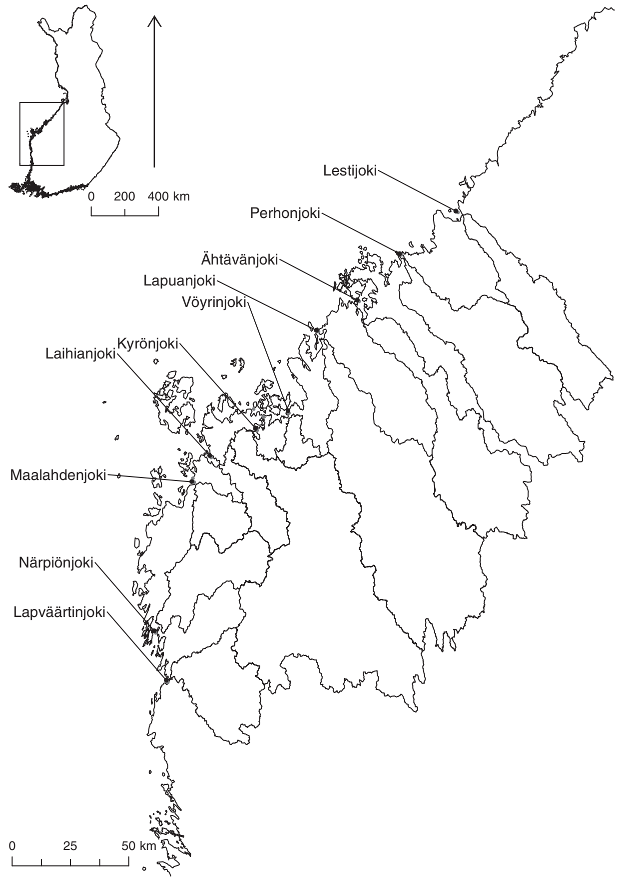

Fig. 1. Study area, estuaries and their upstream river catchment areas. Base map: © MML, 2013 Catchment areas: © SYKE, 2013.

2.2. Problem formulation

We followed the ecological risk assessment (ERA) procedure of US EPA (1998). Problem formulation included gathering the available information, evaluating nature and significance of the risk and developing a conceptual model for risk assessment (Fig. 2). Data and risk characterization plans were developed. Good ecological and chemical status of estuaries was defined as assessment endpoints, *i.e.* the environmental values to be protected.

2.3. Exposure and effect assessment

2.3.1. Water and sediment quality

Water quality data was obtained from the database of Finnish Environment Institute (HERTTA version 5.6). Water quality monitoring results for Al, As, Cd, Co, Cr, Cu, Pb, Ni, Zn, alkalinity, conductivity, total organic carbon (TOC), salinity and pH were collected from March 2009 to September 2010 to represent exposure conditions prevailing during the field work period of our study, and preceding time frame covering 19 months and three flood periods. Monitoring locations near river mouths were selected to represent estuary conditions in case no water quality measurements in the estuary area existed.

Sedimentation rates have been determined from the estuaries of R. Kyrönjoki and R. Vöyrinjoki, and they are approximately 1 cm/a and 4–5 cm/a, respectively (Heikkilä, 1999; Nordmyr et al., 2008a). Surface sediment samples (0–3 cm) were collected during the summer and autumn of 2010 from 10 different estuaries and 14 sample locations to

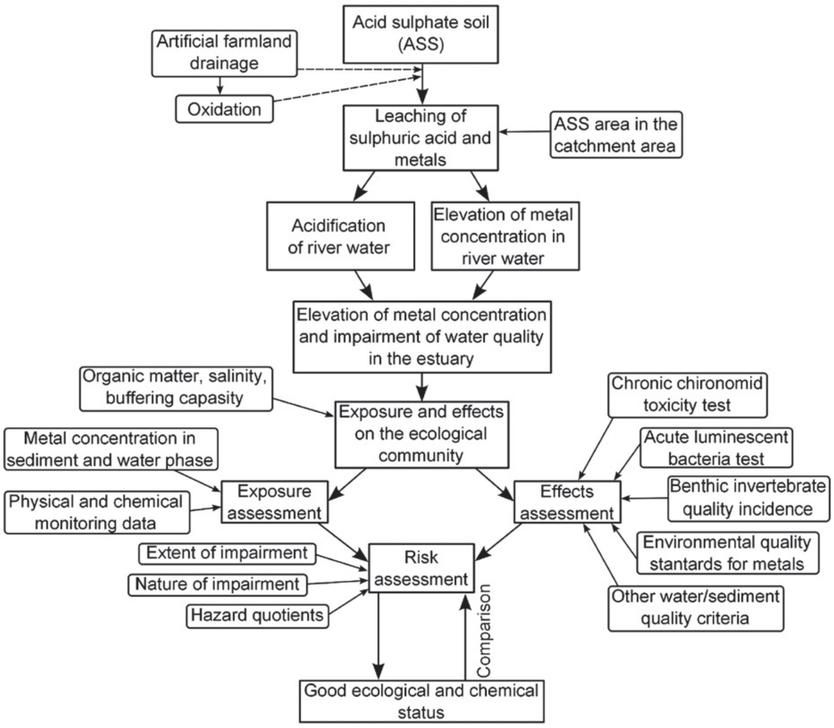

Fig. 2. Conceptual model of risk assessment.

represent three years before sediment sampling at the most. In the rivers Kyrönjoki and Maalahdenjoki samples were taken from three different distances from the river mouth towards the sea (at the river mouth, 6 km and 12 km distances at Kyrönjoki and at the river mouth, 0.5 km and 2 km distances at Maaladenjoki) in order to identify potential gradient of pollution impact. In every location three ca 100 ml replicate samples were taken from the first accumulation basin in the estuary with a Sandman corer for metal analysis and the luminescent bacteria test. An additional sediment sample was taken with an Ekman grab for the chronic Chironomid toxicity test. All the sediment samples were stored in plastic polyethylene containers in a cooler in the field, transported to laboratory in 7 h and stored at −20 °C.

Dry weight (dw) and loss on ignition (LOI) were determined at 105 °C in 24 h, and at 800 °C in 1 h, respectively. LOI was used as an approximate for sediment organic matter (SOM). Concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the sediment samples were determined by Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) technique (Perkin-Elmer Elan DRC II) after microwave digestion with HNO_3. Sampling and analysis for all water quality parameters were carried out in accredited laboratory of the Finnish Environment Institute. Analyses of Co and Fe were not included in the accreditation.

Background metal concentrations for sediment and water phase were estimated from the literature (Government Decree 868/2010; Nordmyr et al., 2008a; Tenhola and Tarvainen, 2008; Verta et al., 2010; Åström and Björklund, 1997). Water metal concentrations were compared with national EQSs during the observation interval (3/2009–9/2010) and minimum pH values in the estuaries were tabulated. Sediment metal concentrations were compared both with the Swedish SQCs (Naturvårdsverket, 1999) since they were estimated to resemble Finnish coastal sediments and with the Finnish dredging guideline (Ministry of the Environment, 2004). Clay content of the sediment samples was approximated to 25% from literature (Nordmyr et al., 2008a) for comparison with national dredging guideline (Ministry of the Environment, 2004) that applies organic matter (LOI) and clay fraction in assessment.

2.3.2. Ecotoxicological effects

We tested the sediment samples for acute and chronic toxicity by luminescent bacteria *Vibrio fischeri* (ISO 21338, 2010) and *Chironomus riparius* midge (OECD, 2004), respectively. For the *V. fischeri* test, 1 g of freeze dried sediment sample was extracted with 5 ml 2% sodium chloride (NaCl) solution in two parallel extractions, and bacteria were exposed to the extract at concentrations ranging from 50% to 0.10% in two replicates. The test was performed as described in ISO standard 21338 (2010) standard, except that the extract was buffered with sodium bicarbonate ($NaHCO_3$) to reduce the variation of pH, and to yield a test pH of 7. Extracts were diluted with 2% NaCl as two parallel dilutions series and each dilution step was tested for toxicity. Effective concentrations (EC_{50}-values) expressed as percentage concentrations of the extract in the test were calculated with probit analysis using SPSS software version 20.0.0 (IBM, 2013).

The laboratory reared *C. riparius* culture of Finnish Environment Institute was set up in June 2011 from six egg masses and it is a mixture of Dutch and German laboratory strain originating from the Institute of environmental studies TNO and the University of Tübingen. The culture was maintained at 20 ± 2 °C temperature in aquariums with sediment to water ratio 1:4 (v/v). One third of the water was changed monthly and water quality (pH, t, O_2 saturation, hardness) was monitored. Water was modified Elendts M7 (without RbCl) artificial freshwater (OECD, 2004). The culture was fed with approximately 1 g of TetraMin, suspended in ultrapure water, 3 times per week per aquarium.

C. riparius test was conducted according to OECD 218 (2004) test guideline as modified in two non-simultaneous tests. In addition to the emerging time of adults and proportion of emerged adults, also mentum deformities of the 4th instar larvae mouthparts was used as an endpoint. Mentum deformities are commonly used as toxicity indicators in field surveys (Groenendijk et al., 1998) and they have also been studied in standard laboratory tests (*e.g.* Janssens de Bisthoven et al., 2001). Temperature, sediment and water pH (VWR pHenomenal), oxygen saturation (VWR DO200), total ammonium (NH_4^+/NH_3) (Tetra test NH_3/NH_4^+) and water hardness (Tetra test GH/KH) was monitored during the tests. Modified artificial sediment (OECD, 2004) with 17% kaolinite clay, 70–75% quartz sand and 2–2.5% SOM (nominal proportions) was used as a control sediment. Natural sediment from Lake Palosjärvi (OC 23%) from unindustrialized area was used as a source of organic material in the artificial sediment. Control or decanted and blended test sediment was weighed 24–52 g into the test vessels to reach 1.5 cm sediment pillars. The test vessels were filled with Elendts M7 (without RbCl) artificial freshwater (OECD, 2004) to reach sediment to water ratio of 1:4 (v/v). Number of replicates per treatment varied from two to five depending on the amount of test sediment available. Control sediments constituted from six replicates. Sediments were conditioned in test vessels for one week after which the conditioning water was removed in order to remove potential ammonia (Moore et al., 1997) and sediment pH was measured. Test water (M7) was added and it was gently aerated with glass Pasteur pipettes for two days prior to start of the test. Evaporated water was compensated with deionised water (Milli-Q®) periodically. The 1st instar (2–3 d old) *C. riparius* larvae were introduced with glass Pasteur pipettes to the test vessels. Aeration of overlying water was resumed one day after introducing the larvae to the test vessels. From the 12th to the 28th day test vessels were observed daily and emerged adults were counted, gender determined and removed. The larvae were fed with 0.5 ml of ground TetraMin® suspension (10 mg/ml) 3 times per week and after 10 days the amount of food suspension was increased to 1.0 ml. At the end of the test organic matter was eliminated with 10% KOH and sediments were sieved through 250 μm mesh. Head capsules of emerged adults and 4th instar larvae that failed to emerge were collected and mounted to glass microscope slides with Euparal mounting medium. Mentum deformities were inspected under light microscope with 40-fold magnification and deformities were classified either as mechanical damage (broken teeth) or as developmental deformities (extra or missing teeth and gaps). Deformity index (DI, Hämäläinen, 1999) was calculated as follows:

$$DI = \frac{d}{n}$$

where *d* is the number of deformed or damaged larvae and *n* is the number of analyzed menta.

Mechanical damage index (MI) was calculated similarly.

2.3.3. Ecological effects on benthic macroinvertebrates

Ecological status of benthic macroinvertebrate communities was assessed using brackish water benthic index (BBI) described by Perus et al. (2007). Monitoring results covering years 2010–2012 and reported in the second national River Basin Management Plan according to WFD were compiled from the Water Body database of HERTTA (version 5.6). If brackish water benthic indices (BBI) were not determined to the estuary site, we used the nearest river benthic invertebrate quality indices defined for a site nearest to the river mouth. Benthic quality index encompasses data from several follow-up sites along the estuary area and it is an average representing situation on the larger area. Thus only one benthic invertebrate quality index was available for study sites for different distances in the estuaries of R. Kyrönjoki and R. Maalahdenjoki although there were three sampling locations for sediment and water in these estuaries.

2.4. Risk characterization

Weight-of-evidence approach was used to characterize the probability and severity of risks for adverse impacts on ecotoxicological status of estuaries impacted by ASS runoff. In addition to the laboratory tests, we compiled acute toxicity data (EC/LC_{50}) of selected metals (Al, Cd, Zn) from the database of United States Environmental Protection Agency (EPA ECOTOX version 4.0). Relevant toxicity test results corresponding to Finnish water chemistry (alkalinity, humic content) and acidity conditions at high discharge in the ASS affected river estuaries were selected. An average between the lower and upper limit values of variable was selected if only a range (*e.g.* confidence limits) was reported to a variable. The selection criteria were: water hardness ≤ 0.505 mmol/l (50.5 mg/l) as $CaCO_3$, pH < 5.9, temperature between 8–25 °C, and DOC undefined − 6.7 mg/l. After the selection the EC/LC_{50} values included in the WoE constituted of the following test species: mayflies (*Ephemeroptera*), caddisflies (*Trichoptera*), midge diptera (*Chironomidae*), rainbow trout (*Oncorhynchus mykiss*), crustacean amphipod (*Hyalella azteca*), waterflea (*Ceriodaphnia dubia*) and unicellular algae (*Pseudokircheneriella subcapitata*). Estimated background concentration from EQS (Verta et al., 2010; Government Decree 868/2010) for Cd and from stream water survey of Geological Survey of Finland (GTK) (Tenhola and Tarvainen, 2008) for Al and Zn was added to the fifth percentile of the EC/LC_{50} values. Hazard quotients (HQs) were calculated as follows:

$$HQ = \frac{EEC_{max}}{P_{5_{EC_{50}}} + C_b}$$

where EEC_{max} is the maximum environmental concentration of selected metal in estuary water or river water if estuary metal concentration was not available, P_{5EC50} is the 5th percentile of selected metal derived from the included EC/LC_{50}-values from the EPA ECOTOX database and C_b is the estimated background concentration. The 5th percentile was derived with the weighted average method of SPSS statistical software (IBM, 2013) (Al: n = 2; Cd: n = 30; Zn: n = 13).

To assess the quality and quantity of ASS related impacts in the study sites, different LoE were ranked using categories (EPA, 1998). Weight was assigned as an expert judgement to every LoE in a following rationale:

Swedish SQC: 1, 2 or 3 scores were assigned if sediment As, Cd, Co, Cr, Cu, Ni, Pb or Zn concentration exceeded class III, IV or V of SQCs (Naturvårdsverket, 1999), respectively. Sum of scores for one estuary and all the metals with weights in three categories based on the range of minimum and maximum scores → weight: 1–8 → 1; 9–16 → 2; 17–24 → 3.

