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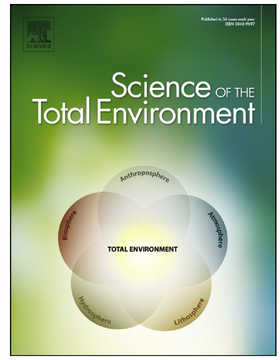

Ecotoxicity assessment of boreal lake sediments affected by metal mining: Sediment quality triad approach complemented with metal bioavailability and body residue studies

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Title Ecotoxicity assessment of boreal lake sediments affected by metal mining: Sediment Quality Triad approach complemented with metal bioavailability and body residue studies.

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ABSTRACT

There are several methods for studying metal-contaminated freshwater sediments, but more information is needed on which methods to include in ecological risk assessment. In this study, we compliment the traditional Sediment Quality Triad (SQT) approach – including information on chemistry, toxicity and ecological status – with studies on metal bioavailability and metal body residues in local organisms.

We studied four mining-affected boreal lakes in Finland by conducting: chemical analyses of sediment and water, toxicity tests (*L. variegatus*, *V. fischeri, C. riparius*, *L. stagnalis*: growth and mortality), and analysis of benthic organism community structure. In addition, we studied the relationships between metal loading, toxicity, metal bioavailability, and metal body residues in the field-collected biota.

Chemistry and benthic organism community structures show adverse effects in those lakes, where the metal concentrations are the highest. However, toxicity was connected to low sediment pH rather than high metal concentrations. Toxicity was observed in 4 out of 6 toxicity tests including growth test with *L. variegatus*, bulk sediment test with *V. fischeri*, and the *L. stagnalis* toxicity test. The *C. riparius* test did not show toxicity. Metal body residues in biota were not high enough to induce adverse effects (0.1–4.1 mg Cu/kg fw, 0.01–0.3 mg Ni/kg fw, 2.9–26.7 mg Zn/kg fw and 0.01-0.7 mg As/kg fw).

Chemical analyses, metal bioavailability assessment and benthic community structures survey revealed adverse effects in the sediments, where metal concentrations are highest (Lake SJ and Lake KS). Standard toxicity tests were not suitable for studying acid, sulfide-rich sediments and, therefore, benthic structure study and chemical analyses are believed to give more reliable results of the ecological status of these sediments.

Keywords

Sediment, metal, ecological risk assessment, triad, bioavailability, body residue

1 INTRODUCTION

Increased metal concentrations in the waterbodies close to metal mines may cause adverse effects in the aquatic ecosystems. Metals often accumulate in bed sediments leading to increased exposure and possible toxicity to benthic organisms (Besser et al., 2015). The evaluation of these effects is challenging. For successfully conducting ecological risk assessment of metals in sediments, there are several aspects that need to be considered: the level of contamination, the bioavailability of the contaminants, the exposure to the contaminants, the responses in the organisms and the question, of whether there are other simultaneous cofactors causing the observed responses (Riba et al., 2004). The uptake routes, mechanisms and effects of metals are dependent on the characteristics of the sediment and the overlying water, as well as on the metal mixture composition (Péry et al., 2008, Camusso et al., 2012, Campana et al., 2013). In addition, there is a lack of knowledge on metal toxicity in soft and acidic water ecosystems, which are sensitive to metal toxicity (Bielmyer et al., 2007, Dam et al., 2008, Hoppe et al., 2015, Ryan et al., 2009). Besides Scandinavia, there are remarkably soft waters at least in Scotland, Australia, eastern North America and Southeast Asia (Howarth et al., 2012, UNEP GEMS, 2006).

Metal toxicity is induced mostly by the bioavailable metal fraction. In water, metal bioavailability is dependent of several factors, including pH, dissolved organic carbon (DOC) and cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) (Di Toro et al., 2005). In (anaerobic) sediments, metal bioavailability is mainly controlled by sulfides, organic matter, Fe-Mn oxides and sediment particle size (Zhang et al. 2014). For comprehensive risk assessment, knowledge of the metal concentrations, bioavailability and biological responses are needed, and, thus, several types of studies are required to attain the required information. The biological responses can be studied in laboratory and in field. In laboratory, standard acute and chronic toxicity tests methods are widely used (ECHA, 2016, U.S. EPA, 1996). Benthic invertebrates are often used for sediment toxicity tests, as they are exposed to contaminants both through dissolved (pore water and overlying water) and particulate phases (ingested sediment particles). Besides the standard toxicity tests, there are novel early warning systems based on animal behavioral responses (Sardo and Soares, 2010, Wallin et al. 2018). In the field, the ecological risk assessment can include analysis of the local conditions, like concentrations and bioavailability of the contaminants, or bioaccumulation (U.S. EPA 1994). The adverse effects can be further evaluated by assessment of benthic organism community structures in the study lakes (Swedish Environment Protection Agency, 2000).