National dredging guideline: 1 or 2 scores were assigned if normalized sediment As, Cd, Cr, Cu, Ni, Pb or Zn concentration exceeded first or second threshold of national dredging guideline (Ministry of the Environment, 2004). Sum of scores → weight: 1–7 → 1; 8–14 → 2; 14–21 → 3.

AA-EQS: 1 score for every exceeding of AA-EQS for Cd, Ni and Pb. Sum of scores → weight 1 → 1; 2 → 2; 3 → 3.

HQ: 1 score for every HQ > 1 for Al, Cd and Zn. Sum of scores → weight: 1 → 1; 2 → 2; 3 → 3.

Minimum pH → weight: 5.5–5.1 → 1; 5.0–4.6 → 2; ≤4.5 → 3.

BBI → weight: Moderate → 1; Poor → 2; Bad → 3.

For *V. fischeri* toxicity test results weight was assigned as 1 (all samples toxic, no significant differences) and for *C. riparius* weight was assigned as 0 (no explicit responses).

The eventual summary was defined by adding up weights assigned to each LoE. Sum of LoE → Final risk class: 0–2.5 → 0; 2.6–5.0 → 1; 5.1–7.5 → 2; 7.5–10.0 → 3. Final weights and risk classes from 0 to 3 were ranked as negligible (+/−), low (+), moderate (++) or high (+++).

2.5. Statistical tests

Statistical tests were conducted with the SPSS statistical software version 20.0.0 (IBM, 2013). Spearman's correlation analysis was conducted to find significant correlations between sediment metal concentrations, sediment LOI and *V. fischeri* EC_{50}. Proportions of emerged adults, sex ratios and mentum deformities were compared with one-

Table 1
Water quality of estuaries on observation interval 3/2009 – 9/2010. Highlighted Cd concentrations indicate overrun of national AA-EQS (Cd: 0.10 μg/l). Minimum pHs are highlighted from lightest to darkest indicating pH levels under 5.5, 5.0 and 4.5.

Estuary		Alkalinity mmol/l	Al μg/l	As μg/l	Cd μg/l	Co μg/l	Cr μg/l	Cu μg/l	Pb μg/l	Ni μg/l	Zn μg/l	Conductivity mS/m	TOC mg/l	Salinity ‰	pH
R. Lestijoki	n	26	22	12	12	0	12	12	12	12	12	29	23	0	29
	min - max	0.04 - 0.26	92 - 2440	0.74 - 1.30	0.01 - 0.04		0.4 - 1.8	0.7 - 6.0	0.1 - 0.6	0.6 - 24	2 - 11	4 - 56	13 - 32		5.7 - 7.1
	mean	0.15 (± 0.07)	609 (± 534)	0.88 (± 0.16)	0.02 (± 0.01)		1.1 (± 0.4)	2.2 (± 1.4)	0.3 (± 0.1)	5 (± 7)	6 (± 3)	7 (± 10)	20 (± 5)		6.3
R. Perhonjoki	n	22	22	12	12	0	12	12	12	12	12	22	22	0	22
	min - max	0.04 - 0.18	190 - 850	0.88 - 1.50	0.01 - 0.09		0.8 - 6.3	0.9 - 3.9	0.3 - 0.7	1.3 - 28	3 - 14	4 - 8	16 - 25		5.4 - 6.7
	mean	0.10 (± 0.05)	535 (± 207)	1.08 (± 0.20)	0.03 (± 0.02)		2.3 (± 1.6)	2.0 (± 0.9)	0.4 (± 0.1)	9 (± 8)	9 (± 4)	6 (± 1)	20 (± 3)		5.9
R. Ähtävänjoki /	n	7	7	0	0	0	0	0	0	0	0	7	7	0	7
L. Luodonjärvi	min - max	0.06 - 0.26	170 - 1800									8 - 11	13 - 17		6.2 - 7.0
	mean	0.17 (± 0.07)	536 (± 574)									10 (± 1)	15 (± 2)		6.5
R. Lapuanjoki	n	22	22	22	22	0	22	22	22	22	22	22	22	0	22
	min - max	0.01 - 0.37	350 - 2300	0.39 - 1.30	0.01 - 0.19		0.6 - 3.7	1.5 - 9.6	0.1 - 1.4	3.3 - 27	4 - 59	10 - 21	14 - 25		4.9 - 6.9
	mean	0.16 (± 0.13)	1099 (± 627)	0.79 (± 0.22)	0.08 (± 0.05)		1.3 (± 0.7)	3.4 (± 1.7)	0.5 (± 0.02)	13 (± 8)	24 (± 16)	14 (± 3)	20 (± 2)		5.6
R. Vöyrinjoki	n	9	9	0	1	0	1	1	1	1	1	9	0	0	9
	min - max	0.01 - 0.23	1600 - 7300		0.41 - 0.41		2.2 - 2.2	11 - 11.0	0.7 - 0.7	67 - 67	140 - 140	29 - 63			4.3 - 6.5
	mean	0.04 (± 0.07)	5222 (± 2093)		0.41 (± 0.00)		2.2 (± 0.00)	11.0 (± 0.0•)	0.7 (± 0.0)	67 (± 0)	140 (± 0)	41 (± 11)			4.4
R. Kyrönjoki 1	n	14	14	14	14	14	14	0	14	14	14	14	6	0	14
	min - max	0.01 - 0.46	500 - 2100	0.71 - 1.80	0.01 - 0.22	0.7 - 13.0	0.6 - 11.0		0.2 - 0.9	7 - 31	4 - 230	14 - 63	16 - 22		5.0 - 7.2
	mean	0.16 (± 0.16)	1281 (± 503)	1.03 (± 0.38)	0.11 (± 0.06)	6.5 (± 3.8)	3.1 (± 3.4)		0.5 (± 0.2)	18 (± 6)	46 (± 55)	23 (± 15)	18 (± 2)		5.8
R. Kyrönjoki 2	n	14	14	14	14	14	14	0	14	14	14	72	12	6	72
	min - max	0.01 - 0.70	330 - 2000	0.47 - 3.70	0.01 - 0.27	0.6 - 14.0	0.6 - 15.0		0.01 - 0.9	5 - 30	4 - 62	12 - 450	10 - 22	0.3 - 2.20	5.0 - 7.5
	mean	0.22 (± 0.24)	1184 (± 599)	1.36 (± 0.93)	0.12 (± 0.06)	6.4 (± 4.0)	4.9 (± 4.0)		0.5 (± 0.2)	16 (± 8)	28 (± 17)	115 (± 129)	16 (± 3)	1.0 (± 1.0)	6.1
R. Kyrönjoki 3	n	15	15	15	15	15	15	0	15	15	15	25	12	12	25
	min - max	0.18 - 0.88	26 - 1300	0.05 - 3.80	0.01 - 0.15	0.1 - 8.5	0.2 - 16.0		0.01 - 0.5	2 - 20	1 - 230	120 - 660	6 - 17	0.9 - 3.7	6.4 - 7.8
	mean	0.56 (± 0.27)	470 (± 464)	1.90 (± 1.32)	0.08 (± 0.05)	3.0 (± 2.9)	4.6 (± 4.5)		0.3 (± 0.1)	9 (± 6)	29 (± 57)	446 (± 174)	9 (± 3)	2.9 (± 0.8)	7.1
R. Laihianjoki	n	9	0	0	0	0	0	0	0	0	0	14	0	0	14
	min - max	-0.43										25 - 49			4.4 - 6.7
	mean	0.16 (± 0.17)										34 (± 8)			5.1
R. Maalahdenjoki 1	n	15	15	15	15	15	15	0	15	15	15	15	6	0	15
	min - max	0.01 - 1.10	110 - 3400	0.05 - 4.90	0.01 - 0.31	0.4 - 14.0	0.2 - 12.0		0.01 - 0.9	3 - 32	2 - 65	17 - 780	10 - 28		4.6 - 7.7
	mean	0.48 (± 0.44)	1597 (± 1258)	2.19 (± 1.70)	0.14 (± 0.10)	6.6 (± 5.3)	5.2 (± 3.7)		0.4 (± 0.2)	17 (± 11)	31 (± 25)	337 (± 289)	18 (± 7)		5.2
R. Maalahdenjoki 2	n	15	15	15	15	15	15	0	15	15	15	15	6	0	15
	min - max	0.11 - 1.10	95 - 3000	0.05 - 7.00	0.03 - 0.17	0.5 - 10.0	0.2 - 28.0		0.01 - 1.0	3 - 23	1 - 46	84 - 810	9 - 27		5.8 - 8.0
	mean	0.69 (± 0.35)	1109 (± 979)	3.10 (± 1.74)	0.10 (± 0.05)	4.2 (± 3.5)	6.9 (± 7.4)		0.4 (± 0.2)	11 (± 8)	18 (± 16)	507 (± 224)	15 (± 7)		6.6
R. Maalahdenjoki 3	n	15	15	15	15	15	15	0	15	15	15	15	6	0	15
	min - max	0.32 - 1.10	52 - 1700	0.05 - 7.30	0.01 - 0.18	0.5 - 5.7	0.2 - 36.0		0.01 - 1.8	2 - 15	1 - 29	280 - 770	9 - 18		6.8 - 8.3
	mean	0.74 (± 0.26)	566 (± 541)	3.20 (± 1.79)	0.07 (± 0.06)	2.2 (± 1.8)	7.1 (± 9.4)		0.34 (± 0.4)	8 (• ± 4)	10 (± 9)	523 (± 155)	14 (± 4)		7.3
R. Närpiönjoki	n	19	13	12	12	0	12	12	12	12	12	20	12	0	20
	min - max	0.05 - 0.67	530 - 2300	0.74 - 1.80	0.03 - 0.16		1.1 - 2.4	3.8 - 6.7	0.4 - 0.7	6 - 26	6 - 45	8 - 20	15 - 29		5.5 - 7.1
	mean	0.26 (± 0.20)	1462 (± 574)	1.00 (± 0.37)	0.10 (± 0.04)		1.8 (± 0.4)	5.4 (± 1.0)	0.5 (± 0.1)	15 (± 6)	26 (± 12)	14 (± 3)	22 (± 4)		6.1
R. Lapväärtinjoki	n	22	22	22	22	0	22	22	22	22	22	23	22	0	23
	min - max	0.05 - 0.56	180 - 1500	0.45 - 1.20	0.01 - 0.05		0.4 - 3.7	0.6 - 3.3	0.2 - 1.0	0.9 - 5	2 - 48	3 - 9	8 - 32		5.5 - 7.3
	mean	0.21 (± 0.16)	739 (± 342)	0.63 (± 0.17)	0.03 (± 0.01)		1.2 (± 0.7)	2.0 (• ± 0.6)	0.4 (± 0.2)	3 (± 1)	10 (± 9)	6 (± 2)	18 (± 6)		6.2

way ANOVA or nonparametric Kruskal–Wallis test if normal distribution and equality of variance were not achieved. Kaplan–Meier survival tables and logrank correlation were used to estimate and compare median emergence times. Kaplan–Meier survival functions represent pooled data from different replicates in a group. Statements of significant differences are based on $p \leq 0.05$.

3. Results

3.1. Exposure profiles

Metal concentrations in water indicated clear contamination of the estuary waters. The mean Cd and Ni concentrations were 1.5 to 7.0 times higher, and 3 to 18 times higher than average national background concentrations of 0.02 μg/l and 1 μg/l, respectively (Table 1) (Verta ym. 2010). Instead, Pb concentrations were on average at the background concentration level of 0.5 μg/l. The water Cd concentrations exceeded national annual average (AA) EQS in 5 of the studied estuaries (Table 1). AA-EQS was not exceeded for Ni and Pb during the observation interval. The national maximum annual concentration (MAC) EQS for Cd is 0.45 μg/l (Government Decree 868/2010) and it was not exceeded in the studied estuaries during observation interval. MAC-EQS is not applied to Ni and Pb concentrations. In addition, acid conditions were also observed in most of the studied estuaries and pH minimum varied from 4.3–6.8.

The characteristics of the sediment samples were different among estuaries and LOI varied from 4.5 to 24%, the mean value being 15% (Table 2). Organic matter content (LOI) was lowest in the estuaries of R. Lestijoki, R. Perhonjoki, R. Kyrönjoki 1 and R. Maalahdenjoki 2 which corresponded to that of sulphidic postglacial brackish water parent sediments (Nordmyr et al., 2006). Correlation coefficients were significant ($p < 0.01$) between LOI and Al ($r = 0.77$), Cd ($r = 0.72$), Co ($r = 0.56$), Cu ($r = 0.86$), Fe ($r = 0.53$), Mn ($r = 0.57$), Ni ($r = 0.65$), Pb ($r = 0.69$) and Zn ($r = 0.60$). In general, sediment Cd, Co, Cu, Ni and Zn concentrations were elevated in the estuaries of ASS hotspot areas and indicated clear deviation (Class III) from background concentrations when classified according to Swedish SQCs (Table 2). Elevated As concentration was observed in one sample location (R. Kyrönjoki 3).

The normalized sediment metal concentrations (10% OM, 25% clay) and their comparison to the national concentration thresholds of dredging guideline (Ministry of the Environment, 2004) are presented in Fig. 3. Most commonly the first threshold of national dredging guideline was exceeded by Cd, Ni and Zn concentrations and these sediments were classified as potentially contaminated. The mean concentrations of As, Cr and Cu also exceeded the first threshold of national dredging guideline in the R. Kyrönjoki 3, R. Perhonjoki and R. Laihianjoki study sites, respectively. Only Ni concentrations exceeded the second threshold of dredging guide in three estuary sites and those sediments were classified as contaminated. Sediment Pb concentrations exceeded neither the first (40 mg/kg) nor the second threshold (200 mg/kg) of national dredging guideline.

3.2. Effect profiles

The sediment extracts were toxic to luminescent *V. fischeri* 30 minute EC_{50}-values ranging from 0.03% to 9.4% (Table 2). Correlation analysis indicated a significant but weak negative association between individual metal concentrations and *V. fischeri* EC_{50}-values and the significant correlation coefficients were detected for Co ($r = -0.38$; $p < 0.05$), Fe ($r = -0.48$; $p < 0.01$), Pb ($r = -0.46$; $p < 0.01$) and Zn ($r = -0.32$; $p < 0.05$).

Although the sediment samples indicated toxicity to *V. fischeri*, no explicit adverse responses were observed in chronic *C. riparius* toxicity test. Adult emergence varied from 50–100% (Table 3) but significant differences were not observed between exposures in the first experimental set up (Kruskal–Wallis test, $p = 0.139$) or the second experimental set up of the test (Kruskal–Wallis test, $p = 0.239$). Valid conditions for a standard test (OECD, 2004) were not fully achieved in the control

Table 2
Sediment metal concentrations, LOIs and bioluminescence EC_{50}-values. Colour codings from lightest to darkest indicate concentrations that exceed limits of classes 3 to 5 of Swedish SQCs respectively.
1 = nearest sample point from the estuary 3 = farthermost sample point towards sea.

Estuary	Distance from estuary	Sediment metal concentration mg/kg dw (± SD)											LOI (SD)	EC50 (SD)
		Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	%	%
R. Lestijoki		9572 (± 202)	10.4 (± 0.6)	0.23 (± 0.02)	8.0 (± 0.2)	21.2 (± 0.2)	9.0 (± 0.3)	20364 (± 598)	252 (± 11)	11.3 (± 0.3)	5.5 (± 0.2)	71 (± 2)	4.5 (± 0.2)	9.34 (± 1.27)
R. Perhonjoki		9656 (± 2264)	9.5 (± 2.7)	0.35 (± 0.08)	10.7 (± 2.6)	81.6 (± 30.9)	12.2 (± 3.5)	23615 (± 6466)	330 (± 91)	11.5 (± 2.6)	7.5 (± 2.1)	101 (± 23)	7.0 (± 1.8)	0.52 (± 0.34)
R. Ähtävänjoki/L. Luodonjärvi		26571 (± 223)	15.5 (± 0.4)	0.76 (± 0.01)	44.4 (± 2.4)	40.2 (± 0.2)	24.1 (± 0.1)	52574 (± 2275)	1492 (± 188)	42.9 (± 0.9)	17.1 (± 0.3)	230 (± 2)	18.3 (± 0.3)	0.03 (± 0.004)
R. Lapuanjoki		25986 (± 4242)	11.9 (± 1.8)	0.46 (± 0.08)	17.8 (± 2.9)	36.1 (± 0.55)	26.1 (± 4.1)	38116 (± 1595)	507 (± 87)	28.4 (± 4.5)	9.9 (± 1.5)	147 (± 23)	17.5 (± 0.8)	5.54 (± 9.41)
R. Vöyrinjoki		43872 (± 6429)	8.1 (± 1.4)	0.59 (± 0.15)	22.9 (± 6.8)	39.7 (± 6.0)	50.5 (± 6.9)	34293 (± 4170)	885 (± 159)	45.5 (± 9.4)	11.1 (± 1.9)	173 (± 54)	22.3 (± 2.4)	1.97 (± 3.22)
R. Kyrönjoki	1	25586 (± 1732)	8.3 (± 0.18)	0.27 (± 0.04)	23.0 (± 1.9)	48.9 (± 4.4)	19.9 (± 2.2)	34950 (± 2659)	444 (± 40)	36.4 (± 2.6)	10.2 (± 0.7)	146 (± 8)	7.3 (± 0.4)	0.13 (± 0.04)
R. Kyrönjoki	2	30605 (± 1661)	9.3 (± 0.7)	0.66 (± 0.07)	31.3 (± 2.8)	49.3 (± 2.6)	35.5 (± 1.8)	35967 (± 2028)	596 (± 75)	53.2 (± 3.8)	10.8 (± 0.6)	210 (± 17)	12.0 (± 0.6)	0.22 (± 0.21)
R. Kyrönjoki	3	35552 (± 198)	27.2 (± 0.8)	0.72 (± 0.04)	58.8 (± 1.9)	53.3 (± 0.1)	35.7 (± 1.1)	41133 (± 601)	4193 (± 323)	64.5 (± 2.5)	14.0 (± 0.3)	315 (± 9)	14.7 (± 0.2)	7.97 (± 3.27)
R. Laihianjoki		59900 (± 1728)	10.2 (± 0.3)	0.92 (± 0.04)	74.3 (± 2.8)	47.1 (± 1.9)	63.1 (± 1.9)	38355 (± 1379)	788 (± 10)	130.5 (± 3.4)	13.9 (± 0.4)	461 (± 10)	18.3 (± 0.2)	0.15 (± 0.16)
R. Maalahdenjoki	1	30526 (± 772)	5.3 (± 0.1)	0.48 (± 0.03)	15.4 (± 0.4)	39.1 (± 0.7)	37.1 (± 0.4)	28015 (± 185)	302 (± 8)	38.4 (± 1.0)	10.2 (± 0.1)	123 (± 5)	18.2 (± 0.6)	2.36 (± 2.60)
R. Maalahdenjoki	2	18284 (± 389)	3.8 (± 0.3)	0.36 (± 0.03)	11.0 (± 0.6)	26.6 (± 0.8)	22.3 (± 1.1)	17855 (± 966)	472 (± 83)	23.5 (± 0.9)	6.8 (± 0.3)	78 (± 3)	10.7 (± 2.0)	5.84 (± 1.74)
R. Maalahdenjoki	3	40189 (± 1973)	14.1 (± 0.4)	1.67 (± 0.14)	121.9 (± 6.0)	44.5 (± 0.8)	44.2 (± 1.2)	38360 (± 409)	4008 (± 409)	125.6 (± 5.2)	16.9 (± 0.4)	500 (± 17)	19.6 (±0.1)	2.26 (± 1.84)
R. Närpiönjoki		46827 (± 953)	10.1 (± 0.7)	0.94 (± 0.02)	33.7 (± 0.1)	62.4 (± 1.7)	59.2 (± 1.6)	38619 (± 1473)	546 (± 14)	81.0 (± 0.8)	15.7 (± 0.2)	248 (± 6)	24.0 (± 0.4)	0.29 (± 0.26)
R. Lapväärtinjoki		30480 (± 333)	7.1 (± 0.2)	0.96 (± 0.06)	33.7 (± 0.9)	52.3 (± 0.5)	27.4 (± 0.4)	35934 (± 911)	533 (± 47)	51.2 (± 1.4)	14.8 (± 0.4)	235 (± 4)	15.5 (± 0.6)	0.71 (± 0.52)

sediments; *i.e.* 70% emergence between 12–23 days. On valid time interval the mean emergence was 62% (range 40–80%) in the first experimental set up and 60% (range 50–80%) in the second experimental set up of the test. The Kaplan–Meier survival analysis indicated that median emergence times were consistent between the exposures. Significant differences were not observed between sex ratios of emerged adults on either experimental set up of the *C. riparius* toxicity test (Kruskal–Wallis test, experimental set up 1: $p = 0.600$; experimental set up 2: $p = 0.092$). Mechanical damage was observed in several menta of IV instar *C. riparius* but deformity indices were low (Table 3). Broken teeth were the typical mechanical damages. Observed deformity types were splitted mid tooth or additional lateral teeth. Mentum deformity, mechanical damage and both indices were uniform throughout both experimental set ups of the *C. riparius* toxicity test (experimental set up 1: DI: Kruskal–Wallis test, $p = 0.57$; MI: ANOVA, $p = 0.678$; MI + DI: ANOVA, $p = 0.468$ and experimental set up 2: DI: Kruskal–Wallis test, $p = 0.626$; MI: Kruskal–Wallis test, $p = 0.813$; MI + DI: Kruskal–Wallis test, $p = 0.555$).

3.3. Risk characterization

Acute hazard quotients revealed that Al concentrations were high and readily in a toxic level in many river estuaries (Table 4). Also the maximum concentrations of Zn were on an effective level and the HQ values exceeded one in the estuaries of R. Vöyrinjoki, R. Kyrönjoki 1 and 3 and R. Laihianjoki.

Benthic invertebrate quality index indicated deterioration of ecological status in most estuaries. The BBI was determined in the estuaries of R. Lapuanjoki, R. Kyrönjoki, R. Laihianjoki, R. Maalahdenjoki, R. Närpiönjoki and R. Lapväärtinjoki. Quality index determined for the lowest part of subject river was used for the rest of the studied estuaries. Benthic invertebrate quality was high at the estuary of R. Lestijoki and R. Närpiönjoki, good at R. Perhonjoki, R. Ähtävänjoki, R. Maalahdenjoki and R. Lapväärtinjoki, moderate at R. Lapuanjoki and R. Laihianjoki and poor at R. Vöyrinjoki and R. Kyrönjoki.

The WoE risk assessment indicated that R. Vöyrinjoki, R. Kyrönjoki 1, 2 and 3 and R. Laihianjoki were the ones most likely suffering from ASS related metal leaching and acid run-off in case no remediation activities will be implemented. The final risk class was high in these estuaries (Table 5), thus probability of considerable deviation from the environmental objectives (good ecological and chemical status) is high. Moderate risk class was assigned to the estuaries of R. Lapuanjoki, R. Maalahdenjoki 1 and R. Närpiönjoki. Risk class was assigned as low in the estuaries of R. Perhonjoki, R. Ähtävänjoki, R. Maalahdenjoki 1 and 2 and R. Lapväärtinjoki. Estuary of R. Lestijoki was the only subject area where ASS related effects were estimated to be negligible.

4. Discussion

4.1. Water phase

Estuary water resembles river water at high discharge. There is no national guidance on ecological classification of coastal waters in relation to pH and acidity but in river water the national threshold value between good and satisfactory water quality is set to 5.5 (Vuori et al., 2009). Water pH was lower than 5.5 in the estuaries of R. Perhonjoki, R. Lapuanjoki, R. Vöyrinjoki, R. Kyrönjoki 1 and 2, R. Laihianjoki, R. Maalahdenjoki 1, R. Närpiönjoki and R. Lapväärtinjoki which indicated that acidic river water pulses were observed in the estuaries and water quality was deteriorated. Critically low pH (≤4.5) was observed in the estuaries of R. Vöyrinjoki and R. Laihianjoki. Dilution of river water to the brackish water of the Baltic Sea was observed in the estuaries of R. Kyrönjoki and R. Maalahdenjoki. In the estuary of R. Kyrönjoki acidic river water was neutralized approximately 12 km from the mouth of the river (Kyrönjoki 3, pH min. 6.4) and in the estuary of R. Maalahdenjoki approximately 0.5 km from the mouth of the river

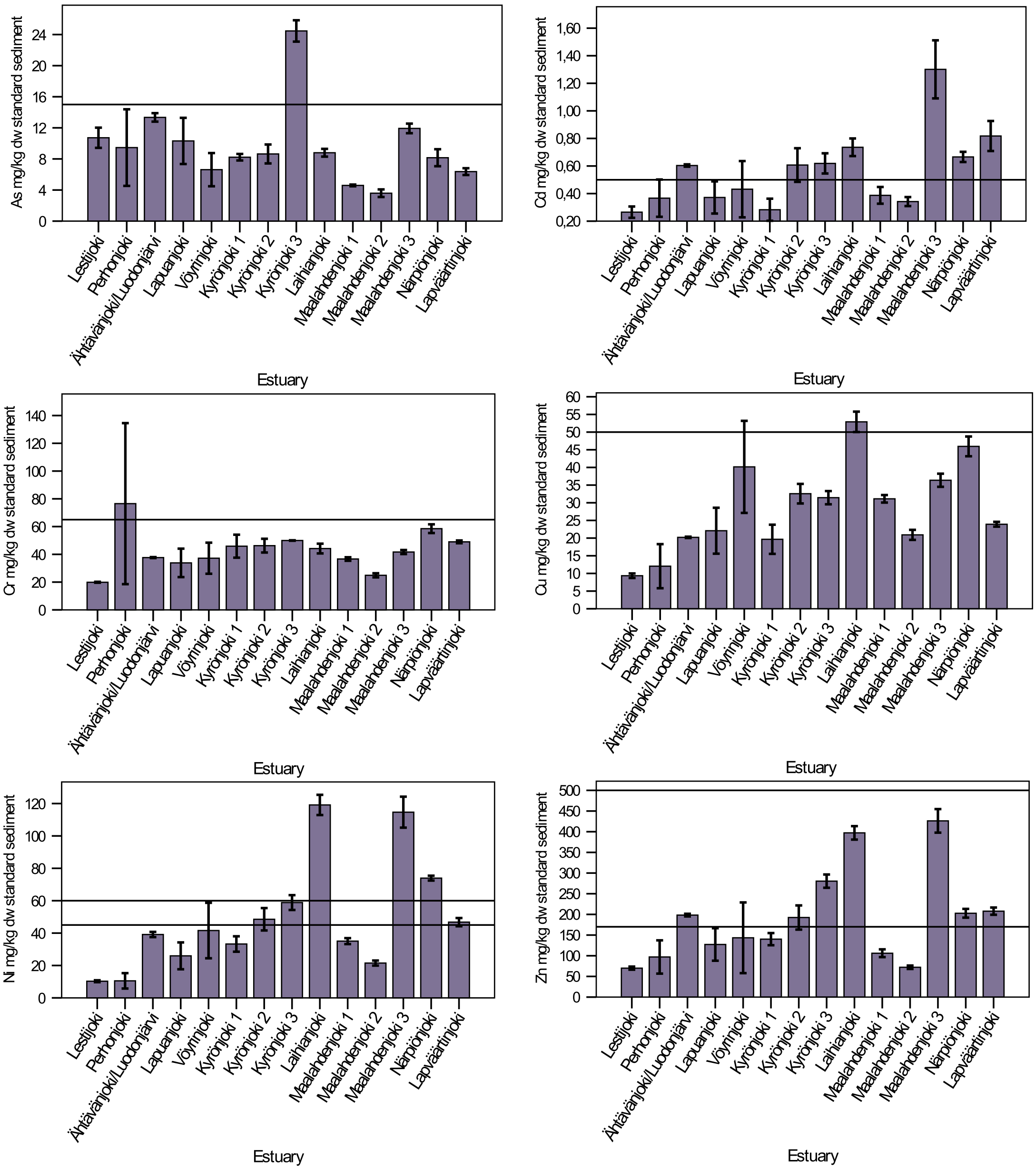

Fig. 3. National thresholds of normalized (10% LOI, 25% clay) dredged sediments (Ministry of the Environment, 2004) and the sediment metal concentrations. Sediments with concentrations above first threshold and second threshold are considered potentially contaminated and contaminated, respectively.

(Maalahdenjoki 2, pH min. 5.8). The estuary of R. Maalahdenjoki is more open than the estuary of R. Kyrönjoki, and thus acidity declines earlier in the estuary.

The greatest metal concentrations in the water column were observed in metals that generally associate to ASS runoff (Al, Cd, Co, Cu, Mn, Ni and Zn). Mean Cd concentration exceeded the annual average EQS (Government Decree 868/2010) on the observation interval (3/2009–9/2010) in the estuaries of R. Kyrönjoki 1 and 2, R. Maalahdenjoki 1 and 2 and R. Närpiönjoki (Table 1). Chronic EQS threshold is founded on protecting 95% of species, and thus Cd concentrations exceeding the EQS can have toxic effects in these estuaries. Although Ni concentrations were below the annual average EQS, but several folds greater than the background Ni concentrations, they indicated that Ni was mobilized from the ASSs. Also, sampling can affect the results since traditional sampling with low frequency (e.g. once per month) may not always represent the actual mean concentration nor the concentration variation in nature. Concentration peaks during spring and autumn may become overlooked if water samples are taken infrequently. Water HQs indicated that Al had the highest hazard in all of the studied estuaries (HQs > 1) and Al concentration peaks have probable effects on the estuary ecosystems during flood period on the study sites (Table 4). According to HQs there was a risk for toxic effects of Zn in the estuaries of R. Vöyrinjoki, R. Kyrönjoki 1 and 3 and R. Laihianjoki. HQs for Cd were smaller than one in all of the studied estuaries but the safety margin was low in the estuary of R. Vöyrinjoki (HQ = 0.6). Thus observed maximum Cd concentrations alone were not likely to induce adverse effects in the studied estuaries. Although single maximum Cd concentrations did not pose an elevated risk in the estuaries during overflow period, continuous Cd concentrations that deviate from background concentration and exceed AA-EQS may have harmful effect in the long run. For example, Brix

Table 3
Mean and standard deviation (±S.D.) of adult emergence (%) in 28th day of the test, median emergence time and 95% confidence limits (± CL), sex ratios (f/m) of emerged adults and mentum deformity indices (MI, DI and MI + DI) of IV instar larvae head capsules of chronic *C. riparius* toxicity test.

Estuary	Emergence, %	Emergence time, d	Sex ratio, f/m	MI	DI	MI + DI
Control 1	90.0 (±12.6)	21.5 (±1.2)	2.2 (±2.3)	0.32 (±0.12)	0.11 (±0.14)	0.35 (±0.10)
R. Kyrönjoki 1	93.3 (±11.5)	19.5 (±0.7)	2.6 (±1.7)	0.44 (±0.06)	0.05 (±0.08)	0.48 (±0.10)
R. Kyrönjoki 2	100.0 (±0.0)	19.5 (±1.8)	1.0 (±0.0)	0.34 (±0.32)	0.00 (±0.00)	0.34 (±0.32)
R. Kyrönjoki 3	100.0 (±0.0)	18.5 (±2.1)	0.7 (±0.3)	0.18 (±0.22)	0.00 (±0.00)	0.18 (±0.22)
R. Maalahdenjoki 1	90.0 (±8.2)	19.5 (±0.6)	1.4 (±0.7)	0.36 (±0.25)	0.03 (±0.06)	0.39 (±0.25)
R. Maalahdenjoki 2	84.0 (±11.4)	20.5 (±1.2)	1.4 (±1.0)	0.25 (±0.11)	0.02 (±0.05)	0.27 (±0.11)
R. Maalahdenjoki 3	96.7 (±5.8)	19.5 (±0.9)	1.3 (±0.6)	0.39 (±0.21)	0.03 (±0.05)	0.42 (±0.17)
R. Lapväärtinjoki	100.0 (±0.0)	19.5 (±2.0)	0.7 (±0.3)	0.24 (±0.13)	0.00 (±0.00)	0.24 (±0.13)
Control 2	61.7 (±11.7)	18.5 (±1.3)	1.4 (±1.3)	0.28 (±0.15)	0.03 (±0.07)	0.31 (±0.14)
R. Lestijoki	80.0 (±14.1)	19.5 (±0.5)	0.4 (±0.4)	0.25 (±0.04)	0.00 (±0.00)	0.25 (±0.04)
R. Perhonjoki	60.0 (±0.0)	18.5 (±0.8)	3.5 (±2.1)	0.35 (±0.21)	0.00 (±0.00)	0.35 (±0.21)
L. Luodonjärvi/R. Ähtävänjoki	70.0 (±28.3)	18.5 (±0.9)	1.6 (±0.5)	0.17 (±0.24)	0.00 (±0.00)	0.17 (±0.24)
R. Lapuanjoki	95.0 (±7.1)	20.5 (±1.1)	0.5 (±0.1)	0.44 (±0.31)	0.11 (±0.16)	0.56 (±0.16)
R. Vöyrinjoki	65.0 (±21.2)	20.5 (±2.4)	0.6 (±0.0)	0.22 (±0.31)	0.00 (±0.00)	0.22 (±0.31)
R. Laihianjoki	50.0 (±14.1)	18.5 (±2.1)	1.0 (±0.0)	0.17 (±0.04)	0.00 (±0.00)	0.17 (±0.04)
R. Närpiönjoki	50.0 (±14.1)	19.5 (±0.9)	0.3 (±0.1)	0.33 (±0.24)	0.00 (±0.00)	0.33 (±0.24)

f/m = females/males.
MI = mechanical damage index.
DI = deformity index.
MI + DI = mechanical damage and deformity index.

et al. (2011) suggested that some benthic invertebrates generally insensitive to acute metal exposure may be affected by chronic exposure.

4.2. Sediment phase

As in the water phase, the greatest concentrations in the sediment samples were observed in metals that generally associate to ASS runoff. Concentrations of Al, Cd, Co, Cu, Mn, Ni and Zn were high in the majority of studied estuaries when compared to Swedish SQCs (Naturvårdsverket, 1999), national dredging guidelines (Ministry of the Environment, 2004) and background concentration (Åström and Björklund, 1997; Nordmyr et al., 2008a; Tenhola and Tarvainen, 2008) (Fig. 3, Tables 2, 3). In the estuaries of R. Lapuanjoki, R. Vöyrinjoki, R. Kyrönjoki 1 and R. Maalahdenjoki 1 mean pH were under 6.0 and minimum pH under 5.5, which may indicate that metals were in dissolved form in the mouth of the river and were deposited further down in the estuary where the influence of brackish water increased (Nordmyr et al., 2008b). In total, sediment Al, Cd, Co, Cu, Mn, Ni and Zn concentrations were 0.3 to 16-fold higher when compared to the ASS parent material metal concentrations (Åström and Björklund, 1997). Metal concentrations above that of ASS parent material concentrations indicated that these metals were enriched in estuary sediments. Total metal concentrations of the sediment samples were similar to previous study of Nordmyr et al. (2008a) showing that massive amounts of Al, Cd, Co, Cu, Mn, Ni and Zn are leached from ASSs and transported to the estuaries. These metals are co-precipitated in the estuaries with organic fraction or Al-, Fe- and Mn-oxyhydroxides when pH and salinity increases, and they end up in the sediment (Åström and Corin, 2000; Åström et al., 2012). Sedimentation is presumably the main process influencing the fate and toxicity of metals in estuaries (Kuivikko et al., 2010). Precipitated metals in sediments may be bioavailable to benthic invertebrates *via* gastrointestinal tract and skin. Metals may also be redissolved from sediments to the aqueous phase if physical-chemical characteristics are altered or sediment is bioturbated.

Table 4
Hazard quotients of selected metals.

Estuary	Al	Cd	Zn
R. Lestijoki	14.1	0.1	0.1
R. Perhonjoki	4.9	0.1	0.1
R. Ähtävänjoki/L. Luodonjärvi	10.4	0.0	0.0
R. Lapuanjoki	13.2	0.3	0.6
R. Vöyrinjoki	42.1	0.6	1.4
R. Kyrönjoki 1	12.1	0.3	2.3
R. Kyrönjoki 2	11.5	0.4	0.6
R. Kyrönjoki 3	7.5	0.2	2.3
R. Laihianjoki	19.6	0.1	1.2
R. Maalahdenjoki 1	19.6	0.4	0.6
R. Maalahdenjoki 2	17.3	0.2	0.5
R. Maalahdenjoki 3	9.8	0.3	0.3
R. Närpiönjoki	13.2	0.2	0.4
R. Lapväärtinjoki	8.6	0.1	0.5

Although all the sediment samples indicated toxicity to *V. fischeri*, the cause of toxicity remains obscure. The EC_{50}-values showed no strong correlation with the sediment metal concentrations thus there has been something else besides metals that have affected the toxic outcome. Results indicated that the acute *V. fischeri* test is suitable tool as a part of screening level risk assessment of boreal ASS impacted estuaries and it should be used together with other risk assessment tools. In contrary to the acute *V. fischeri* test, the sediments were not toxic to the chironomid midge *C. riparius*. Chironomids are able to survive in challenging conditions and *C. riparius* is considered as an opportunistic species, since its response to various toxicants can be dependent on the quantity and quality of food available (de Haas et al., 2002). It is also recognized that species sensitivity to toxicants varies and toxicity tests should be conducted with multiple species in risk assessment (Tuikka et al., 2011). Larger number of replicate sediment samples applied after shorter storage time might have given us more reliable view on the toxicity of the sediments. The stabilization period in *C. riparius* test has probably also altered for example partitioning of metals between sediment and water phase. Fresh sediments should be used in *C. riparius* test in order to avoid sediment alteration during storage (Sae-Ma et al., 1998).

4.3. WoE approach

This WoE approach indicated that metals and acidity can have long-term effects on the ASS affected estuaries. Several LoE suggest that good ecological and chemical status is not attained in most of the studied estuaries. Strongest evidence came from exposure profiles while results of acute and chronic toxicity tests provided somewhat inconsistent evidence. Uncertainties in the used WoE approach arose from limited data available for site specific risk assessment. The risk assessment was partly time-conditional, since the sediment samples were collected only once during summer 2010. The observation interval for water

Table 5
Summary table of risk assessment endpoints and final risk classification. Risk class was assigned as negligible (+/−), low (+), moderate (++) or high (+++).

	Endpoint								Summary
Estuary	Swedish SQCs	Finnish dredging guideline	Water EQSs	Water HQs	pH minimum	*V. fischeri*	*C. riparius*	Benthic invertebrate quality index	
R. Lestijoki	+/−	+/−	+/−	+	+/−	+	+/−	+/−	+/−
R. Perhonjoki	+/−	+	+/−	+	+	+	+/−	+/−	+
L. Luodonjärvi/R. Ähtävänjoki	+	+	+/−	+	+/−	+	+/−	+/−	+
R. Lapuanjoki	+	+/−	+/−	+	++	+	+/−	+	++
R. Vöyrinjoki	+	+/−	+/−	++	+++	+	+/−	++	+++
R. Kyrönjoki 1	+	+/−	+	++	++	+	+/−	++	+++
R. Kyrönjoki 2	+	+	+	+	++	+	+/−	++	+++
R. Kyrönjoki 3	++	+	+/−	++	+/−	+	+/−	++	+++
R. Laihianjoki	++	+	+/−	++	+++	+	+/−	+	+++
R. Maalahdenjoki 1	+	+/−	+	+	++	+	+/−	+/−	++
R. Maalahdenjoki 2	+/−	+/−	+	+	+/−	+	+/−	+/−	+
R. Maalahdenjoki 3	++	+	+/−	+	+/−	+	+/−	+/−	+
R. Närpiönjoki	+	+	+	+	+	+	+/−	+/−	++
R. Lapväärtinjoki	+	+	+/−	+	+	+	+/−	+/−	+

quality was 19 months (3/2009–9/2010), but the BBI expressed the quality of benthic invertebrate community over several years and can therefore be taken as the most distinct evidence on the long-term negative effects of ASSs in the estuaries.

There were some variations in the final risk classes between the study sites. The risk class was the highest in the estuaries of R. Vöyrinjoki, R. Kyrönjoki and R. Laihianjoki which catchment areas are situated in the ASS hotspot area (Nordmyr et al., 2008a; Saarinen et al., 2010). There are ASSs to a lesser extent in the catchment area of R. Lapväärtinjoki (Nordmyr et al., 2008a) and the risk was assigned as low in the estuary of R. Lapväärtinjoki. On the contrary, the risk was negligible in the estuary of R. Lestijoki although ASS related effects have been observed in the upstream river occasionally (Edén et al., 1999). Effects of ASSs vary on a spatial and temporal scale (Nyberg et al., 2012) and there are both hydrological and geochemical processes that have an impact on the observed effects (Palko, 1994; Österholm and Åström, 2008; Nyberg et al., 2012). No extremely long and dry periods were observed during summers 2009 or 2010 in the Ostrobothnia of the Baltic Sea. Thus the deepest layers of ASSs were not oxidized. This may account for relatively good water and sediment quality for example in the estuary of R. Lestijoki. It is expected that in the future acidity peaks occur more frequently during winter periods if rainfall and mean temperatures increase due to projections of climate change (Saarinen et al., 2010).

This assessment revealed some shortcomings in data and complementing monitoring needs in the estuaries of the Baltic Sea. For example, besides determining the total metal concentrations from water and sediment, it would be advantageous to determine metal speciation since speciation affects metal toxicity (Gerhardt, 1993; Nystrand et al., 2012; Riba et al., 2003). It should also be noted that the exposure exists as multi-metal exposure in the studied estuary sites and separating individual metals for risk assessment is almost unfeasible since the exposure consists of multiple variables in nature and assessment of combined effects is commonly challenging. In overall, metals and low pH together have a negative impact on the benthic invertebrate community (Gerhardt, 1993). In our study the quality of benthic invertebrate community was generally deteriorated in the estuaries where the overall sediment and water quality exceeded guideline values.

5. Conclusions

Monitoring data showed that concentrations of metals abundantly leached from ASSs (Al, Cd, Co, Cu, Mn, Ni and Zn) were mostly elevated in the water column. The concentrations of Al, Cd and Zn were at levels likely to have adverse effects on the estuary ecosystems in the ASS hotspot areas. Low pH values (<5.5) were also observed in the studied estuaries. Acidic and metal rich river waters clearly impaired water quality during flood period.

Metals that were elevated in the water column were also enriched in the surface sediments of the studied estuaries. Concentrations of Al, Cd, Co, Cu, Mn, Ni and Zn were several folds greater in the sediment samples than the background concentrations indicating that several Baltic Sea estuary ecosystems are under stress due to ASS effects. This was supported by the observed deteriorated benthic invertebrate community in the estuaries. Instead metals that are not generally abundantly leached from ASSs (As, Cr, Fe and Pb) were not typically enriched in the surface sediments.

Although this risk assessment included some uncertainties the WoE approach showed that the Baltic Sea estuaries under the influence of ASS catchments were likely to experience deterioration of ecosystems if no remedial actions are considered. This is especially important due to changing climate and forecasted increase in the precipitation in the Northern Europe. More information is needed especially concerning low salinity (1–2‰ S) estuaries where water temperature and pH are also low.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.11.073.

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II

ECOTOXICITY ASSESSMENT OF MINING-AFFECTED LAKE SEDIMENTS BY *LUMBRICULUS VARIEGATUS* BIOTEST

by

Jaana Wallin, Juha S. Karjalainen, Ari Väisänen, Jussi V.K. Kukkonen & Anna K. Karjalainen 2018

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III

LUMBRICULUS VARIEGATUS (ANNELIDA) BIOLOGICAL RESPONSES AND SEDIMENT SEQUENTIAL EXTRACTIONS INDICATE ECOTOXICITY OF LAKE SEDIMENTS CONTAMINATED BY BIOMINING

by

Jaana Wallin, Kari-Matti Vuori, Ari Väisänen, Johanna Salmelin & Anna K. Karjalainen 2018

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Lumbriculus variegatus (Annelida) biological responses and sediment sequential extractions indicate ecotoxicity of lake sediments contaminated by biomining

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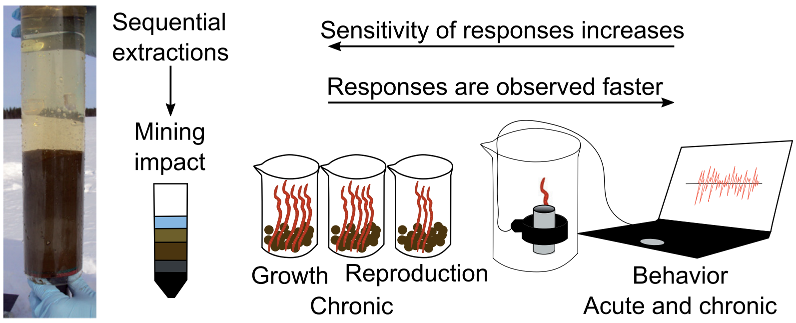
HIGHLIGHTS

- Effects of biomining on lake sediments were studied with *Lumbriculus variegatus* biotests.
- Sediment sequential extractions supplemented characterization of the mining effect.
- Growth and reproduction of *L. variegatus* were more sensitive bioindicators than behavior.
- Behavioral responses outdid traditional endpoints for fast screening purposes.

GRAPHICAL ABSTRACT



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ABSTRACT

We assessed potential ecotoxicity of lake sediments affected by biomining effluents in northeastern Finland. Growth, reproduction and behavior of the sediment-dwelling oligochaete *Lumbriculus variegatus* (Müller 1774) were used as ecotoxicity endpoints. Standardized chronic bioassays were used for growth and reproduction, and acute and chronic tests with Multispecies Freshwater Biomonitor (MFB) for behavior assessments. Sequential extractions were used to characterize metal bioavailability and exposure conditions in the sediments, which indicated mining-induced contamination gradients of S, Cu, Ni and U and also bioavailability gradients of S and Ni. Among the ecotoxicity endpoints, growth and reproduction responses of the standard bioassays appeared more sensitive than the behavioral responses at 21 d. In the two most mining-affected test sediments, mean number of worms and dry biomass decreased 35–42% and 46–51% in comparison to the reference sediment, respectively. The behavioral changes of worms, i.e. peristaltic and overall locomotory activity, decreased on average 20–70% and 2–61% at 21 d in the same sediments. However, these behavioral changes were observed at the onset of exposure indicating MFB technique is a suitable and rapid screening level ecotoxicity assessment tool.

1. Introduction

The global mining industry and its emissions are expected to grow substantially in the coming decades despite the introduction of more resource-efficient metal industrial technologies (UNEP, 2013). Recently,

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alternative "green mining" techniques, such as bioheapleaching (Riekkola-Vanhanen, 2013; Saari and Riekkola-Vanhanen, 2012), have been developed to increase the energy efficiency of mines and minimize their emissions. The Terrafame (Talvivaara) mine in north-eastern Finland is one of the first large-scale commercial mines in Europe exploiting bioheapleaching technology. A special feature of the mining area is sulfide-rich black shale with a high potential for weathering and acidification (Gustavsson et al., 2012; Loukola-Ruskeeniemi et al., 1998).

Accidental spills from the Terrafame mine have caused adverse ecological impacts on the recipient water bodies. One of the major impacts has been the formation of a metal contamination gradient in the lake sediments, as well as fundamental changes in the overall water quality, including extreme salinity and acidity (Kauppi et al., 2013; Leppänen et al., 2017; Väänänen et al., 2016). Biomining impacts of the area resemble typical acid mine drainage (AMD) effects: runoff waters are acidic, with highly elevated concentrations of sulfur, iron, nickel and other metals (Johnson and Hallberg, 2005; Salmelin et al., 2017). While ecotoxicity of AMD is well documented in several stream water studies (e.g. DeNicola and Stapleton, 2002; Gerhardt et al., 2004; Lin et al., 2007; Salmelin et al., 2017), knowledge of AMD lake sediment ecotoxicity is more limited (Väänänen, 2017).

The metal enriched mining effluents are diluted in the recipient water bodies and metals are commonly precipitated to sediments. Sediments as sinks of contaminants are highly complex and dynamic systems and their quality is influenced by multiple anthropogenic activities such as metal mining (Burton, 2013; P.M. Chapman et al., 1999; Luoma, 1989; Väänänen et al., 2018). Due to several interacting biotic and abiotic factors affecting metal bioavailability and final uptake by aquatic organisms (Burton, 2013; Chapman et al., 1998; De Jonge et al., 2013; Luoma, 1989), ecotoxicity assessment of sediments is not straightforward.

Standardized toxicity tests using the growth and reproduction of oligochaetes (OECD, 2007) are routinely used in sediment ecotoxicity studies. However, they have been utilized in only a few AMD lake sediment studies. In addition to the chronic oligochaete toxicity tests, a number of studies have used the Multispecies Freshwater Biomonitor (MFB) impedance conversion technique (Gerhardt, 2007; Gerhardt et al., 1994) as a tool for quantitative recording of behavioral responses of oligochaetes in polluted sediments (Gerhardt, 2007; Sardo and Soares, 2011, 2010). Both inorganic and organic pollutants may alter behavior of aquatic organisms in various ways (Melvin and Wilson, 2013; Weiss et al., 2001). For example, reduced foraging behavior or ability to escape from the predators can have effects on the population level (Gerhardt et al., 1994; Weiss et al., 2001). Behavior is thus considered as an early-warning indication of more severe ecotoxic effects. However, there are no comparative studies on the standardized chronic bioassay endpoints versus acute behavioral responses in sediments influenced by AMD.

Benthic macroinvertebrates are an essential part of aquatic foodwebs, and they may act as a route of many contaminants to the higher trophic levels (Camusso et al., 2012; Rainbow, 2002). Some species spend their entire life cycle within the sediment, whereas others have variable proportions of sediment-dwelling phases. Benthic oligochaetes, like *Lumbriculus variegatus*, are predominantly sediment-dwellers, serve as prey for fish and are a crucial part of sediment decomposers (Luoma, 1989). In the sediments, oligochaetes are exposed to metals via multiple routes, including overlying water, pore water and ingested sediment (Burton, 2010; Camusso et al., 2012; Rainbow, 2002).

Our aim was to investigate (a) the overall applicability of the standard *Lumbriculus variegatus* toxicity test and (b) the behavioral responses of the worms using the MFB technique in the AMD sediment ecotoxicity assessment. In addition, our purpose was to evaluate usability of the traditional sequential extraction techniques (SEs, Tessier et al., 1984; Väisänen and Kiljunen, 2005) in the characterization of the bioavailability of metals along the contamination gradient in the Terrafame mine region. Furthermore, our objective was to compare, for the first time, evidence from the sediment bioassays and SEs and their integrated use in AMD sediment assessments.

2. Materials and methods

2.1. Study sites

The study sites were located in northeastern Finland (Fig. 1). Four lakes (Salminen, Kalliojärvi, Kolmisoppi and Jormasjärvi) downstream (northern waterway) from the mine effluent discharge and one reference lake were sampled on March 2016. The Terrafame mine (formerly Talvivaara) started operation in 2008 and its main products are nickel (Ni), zinc (Zn), copper (Cu) and cobalt (Co). The Terrafame mine uses bacterial bioheapleaching method to recover metals form low-grade ore (Riekkola-Vanhanen, 2013).

2.2. Water quality and sediment sampling

Water depth, temperature, oxygen concentration and saturation, specific conductivity and pH were measured from epilimnion (1 m below the water surface) and hypolimnion (1 m above the lake bottom) on site with a multiparameter water quality sonde (YSI V2 6600).

Eight to nine parallel surface sediment (0–5 cm) samples were taken with Sandman gravity corer from the accumulation basins. Samples were combined immediately after sampling to one bulk sediment. Two subsamples were separated from the bulk sediment for metal analysis and pH measurement and subsample for metal analysis was frozen (−21 °C). Sediment pH was measured with a portable pH meter after sediment sampling (VWR pH 100). Sediment dry weight (dw) and loss on ignition (LOI) were determined prior to the ecotoxicity test from the bulk sample according to SFS-standard 3008 (2000). Bulk sediments were stored in plastic polyethylene containers in the dark at +4 °C prior to experiments and frozen samples were vacuum freeze-dried (Christ Alpha).

2.3. Test organisms and ecotoxicity test design

Test organisms were laboratory reared *Lumbriculus variegatus* (Müller 1774) from the University of Jyväskylä (Department of Biological and Environmental Science). The Oligochaeta worms were cultured in aerated 20 l glass aquaria containing artificial freshwater (AFW, Ca + Mg hardness 1.0 mM, pH 8.7) (ISO, 2012) at 20 °C (SD = 1) in a 16:8 h light:dark cycle (illuminance <500 lx at water surface). Shredded soft-paper towels were used as a substrate in the aquaria and the worms were fed three times a week with Tetramin® (TetraWerke, Melle, Germany) fish food ad libidum. A 24-h gut purging time in AFW was used prior to the test. Only active and medium-sized worms with no signs of recent fragmentation were chosen for the test.

The toxicity test was conducted following the standard methods (OECD, 2007) at 19.7 (SE ± 0.1) °C with a test duration of 21 days. Twenty *L. variegatus* individuals were used per replicate and each lake sediment was tested as six replicates. Sediments were mixed with a plastic spoon and approximately 45 ml of sediments were placed into the beakers (Table S1). Sediment pH was measured, and three replicate samples of approximately 40 ml were taken from the bulk sediment for sequential extractions. Water samples (15 ml) for metal and nutrient analyses were taken at 0 d (AFW) and at 21 d (overlying water) from 3 replicate beakers. Samples were filtered (0.45 μm Whatman® GD/XP) and acidified with suprapur 65% nitric acid (HNO_3; Sigma-Aldrich®). The overlying water was aerated during the test except for one day prior to introducing the *L. variegatus* to the beakers and oxygen saturation ranged from 96.0% to 100.8% during the toxicity test. The ratio between sediment and overlying water was 1:4 (v:v) and the sediments were allowed to settle for two days. At the beginning of the test, one of the 20 *L. variegatus* was separated for behavioral measurements and the rest 19 worms were introduced to each replicate beaker (250 ml; height 120 mm; diameter 60 mm). Artificial sediment (AS) was a control for growth and reproduction of the laboratory culture of *L. variegatus* for validity of the test as required in OECD 225 standard (2007). AFW was used above sediments in all exposures. Detailed

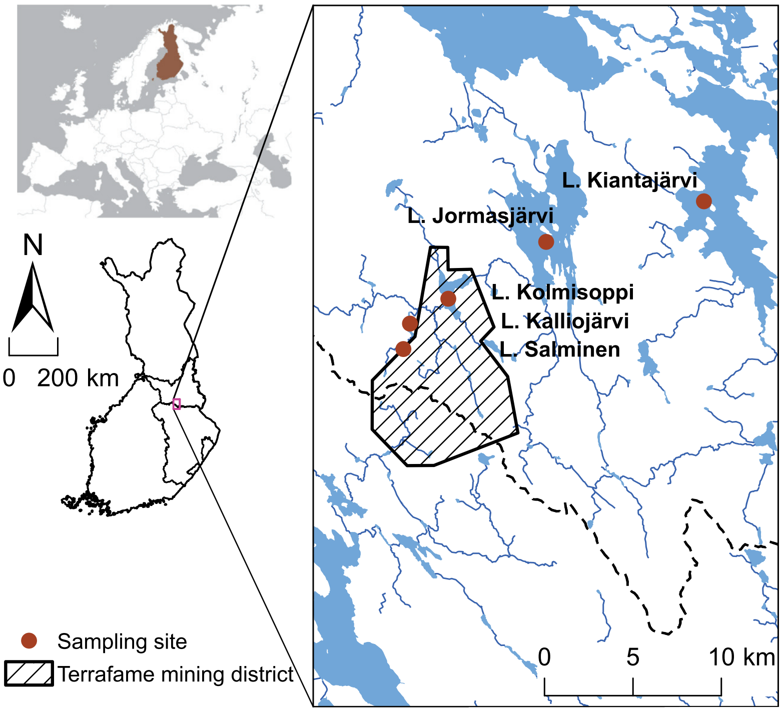

Fig. 1. Map of the study sites downstream from the mine in Finland in Europe. Lake Kiantajärvi is the local reference lake. Map data: General map and topographic map of Finland ©National Land Survey of Finland 6/2017; Catchment areas and Terrafame mining district, National Database of Regional Land Use Plans ©Finnish Environment Institute (SYKE), 6/2017; Maps were constructed with ArcGIS® v. 10.3.1 (ESRI Inc., Redlands, CA).

composition and preparation of AS and AFW is described in the supplementary information (Table S1) and standards (ISO, 2012; OECD, 2007). The initial biomass of laboratory culture was determined by weighing (Mettler Toledo XP56) 9 batches of 20 *L. variegatus* individuals at 0 d. Temperature and oxygen saturation (YSI proODO) were monitored from a reference beaker and pH (VWR pH 100) and total ammonia (ThermoFischer Scientific Orion™ HP Ammonia Electrode) from overlying water from exposures during the tests. Evaporated water was compensated with ultrapure water during the toxicity test.

After 21 days the worms were sieved from the sediment through a 250 μm sieve. One medium-sized *L. variegatus* was separated for MFB measurements and the rest were rinsed twice in AFW, blotted dry and weighed. Dry weight was determined from vacuum freeze-dried *L. variegatus*.

2.4. Behavioral measurements of Lumbriculus variegatus

Exact description of MFB operation is described by, among others, Gerhardt et al. (1994), Macedo-Sousa et al. (2008) and Sardo et al. (2007). The function of the MFB chambers and a suitable number of *L. variegatus* per chamber were verified in a preliminary test. Behavioral responses were recorded at the beginning (0 d) and end (21 d) of the test. The measurement chamber was placed vertically into a wide 1000 ml beaker filled with AFW. The top part was covered with mesh and a cap and the bottom of the chamber was blocked with parafilm and the mesh under the cap to prevent the worms from escaping from the measurement chamber. Six replicate chambers were used for every lake sediment, but for Lake Kalliojärvi one test chamber failed to sufficiently register a signal during the MFB measurements at 21 d and the data were excluded from further analysis. In addition to test chambers, one chamber with corresponding lake sediment but without a worm and another empty chamber in AFW were used as controls for background noise. At the beginning of the test, one of the 20 *L. variegatus* was randomly selected for MFB measurement. At the end of the toxicity test (21 d), one medium-sized worm was separated from the test sediment on a 250 μm sieve. Worms at 0 d and 21 d were placed with a dental probe into the chambers half filled with test sediment and AFW on top of the sediment. The chambers were allowed to settle for 20 to 30 min before the measurements in order to allow *L. variegatus* to burrow properly into the sediment. Behavior was measured for 3 h in ten minute intervals during which the behavior was recorded for four minutes. Peristaltic movements were recorded from a frequency range of 0.5 to 1.0 Hz and overall locomotory activity at 1.0 to 3.0 Hz according to Sardo and Soares (2010, 2011). Oxygen saturation was measured from one replicate beaker at the beginning and at the end of measurement period and it remained over 95% in all beakers.

2.5. Sequential extractions and chemical analyses

Sediment element fractionation was studied with ultrasound assisted sequential extractions (Tessier et al., 1979; Väisänen and Kiljunen, 2005). Fractions included those that were exchangeable (F1), carbonate-bound (F2), Fe and Mn oxides bound (F3), organic matter and sulfide bound (F4) and residual (F5), as described in Tessier et al. (1979). The extraction procedure is described in Väisänen and Kiljunen (2005), except that 0.5 M NH_4Cl instead of $MgCl_2$ was used in the F1 extraction. Briefly, 500 mg of dry sediment sample was weighed into a 50 ml test tube and an extracting solution was added. Ultrapure water (conductivity <0.056 μS/cm, Elga) was used while the extracting solutions used were ≥ 65% nitric acid (p.a., Sigma-Aldrich) (0.02 M in F4), 15% solution (v/v) of ≥37% hydrochloric acid (Sigma-Aldrich), 100% acetic acid (VWR chemicals), 3.2 M ≥ 98% CH_3COONH_4 (Sigma-Aldrich), ≥ 99.5% NH_4Cl (Sigma-Aldrich), 0.1 M 99% $NH_2OH \cdot HCl$ (Sigma-Aldrich), 1 M CH_3COONa (p.a., Merck) and 30% H_2O_2 (VWR chemicals). Samples were sonicated for 3 to 18 min (Väisänen and Kiljunen, 2005) with Elmasonic P (37 kHz) and centrifuged (Thermo Scientificin Labofuge 400, 10 min, 300 r/min). The extractant was separated into a 50 ml

Table 1
Mean sediment elemental concentrations (mg/kg dw) in each fraction of the sequential extractions and standard errors (SE, n = 3). See Materials and methods for fraction descriptions. Control represents the artificial sediment, Kiantajärvi the local reference lake, and Jormasjärvi to Salminen the lake network downstream from the mine. Empty cells in the table are non-detect values.

Lake		S	Fe	Cd	Co	Cr	Cu	Ni	Sr	U	Zn	Mn
		mg/kg dw										
Fraction 1												
Control	Mean	93	0.5	0.006	0	0.065	0.21		1.79	0.3		5
	SE	15	0.2	0.003	0.04	0.015	0.05		0.09	0.3		1.4
Kiantajärvi (ref)	Mean	56	21	0.043	0.96	2	0.2	0.8	5.4	1.4		730
	SE	4	5	0.014	0.12	0.12	0.2	0.3	0.4	0.4		0.4
Jormasjärvi	Mean	560	28	0.054	1.6	2.77	0.3	5.4	8.8	0.6	4.1	970
	SE	30	4	0.011	0.06	0.07	0.05	0.3	0.4	0.2	0.5	30
Kolmisoppi	Mean	2580	51	0.026	1.16	0.84	0.2	7.4	9.2	1.7	1.1	210
	SE	80	5	0.01	0.03	0.09	0.11	0.5	0.14	0.4	1.1	5
Kalliojärvi	Mean	28,000	197	0.023	0.72	3.51	0.2	22.4	21.6	4.5		1243
	SE	400	6	0.011	0.04	0.04	0.3	0.6	0.3	0.4		9
Salminen	Mean	54,000	890	0.414	1.64	15	0.72	84	14.5	42.1	18.1	
	SE	2000	20	0.005	0.03	0.4	0.15	2	0.4	8	0.7	
Fraction 2												
Control	Mean	4	0.95	0.048		0.17	0.43	0.14	0.42	4.6	3	1.02
	SE	4	0.12	0.003		0.03	0.07	0.07	0.05	0.4	4	0.16
Kiantajärvi (ref)	Mean	24.8	25	0.114	0.64	0.95	0.73	0.57	2	4.6	4.5	247
	SE	1.2	3	0.013	0.06	0.19	0.05	0.13	0.3	0.5	0.4	50
Jormasjärvi	Mean	130	62	0.11	2.46	2.3	0.58	11.5	3.91	6.5	32	590
	SE	7	5	0.02	0.02	0.4	0.07	0.13	0.07	0.4	2	8
Kolmisoppi	Mean	360	86	0.15	2.027	0.595	0.38	13.1	4.2	6.7	48.9	118
	SE	14	3	0.05	0.009	0.015	0.09	0.3	0.1	0.8	1.4	3
Kalliojärvi	Mean	3613	724	0.279	1.33	2.56	0.18	65.8	10.06	50.7	49	727
	SE	70	15	0.009	0.01	0.09	0.03	1.4	0.14	1.3	50	7
Salminen	Mean	17,317	142	0.141	0.21	7	2.4	13	1.6	108	24	361
	SE	1200	12	0.004	0.05	0.3	0.8	2	0.4	2	5	110
Fraction 3												
Control	Mean	153	8.2	0.01	0.01	0.14	0.08	0.25	0.38	1.5	0.4	0.4
	SE	3	0.2	0.012	0.03	0.06	0.05	0.13	0.04	0.3	0.4	0.05
Kiantajärvi (ref)	Mean	166	470	0.49	2.9	1.21	0.64	3.5	1.19	22	30	168
	SE	3	40	0.02	0.3	0.12	0.09	0.5	0.09	1.4	3	13
Jormasjärvi	Mean	215	490	1.04	4.5	1.09	0.23	20.3	1.77	22.5	202	295
	SE	4	30	0.06	0.3	0.09	0.03	1.3	0.05	1.3	7	6
Kolmisoppi	Mean	400	1000	1.55	3.6	0.52	0.66	26.3	2.84	32.9	180	66
	SE	200	400	0.08	0.2	0.04	0.14	1.4	0.1	1.3	8	1.3
Kalliojärvi	Mean	520	1050	0.72	1.23	0.87	0.03	51	6.1	51	90	178
	SE	20	50	0.03	0.07	0.03	0.07	3	0.2	3	20	3
Salminen	Mean	1000	570	0.405	0.5	11.2	0.33	18	2.5	48	63	57
	SE	200	40	0.011	0.05	1	0.09	1.1	0.3	7	3	15
Fraction 4												
Control	Mean	7	3.7	0	0.03	0.8	1.1	0.63	0.197	5.1	1.5	0.17
	SE	2	0.4	0.013	0.03	0.1	0.19	0.16	0.014	0.2	0.3	0.03
Kiantajärvi (ref)	Mean	614	373	0.25	4.2	15.5	14.9	13	2.01	21	34	130
	SE	30	60	0.04	0.4	0.9	0.03	1.3	0.13	4	3	9
Jormasjärvi	Mean	3524	847	3.03	13.8	17.5	29.2	67	2.5	44.1	245	215
	SE	120	40	0.13	0.3	0.3	0.9	2	0.13	0.8	11	5
Kolmisoppi	Mean	4573	1379	1.78	12	16.4	43.6	79	2.67	63	145	51
	SE	140	90	0.13	0.6	0.4	0.5	4	0.11	4	8	3
Kalliojärvi	Mean	5987	2139	1	3.4	10.9	25	89.5	5.87	152	57	36.2
	SE	200	200	0.1	0.2	0.3	3	1.3	0.04	10	0.8	1.3
Salminen	Mean	13,519	5308	2.56	2	94.5	74	61.3	4.47	379	262	80
	SE	300	200	0.15	0.3	1.3	4	0.8	0.13	6	20	15
Fraction 5												
Control	Mean	20	100	0.013	0.05	1.6	0.5	0.7	0.09	0.1	1.1	2
	SE	20	80	0.01	0.05	1.3	0.4	0.7	0.08	1.1	0.8	0.2
Kiantajärvi (ref)	Mean	40	7000	0.2	3	10	5	10	0.6	25	18	70
	SE	30	5000	0.2	3	7	2	7	0.4	20	13	60
Jormasjärvi	Mean	510	29,300	1.08	5.4	15.4	7.9	20.5	0.82	115	71	205
	SE	30	400	0.03	0.2	0.3	0.4	0.9	0.02	4	3	7
Kolmisoppi	Mean	220	7100	0.231	2.85	8.9	5.9	9.4	0.86	25.9	19.1	43.6
	SE	30	200	0.004	0.09	0.4	0.4	0.2	0.03	0.7	0.6	1.1
Kalliojärvi	Mean	3100	33,000	1.14	1.04	6.4	3.26	11.7	0.6	124	10.9	17.4
	SE	300	2000	0.07	0.06	0.3	0.13	0.8	0.04	7	0.9	0.9
Salminen	Mean	910	12,000	0.42	0.5	12.3	2.4	8.7	0.39	44	11	9.5
	SE	70	2000	0.06	0.14	0.4	0.3	0.9	0.03	7	3	0.5

volumetric flask and residue was washed once with 5 ml of ultrapure water between each step.

Total concentrations in *L. variegatus* were determined as described in more detail in the supplementary material (Table S2). Freeze-dried worms were digested in *aqua regia* solution by sonicating three times as described above at 50 °C and diluted to 10 ml in metal-free plastic tubes (VWR) with ultrapure water. Metal and nutrient (P, S, Ca, Mg, Al, Fe, K, As, Cd, Co, Cr, Cu, Ni, Sr, U, Zn and Mn) concentrations were analyzed by inductively coupled plasma optical emission spectrometry (ICP–OES; PerkinElmer Optima 8300) (Table S2). Measurement results under the detection limit (Table S2) or relative standard deviations (RSD) over 10% were discarded (nondetect values).

2.6. Statistical tests

Average time in the predefined frequency range was calculated for each worm from the four-minute record periods during the three-hour measuring period at 0 d and 21 d. Shapiro-Wilk test was used to assess the normality of the data and the Levene test to assess the homogeneity of variances. Analysis of variance (ANOVA) was conducted with Tukey HSD post-hoc comparisons to examine the differences in wet weight (ww), dry weight (dw) and number of *L. variegatus* at 21 d between exposures. Behavioral data were normally distributed except for the Lake Kolmisoppi. Equal variances across groups were achieved only in the peristaltic movements at 21 d and ANOVA with Tukey HSD post-hoc comparison was used. Welch test was used to test the differences in the group averages for the rest of the behavioral variables (peristaltic movements at 0 d, overall locomotory activity at 0 d and 21 d) with Games-Howell post-hoc comparisons. Differences in sediment pH at 21 d were examined with nonparametric Kruskal-Wallis test. Correlations between the behavioral, growth and reproduction responses of *L. variegatus* as well as average element concentration in different sediment fractions, sediment LOI and pH were determined using the Pearson correlation coefficient. The nondetect values were replaced with 20% of detection limit (sediments) and elements which had over 30% missing values were excluded (*L. variegatus* and sediment) (Table S2). All the statistical tests were performed with SPSS version 24 (IBM Corp, Armonk, NY).

3. Results

3.1. Element concentrations in water, sediment and L. variegatus

A clear salinization gradient was observed in the epilimnion of the studied lakes during the late winter sediment sampling occasion. Specific conductivity was the highest in the epilimnion of Lake Salminen (11,283 μS/cm) and the lowest in the reference Lake Kiantajärvi (31 μS/cm). Lakes Salminen and Kalliojärvi were also hypoxic with the O_2 concentration in epilimnion at 0.78 and 0.58 mg/L, respectively. Sediment organic carbon content estimated from LOI was the lowest in the AS control (2%) and the reference lake Kiantajärvi (3.6%) and the highest in Lake Salminen (20.7%) (Table S4).

Sequential extractions revealed that total nutrient and trace element concentrations were in general higher in the mining impacted lakes than in the reference lake Kiantajärvi (Table 1, Table S5). Concentrations of S, Cu, Ni and U in the sediments showed decreasing concentration away from the mining site (Table 1). Instead, Co, Cr and Mn did not show a decreasing pattern along the watercourse. Sediment Mn concentrations in the reference lake Kiantajärvi were higher than in the two lakes nearest to the mining site (Lake Kolmisoppi and Lake Salminen). Arsenic was only measured with minor amounts in the exchangeable fraction of AS and reference sediment (Table S5).

Overall, the highest metal concentrations were observed in the sediments of lakes Salminen and Kalliojärvi, closest to the mining site. Concentrations of Fe, Cd, Pb, Zn and Mn peaked in the sediment of Lake Jormasjärvi, which was the furthermost lake from the mining site. The most easily extracted elements were Sr and Mn, which were on average 50 to 60% in the exchangeable form in all the studied lake sediments (Fig. 2, Table 1). The exchangeable and carbonate bound fractions (F1 + F2) together representing the most mobile fraction covered on average 60 to 90% of Ca, K, Sr and Mn total concentration in all field-collected sediments. The third metal binding fraction associated with Fe and Mn oxides was negligible or covered <20% for most metals and sediments except for Cd and Zn. Approximately 30 to 85% of S, Cd, Cr Cu, Ni, Pb and U were associated with organic matter and sulfide bound fraction (F4).

Sequential extractions revealed that not only the total concentrations but also the most bioavailable fraction (F1) of S, Ni and U were elevated in lake sediments nearest to the mine (Fig. 2, Table 1). For Mg, Co and Zn a similar but not as consistent gradient was observed. Cd concentrations in the mining-affected lake sediments were three to five times the concentration in the reference sediment and the proportion of bioavailable fraction varied from 3% to 14%.

Concentrations of the measured elements were generally higher in *L. variegatus* exposed to the field collected sediments in comparison with control (AS) (Table 2, S3). Concentrations of P, S, Cu and Zn were highest in *L. variegatus* exposed to sediments nearest to the mining site. However, Na, Mg, Al, Fe, Sr and Mn body residues in *L. variegatus* exposed to mining-affected test sediments were lower in the sediments nearer to the mine. Significant correlations were found only between element concentration in *L. variegatus* and in the sediment organic matter and sulfide bound fraction for P ($r^2 = 0.828$), Zn ($r^2 = 0.843$) and Mn ($r^2 = 0.820$) (Table S6).

Total and dissolved element concentrations were generally higher in the AFW above the sediments at 21 d in comparison to AFW at 0 d (Table S4), indicating leaching of metals from the sediment during the toxicity test. P, Co, Cr, Cu, Ni, U and Zn were rarely detected from the water; they were mainly recovered in the test vials with sediments from the most contaminated lakes (Lakes Kolmisoppi, Kalliojärvi and Salminen). As, Cd and Pb were not detected from the AFW of the test vials.

3.2. Ecotoxicity test and behavioral responses of L. variegatus

The wet weight of L. variegatus was reduced in the test sediments originating from lakes Kalliojärvi and Salminen ($F = 46.4$, $p < 0.001$), as was the dry weight also in the Lake Jormasjärvi sediment ($F = 46.9$, $p < 0.001$) (Fig. 3). The number of worms retrieved at 21 d was lower in the lake sediments of Jormasjärvi, Kalliojärvi and Salminen in comparison to the reference lake ($F = 34.4$, $p < 0.001$). Instead, there was no statistically significant decrease in the growth or reproduction of worms in the test sediment of Lake Kolmisoppi located within the mining concession, in comparison to the reference sediment (Fig. 3). The *L. variegatus* reproduced sufficiently in the AS control by a factor of 2.4 on average, but in the natural reference sediment the number of individuals was increased only by a factor of 1.6, which is less than the required reproduction factor 1.8 in the OECD 225 standard (OECD, 2007).

The worms expressed normal burrowing behavior in all the test sediments, residing their posterior part in the overlying water. In the AS control, *L. variegatus* expressed different behavioral responses than in the natural sediments (peristaltic 0 d: $F = 7.2$, $p = 0.002$; peristaltic 21 d: $F = 5.9$, $p = 0.006$, overall locomotory activity 0 d: $F = 5.5$, $p = 0.006$; overall locomotory activity 21 d: $F = 7.3$, $p < 0.001$) (Fig. 4). The peristaltic movements were lowered in the sediments of lakes Kolmisoppi, Kalliojärvi, Salminen and AS control at 0 d in comparison to the reference Lake Kiantajärvi. After 21 d, the highest peristaltic movements were observed in the AS sediment and when compared to the reference peristaltic movements they were lowered only in the Lake Salminen sediment (Fig. 4). The activity of *L. variegatus* was the highest in the sediments of lakes Kiantajärvi, Kalliojärvi and Jormasjärvi at 0 d. The overall locomotory activity at 0 d was lower in the Lake

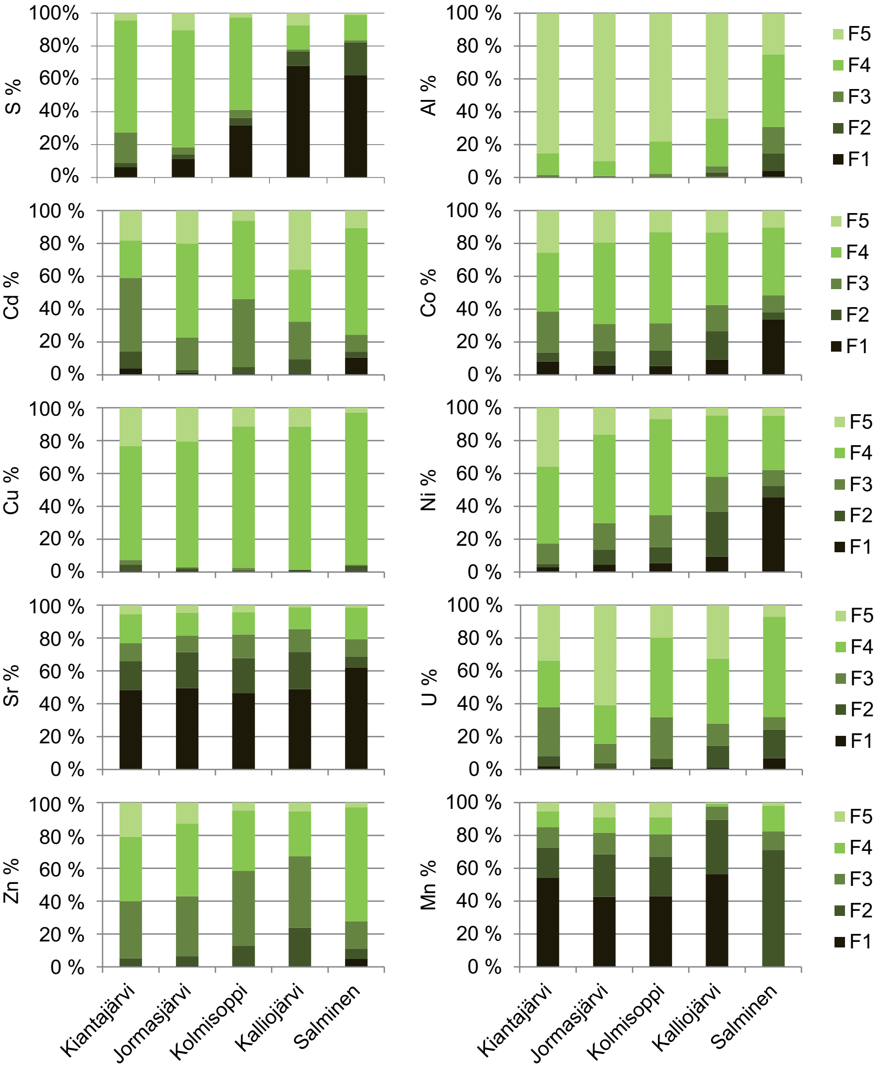

Fig. 2. Percentages of metal concentrations associated with different fractions of sequential extractions (F1: exchangeable; F2: carbonate; F3: Fe and Mn oxides; F4: organic matter and sulfides; F5: residual). Kiantajärvi represents the local reference lake and Jormasjärvi to Salminen the lake network downstream from the mine.

Table 2
Mean elemental concentrations in *L. variegatus* at 21 d (mg/kg) and standard deviations (SD). Control represents the artificial sediment, Kiantajärvi the local reference lake, and Jormasjärvi to Salminen the lake network downstream from the mine. Empty cells in the table are non-detect values.

Lake		S	Ca	Al	Fe	K	Cd	Cu	Ni	U	Zn	Mn
Control	n	3	3	3	3	3	1	3		2	3	3
	Mean	4086.6	980.2	282.4	453.5	4635.1	0.3	12.1		3.3	139.4	9.7
	SD	215.3	22.8	51.7	39.4	261.7		0.8		0.2	6.9	0.7
Kiantajärvi	n	3	3	3	3	3	3	3		2	3	3
	Mean	5026.4	1392.4	3104.5	4924.7	4531.3	1.0	18.1		16.9	160.6	153.4
	SD	49.6	65.0	317.1	510.8	59.0	0.0	0.9		1.9	8.8	11.7
Jormasjärvi	n	3	3	3	3	3	3	3		1	3	3
	Mean	5769.1	1321.7	1807.2	4670.8	4610.5	1.6	18.8		12.6	215.7	130.8
	SD	136.5	64.1	268.5	689.4	114.0	0.2	2.2			27.0	20.5
Kolmisoppi	n	3	3	3	3	3	3	3			3	3
	Mean	5886.2	1785.7	1970.4	3941.8	4544.7	1.1	18.9			195.7	57.6
	SD	247.6	99.5	488.9	751.0	178.9	0.0	0.6			8.5	9.4
Kalliojärvi	n	3	3	3	3	3		3	2		3	3
	Mean	6124.8	921.0	561.5	2660.3	4572.4		19.5	3.3		199.9	24.0
	SD	127.8	15.4	116.1	1074.1	45.6		2.2	0.7		11.5	1.1
Salminen	n	3	3	3	3	3		3	1		3	3
	Mean	5661.2	1150.7	211.0	454.6	4555.4		21.1	3.6		209.5	14.5
	SD	26.9	33.3	55.2	47.3	104.9		1.6			1.0	0.2

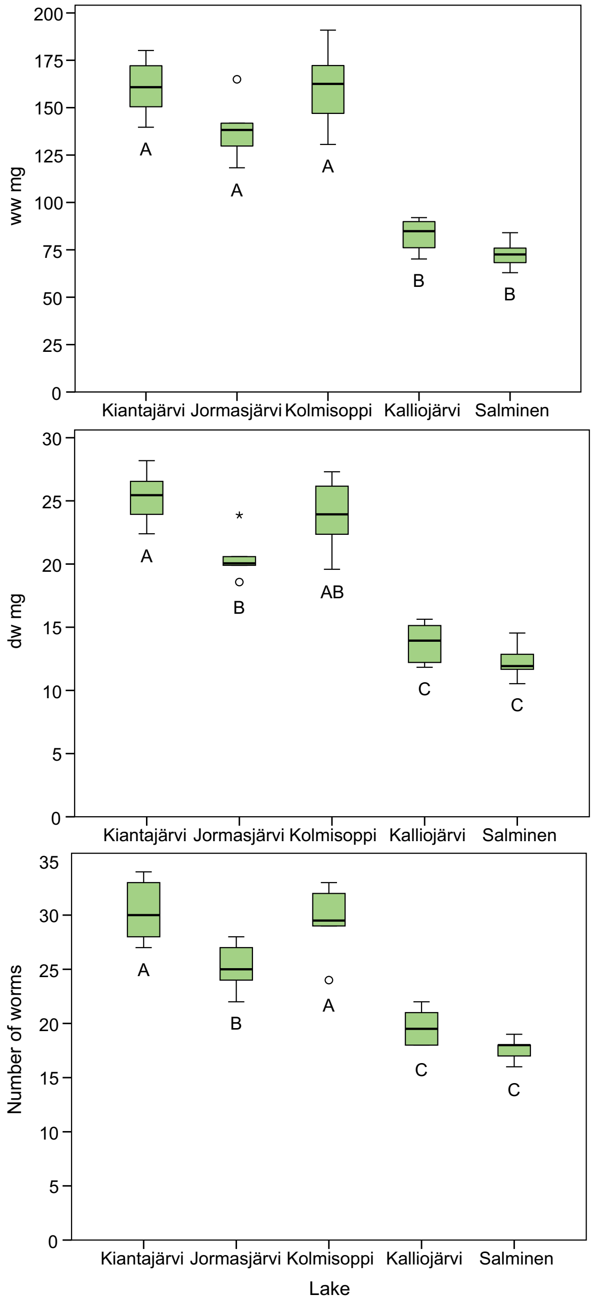

Fig. 3. *Lumbriculus variegatus* wet weight (ww), dry weight (dw) and number of individuals after 21 days of exposure to the mining-affected sediments. Kiantajärvi represents the local reference lake and Jormasjärvi to Salminen the lake network downstream from the mine. Letters denote significant ($p < 0.05$) differences between sites. Open circles and asterisks mark outliers >1.5 and >3 times the interquartile range, respectively.

Salminen sediment than in the reference Lake Kiantajärvi. At the end of the toxicity test period, activity evened out and the only difference was the low *L. variegatus* activity in the Lake Salminen sediment in comparison to the AS control.

The toxicity test duration was 7 days shorter than the 28 d required in the standard toxicity test. Yet a similar decrease in sediment pH as in our previous experiments (Wallin et al. 2018, unpublished) was observed. In the sediments of lakes Jormasjärvi, Kalliojärvi and Salminen pH dropped below 5.0 (Fig. 5). The sediment pH at 21 d was significantly lower in Lake Salminen when compared with the reference Lake Kiantajärvi ($p = 0.003$), AS ($p < 0.001$) and in Lake Jormasjärvi in comparison with AS ($p = 0.015$). Lake Salminen sediment was acidic and the pH was 4.6 in the field, then 4.4 in the beginning of the test after 22 d storage time, and finally 4.0 at 21 d (Table S4).

4. Discussion

4.1. Elemental concentrations in sediment, L. variegatus and water

Sequential extractions revealed that the partitioning of elements in boreal lake sediments was both element- and site-specific. This emphasizes the importance of site-specific assessment of sediments downstream from the mining activities. The majority of the elements were not present in the residual fraction indicating that changes in sediments may alter the speciation of the studied elements because only the residual fraction is considered to be stable in the environment (Tessier et al., 1979; Torres and Auleda, 2013). A decreasing gradient in the residual fraction towards the mine suggested that the origin of the elements in the lake sediments was due to anthropogenic activities (Pagnanelli et al., 2004), such as biomining activities.

Metal partitioning in boreal sediments is not well studied. Several methods for sequential extractions exists, thus comparison of our results with other studies include considerable uncertainty. In the present study, sediments were rich in organic matter and a major part of Cu and Pb, and in some sediments Cd, Cr, Zn, U and Ni were associated with the organic matter and sulfide bound fraction (Fig. 2, Table S5). Some similarities can also be observed with Cu, Pb and Zn in river sediments of an abandoned pyrite mining area in which metals were mainly associated with sulfide bound and residual phases (Pagnanelli et al., 2004). Residual Fe is generally related to primary and secondary minerals and residual fraction originates from the local bedrock and the mine tailings (Gómez-Álvarez et al., 2007; Tessier et al., 1979). Cu expressed similar division in the residual and non-residual fraction as in Gómez-Álvarez et al. (2007). Instead Cd, Pb, Zn and Mn were associated 25 to 80% less in the residual fraction than in Gómez-Álvarez et al. (2007), which may be due to the higher organic matter and sulfur content of our lake sediment samples. The association of Cu, Zn and Pb with organic bound and sulfide fraction of the sediments was also observed by Torres and Auleda (2013). In the present study, Ni and Co also had similar partitioning and they were less associated with organic matter and sulfide fraction than Cu but similarly to Zn, concentrations of which covered on average 40% in the F4 fraction (Fig. 2). Cr was more associated with a residual fraction than the metals above, except for in Lake Salminen, which was the first lake downstream from the mine with lowest sediment pH.

Sulfur was naturally associated in the organic matter and sulfide bound fraction in the reference Lake Kiantajärvi. The proportion of the organic matter and sulfide bound fraction decreased while the proportion of the exchangeable fraction increased towards the lake nearest to the mining site (Fig. 2), which implied a mining-induced gradient in sediment S fractions. Association with the most easily liberated exchangeable fraction (F1) suggested that a major part of S consisted of sulfates in the lake sediments nearest to the mining site. Sulfur concentration in sediments declined when moving away from the mining site, which has been observed also in our previous study (Wallin et al., 2018, unpublished). The fourth extraction step recovered both organic matter and sulfide bound fraction; hence, organic and pyrite forms could not be separated (Torres and Auleda, 2013).

Metal concentrations in *L. variegatus* were lower in the present study than in our previous experiment, which was one week longer but with an otherwise similar test design (Wallin et al. 2018, unpublished). Lower metal concentrations may be due to a shorter exposure period whereupon the net uptake from sediment to *L. variegatus* may have been less steady state (Sardo and Soares, 2011). Monitoring of tissue metal concentration for predicting ecological effect and risks of metals

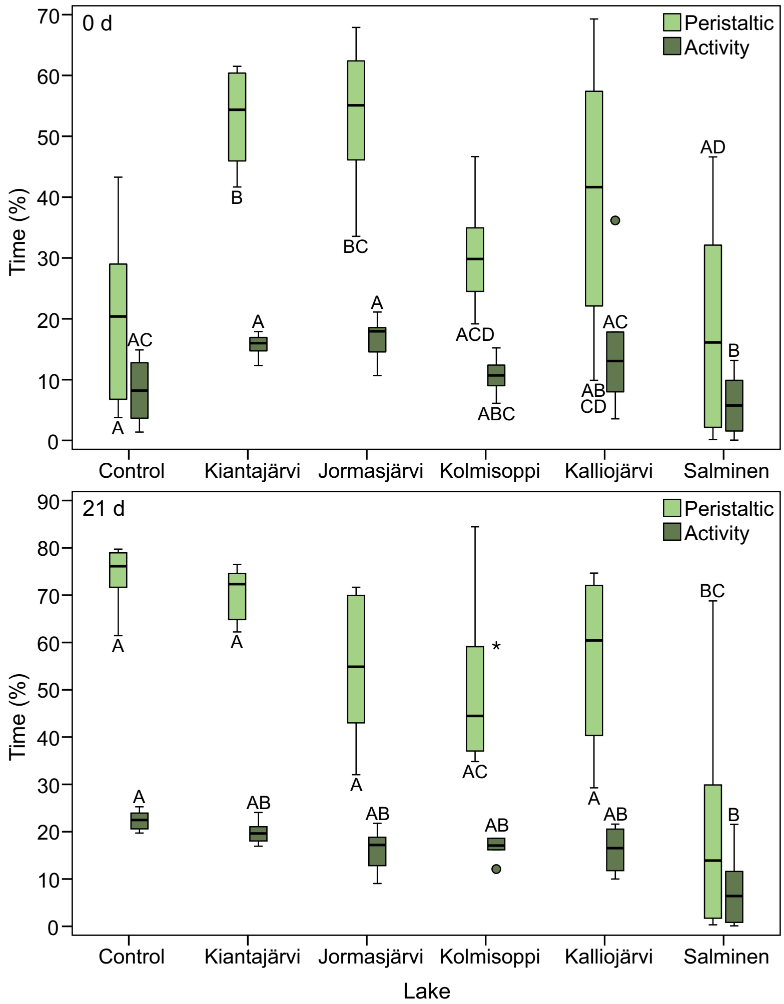

Fig. 4. Behavioral responses of *L. variegatus* as average percentage spent in a predefined frequency range in mining affected sediments at 0 d (upper) and 21 d (lower). Control represents the artificial sediment, Kiantajärvi the local reference lake, and Jormasjärvi to Salminen the lake network downstream from the mine. Letters denote significant ($p < 0.05$) differences between sites in peristaltic and the overall locomotory activity. Open circles and asterisks mark outliers >1.5 and >3 times the interquartile range, respectively.

in aquatic ecosystems has been proposed in many contexts (Bervoets et al., 2016; De Jonge et al., 2013; Méndez-Fernández et al., 2017). There are also observations that contradicts application of tissue concentrations as indication of hazard, because linking tissue concentrations of metals to toxic effects is not straightforward (Burton, 2010; Carmen Casado-Martinez et al., 2010; DeForest et al., 2007; McGeer et al., 2003; Méndez-Fernández et al., 2013). Concentrations of Cd, Cr, Cu, Ni, Pb and Zn in *Tubifex tubifex* exposed for 28 days to mining-affected sediments under standard conditions were on average 35 to 194% of those in field-collected aquatic oligochaetes from same area (Méndez-Fernández et al., 2017). Concentrations of S, Cu and Zn in *L. variegatus* increased along the corresponding concentration in the sediment and against distance from the mining site (Table 2). These elements are known components of Terrafame mine effluents (Marttila, 2017).

Ni was detected from the sediment samples but not from the test water or *L. variegatus* excluding the most contaminated lake sediments of Kalliojärvi and Salminen nearest to the mine. These two lakes also had the highest sediment Ni concentrations. This was also observed by Wallin et al. (2018, unpublished) using a similar test setup with sediments severely affected by mining in the same area. When the test setup included natural hypolimnion above the sediment instead of AFW, Ni was detected from the *L. variegatus* (Wallin et al., 2018, unpublished), indicating that Ni was not highly bioavailable from the Terrafame mine sediments. A corresponding conclusion can be made from the SEs, where over 40% Ni bioavailability was estimated only in the Lake Salminen sediment. Our results are in compliance with Camusso et al. (2012), who suggested that *L. variegatus* accumulated Ni mainly through pore water. The importance of exposure from the pore water has also been observed with *T. tubifex* exposed to metal contaminated sediments (Méndez-Fernández et al., 2014).

For metals, correlation between concentration in *L. variegatus* and separate sediment extraction fractions was significant and positive only with Zn and Mn in organic matter and sulfide bound fraction. This implied that the exchangeable and carbonate bound fractions were not solely responsible for metal mobility from the sediments. Dietary exposure is a significant part of metal uptake in *L. variegatus* and may enable exposure of sediment contaminants to higher trophic level organisms (Camusso et al., 2012; Woodward et al., 1995; Xie et al., 2008). That said, proportionally lower metal concentration in *L. variegatus* in the sediments near the mining site may partly be explained by avoidance of both sediment ingestion and burrowing, which was also supported by the lowered ww and dw of *L. variegatus* exposed to Lake Kalliojärvi and Salminen sediments. For *T. tubifex*, the

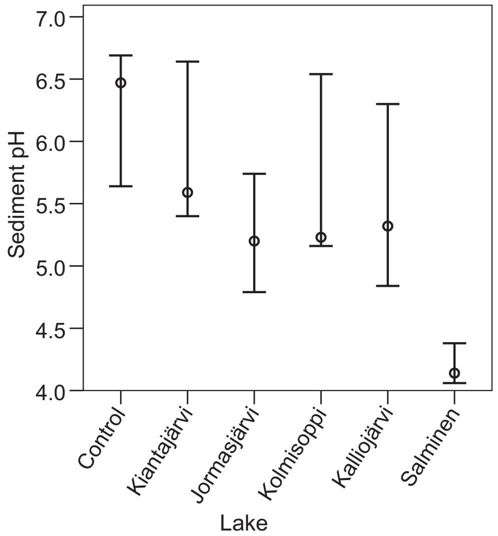

Fig. 5. Sediment minimum and maximum (vertical lines) and median (circle) pH during 21 d toxicity test. Control represents the artificial sediment, Kiantajärvi the local reference lake, and Jormasjärvi to Salminen the lake network downstream from the mine.

ingestion of sediment contaminated with organic compounds or metals was estimated to be 42% of the ingestion of an uncontaminated sediment (Martinez-Madrid et al., 1999; Méndez-Fernández et al., 2014). Some species such as snails were reported to be able to avoid contaminated prey (Guo et al., 2013). Bioaccumulation factors for most metals has been reported to be inversely related to concentration in water and sediment, and accumulation is mainly dependent on the dynamics of metal uptake, detoxification and/or elimination (Carmen Casado-Martinez et al., 2010; DeForest et al., 2007; McGeer et al., 2003). Although correlations between only Zn and Mn concentrations in *L. variegatus* and in sediment organic matter and sulfide bound fractions were significant, this could imply that accumulation potential is underestimated if only the most easily extracted fractions (F1, F2) are considered.

Elements leached partly to the overlying water from the sediment during the toxicity test but the leached proportion was under 2% of the corresponding total concentration in the sediment at 0 d (Table S3, S5). Mn was the most easily leached metal. Leaching had a negligible effect on the sediment total elemental concentration, but the change was proportionally larger in the water phase and leaching could have influenced the exposure route of metals (Luoma, 1989).

4.2. Responses of L. variegatus to contaminated sediments

Dry weight and the number of *L. variegatus* at 21 d were the most sensitive toxicity endpoints in the present study. The most polluted lakes were well identified with the 21-day toxicity tests. Endpoint comparisons with the local reference lake was the most practical approach because the characteristics (e.g. pH, organic carbon and dry matter content) of AS obviously did not correspond with the lake sediments.

The standard chronic toxicity test period for *L. variegatus* is 28 days but 21 days was used in the present study in order to mitigate the pH decrease in the sediment and water during toxicity test and the time that *L. variegatus* was exposed to acidic conditions. Sediment pH nevertheless decreased one unit on average in the field-collected test sediments during the exposure period (Fig. 3). Sediment pH was especially low in Lake Salminen already in the field (4.6), and the responses of *L. variegatus* exposed to sediments are not due solely to metals, because low sediment pH had also effects on the growth, reproduction and behavior.

The number of *L. variegatus* in the AS control increased well during the toxicity test and reproduction was above the requirement of the valid test described in the standard (OECD, 2007). In the reference sediment, however, reproduction was 11% lower than the required (increase by factor 1.8). Similar reproduction in field-collected reference sediment has been observed in, for example, Xie et al. (2016), in which the number of *L. variegatus* increased 59% in two weeks. Although the toxicity test duration was shortened, the *L. variegatus* had enough time to grow and reproduce sufficiently, and the shorter exposure period did not essentially affect the comparability to chronic tests with a normal 28-day exposure.

The behavioral responses of *L. variegatus* were not as consistent across the concentration gradient as the weight and number of worm individuals. Behavior was also different in the AS control compared to the field-collected test sediments and the responses between some individuals varied substantially (Table S7). In the Lake Salminen, Kalliojärvi and AS control sediments, peristaltic movements were distributed widely at 0 d; half of *L. variegatus* showed low peristaltic movement while the other half showed remarkably higher movement. Individual-specific variation in behavioral responses is common and a similar phenomenon has been observed with *L. variegatus* in Pb spiked water and sediment, *Heptagenia dalecarlica* larvae exposed to multimetal contaminated water and with *Lampetra fluviatilis* larvae exposed to persistent organic pollutant and Hg contaminated sediments (Gerhardt, 2007; Salmelin et al., 2017, 2016). This may reflect behavioral plasticity because stress reactions vary both between individuals and different exposure conditions (Gerhardt, 2007; Salmelin et al., 2017, 2016). In the AS control at 0 d, one plausible explanation to the large variation was that for some individuals it took longer to burrow into the dense AS whereas in natural softer sediments burrowing was easier.

The composition of the AS control was not comparable to natural sediments rich in organic matter. Sediment characteristics, such as grain size, have an effect on the behavioral responses of *L. variegatus* (Sardo et al., 2007). Behavioral responses in potentially contaminated sediments should be compared to natural representative sediment. Furthermore, the decreasing gradient in estimated sediment organic matter downstream from the mine impeded the interpretation of the results because the most contaminated sediments had the highest organic carbon content. Organic carbon in the sediment affects the feeding rate of *L. variegatus*, and worms need to ingest higher amounts of sediment to cover a similar energy demand in sediments with low organic carbon in comparison to sediments with higher organic carbon content (Leppänen and Kukkonen, 1998).

The low variability of locomotory activity measurements indicates that it may be a less sensitive endpoint to individual behavioral variation than the peristaltic movement with higher variations. Ding et al. (2001) found that the forward crawling movement of *L. variegatus* showed larger variation than the swimming frequency did, which was consistent among the studied *L. variegatus*. Forward crawling frequency also was inhibited along time and concentration gradient in an antiparasitic drug exposure (Ding et al., 2001). Frequency of that forward crawling movement responded peristaltic movements (0.5–1 Hz) recorded in the present study.

In the present study, *L. variegatus* showed two distinct behavioral responses in the predefined frequency ranges. In contrast to previous studies by Sardo and Soares (2010), peristaltic movements showed more inhibition than locomotion activity in lake sediments polluted by acid mine drainage. However, a decrease in both peristaltic movements and locomotion activity has been observed with *L. variegatus* exposed to metals (Gerhardt, 2009; Sardo and Soares, 2011). O'Gara et al. (2004) found that Cu exposure impaired the helical swimming and body reversal behavior of *L. variegatus*. The behavioral responses of *L. variegatus* are related to their foraging behavior and ability to hide or escape predators. Impaired foraging may lead to impaired vigilance, which may increase vulnerability to predators. Furthermore, changes in the feeding of the bottom-dwelling animals may induce changes in the structure and function of the lake benthic community (Blankson et al., 2017).

Mining-affected sediments reduced the overall locomotory activity and peristaltic activity of *L. variegatus*, as was demonstrated with the MFB measurements. Previous results of *L. variegatus* behavioral responses to sediment metals have been conducted mainly with acidic and heavily polluted natural sediments or sediments spiked to metal concentrations in which mortality occurs (Sardo and Soares, 2011, 2010). One of our aims was to assess the ability of MFB to detect the behavioral early warning responses of *L. variegatus* to multimetal contaminated sediments. We could not distinguish the least mining-affected sediment (Lake Jormasjärvi) from the reference sediment with MFB even though there was a statistically significant difference in *L. variegatus* growth. At the same time, there was no difference between *L. variegatus* wet weight, dry weight or number in the Lake Kolmisoppi sediment compared with the reference at 21 d, despite the difference in peristaltic movements between these two sites at 0 d.

While both the overall locomotory and peristaltic activities were similar at 0 d and 21 d, the MFB appears to be a fast instrument for detecting potential behavioral differences. Instead, when chronic responses are considered, growth was a more sensitive endpoint, as also suggested by Melvin and Wilson (2013). Earlier, the behavioral responses of *L. variegatus* to sediment spiked with Pb were observed after 10 days and 28 days of exposure but not at 1 day (Sardo and Soares, 2011) and to mining contaminated sediments after 10 days of exposure (Sardo and Soares, 2010). However, responses have also been observed in short-term exposures. Cu exposure a produced time-dependent decrease in the helical swimming behavior of *L. variegatus* already under a 3-h examination period (O'Gara et al., 2004). Although the observed responses may be more consistent in chronic exposures, acute exposures may also be useful. In the present study acute responses were indeed more evident than long-term ones. One disadvantage in acute exposures might be that there is more variation in the acute reaction with some individuals or species responding with increased activity while others remain inactive (Gerhardt, 2007; Salmelin et al., 2016). The interpretation of behavioral responses therefore requires caution and extensive expertise. Instead of calculating averages, an analysis of behavioral patterns might be more relevant.

There are neither standards nor validated methods for behavioral assessment with the MFB technique. Although encouraging results are often reported, it is not recommended to use the technique as a replacement for traditional toxicity tests. Instead, it could be used as an additional endpoint in ecotoxicity tests (Gerhardt, 2007; Melvin and Wilson, 2013; Sardo and Soares, 2011). MFB is ~~quite~~ easy to set up and measurements are fast, so MFB could be applied in the screening level assessment. MFB could also be applied in urgent risk assessment scenarios, as our study indicated, since the standardized laboratory tests can be laborious and their test duration long. An additional advantage is that the MFB device is portable and the responses can be also monitored in the field in shallow environments. Although *L. variegatus* is not the most sensitive among the species commonly used in metal toxicity tests (Phipps et al., 1995), it is more sensitive than, for example, *T. tubifex* (K.K. Chapman et al., 1999). The advantage in comparison to locally representative sensitive fauna is its versatility; *L. variegatus* is a good model organism and easily cultured (Chapman, 2001).

5. Conclusions

In the present study, the impacts of a multimetal mine utilizing a biomining technology on the downstream lake sediments were assessed for the first time with both standardized chronic endpoints and behavioral responses of *L. variegatus*. In addition, the anthropogenic impact of metals and major ions were demonstrated with sequential extractions and a gradient of decreasing sediment metal concentrations away from the mine. Sequential extractions appeared suitable for assessing the potential bioavailability of sediment metals. Sequential extractions and *L. variegatus* body residues together indicated both exchangeable fraction and more tightly bound fractions can be predictors for metal bioavailability in worm gut contents. Biological endpoints revealed obvious ecotoxicity impact gradients along the watercourse. The standard toxicity test responses of growth and reproduction were sensitive endpoints in regard to the chronic effects of the biomining-affected sediments. The activity of *L. variegatus* was also reduced in the test lake sediments nearest to the metal mine and responses were observed already under acute exposure. Multispecies freshwater biomonitor showed its usefulness as a screening level tool for urgent risk assessment occasions, and it could also be used as an additional endpoint with traditional toxicity tests when assessing the impacts of mining-affected lake sediments.

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Appendix A. Supplementary data

Supplementary material includes tables S1–S7. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.07.117.

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