The Sediment Quality Triad (SQT) approach combines several important parts: (1) Sediment chemistry, including the levels of the contaminants, (2) Sediment toxicity assessed by biotests and (3) The effects on the local benthic organism structures (Long and Chapman, 1985, Riba et al., 2004). In this study, we add two more elements to the SQT approach: metal bioaccumulation and bioavailability. By our knowledge, there are no SQT studies including all the five aspects. Previous SQT studies have included either metal bioavailability (Lee et al. 2006), bioaccumulation (Riba et al. 2004, Lopes et al. 2014), or neither of them (Khim et al. 2018). Riba et al. (2004) and Lopes et al. (2014) have evaluated the bioavailability based on bioaccumulation, but the bioavailability itself was not analyzed. Borgmann et al. (2001) have conducted a metal toxicity study where they compared observed toxicity to the bioaccumulation and bioavailability of metals (Cd, Co, Cu and Ni), using several species: *Chironomus riparius*, *Hezagenia limbata*, *Hyalella azteca* and *Tubifex tubifex*. They were able to connect the increased bioaccumulation to metal concentrations in the environment for Cd, Co and Ni. In their study, Ni was the only metal accumulating in concentrations high enough to cause toxic responses. In addition, they found the adverse effects of metals to arise from the dissolved metal phase instead of the bulk sediment.

This study focuses on the ecological risk assessment of metal mining on boreal lake sediments, using improved, modern SQT approach. The aim is evaluate the toxicity of the sediments, to test the suitability of different methods, and to study the effects of metal bioavailability and bioaccumulation on ecological risk assessment. We studied four, metal-contaminated lakes in Finland. We complemented the traditional SQT approach (1-3) with additional, applied SQT methods (4-5) as following: (1) Analysis of sediment chemistry, conducted in our earlier study (Väänänen et al., 2016), (2) benthic community structure analyses, where the total number of organisms and the abundancy of specimen were studied, (3) a selection of toxicity tests (luminescence bacteria *Vibrio fischeri*, oligochaete *Lumbriculus variegatus*, snail *Lymnaea stagnalis,* and an early warning behavioral test with midge *Chironomus riparius*), (4) metal bioavailability from our previous study (Väänänen et al. 2016) and (5) metal bioaccumulation as metal body residues in field-collected fish and chironomids

2 MATERIAL AND METHODS

2.1 Sediment samples

Four metal-contaminated natural sediments in Finland were chosen for this study (Lakes Kirkkoselkä=KS, Junttiselkä=JS, Laakajärvi=LJ and Sysmäjärvi=SJ) (Table 1). The sediments were collected using Ekman grab samplers, sieved to particle size < 1 mm and stored at +4 °C. The primary source of the metal pollution in the lakes originates from mining activities. Two of these metal mines are still operating; Pyhäsalmi Mine next to Lakes KS and JS, which quarries Zn and Cu, and Terrafame Group Ldt. (previously Talvivaara Mine) next to Lake LJ, which quarries Ni, Cu, Zn and Co. Lake SJ is located next to the Outokumpu Mine, where Cu mining activities ceased in the 1980s. Thorough chemical analysis of these lake sediments was conducted in our earlier study and the metal concentrations in these lake sediments were elevated for Ni (28–204 mg/kg), Zn (168–863 mg/kg), Cu (19–248 mg/kg) and As (6–53 mg/kg), analyzed by ICP-MS (Table 1) (Väänänen et al., 2016). Several sediments were used as references for the toxicity tests. Main reference sediments is from a non-polluted Lake Parkkimanjärvi (PJ) close to sites KS and JS. For the toxicity tests, additional references were used: Artificial freshwater sediment (AFS), plus laboratory-specific generic reference sediments Lake Palosjärvi (PaJ) at the University of Jyväskylä and Lake Höytiäinen (Hy) at the University of Eastern Finland. Artificial sediments were prepared in the laboratory according to the OECD guideline 225 (OECD, 2007) by mixing kaolinite clay, quartz sand and ground peat.

2.2 Toxicity tests

The toxicity tests were performed with *L. variegatus* (growth and reproduction), *V. fischeri* (at the time, now *Aliivibrio fischeri*) (bioluminescence), *C. riparius* (behavior) and *L. stagnalis* (growth and mortality). *L. variegatus* were reared at the University of Eastern Finland (Department of Biology, Joensuu campus) following the protocol of Leppänen and Kukkonen (1998). For *V. fischeri*, a commercial toxicity kit was used (BioTox kit, Aboatox Finland). *C. riparius* were from the Finnish Environment Institute laboratory stock (Jyväskylä) as were *L. stagnalis*, which originated from a local Lake Konnevesi, in Central Finland. A summary of the used test methods and endpoints are presented in Table 2.

For ***Lumbriculus variegatus***, a 28-day growth and reproduction test was conducted following OECD guideline 225 (OECD, 2007). Reference sediments AFS, PJ and Hy were used. In AFS, urtica powder (*Urtica sp*.) was used as a food source [0.5% of sediment dry weight (dw)]. Growth was measured as increase of biomass over the experiment period, and reproduction was measured as a change in number of individuals (n=4). All the experiments were carried out using 200 ml glass jars, adding a few centimeters thick sediment layer (50 g) and 130 ml of artificial freshwater (1mM, pH of 6.5–7.5) (OECD, 1992). *L. variegatus* were acclimatized to artificial fresh water 24 h prior to starting the experiment, 10 individuals per replicate. The temperature was 20 ± 1 °C during the experiment and a recommended 16:8 h light:dark cycle was used. The temperature, water pH (WTW CellOx 325, Germany), oxygen saturation (WTW SenTix 41, Germany) and ammonia concentration (Schott NH 1100, Germany) were monitored weekly from at least 1/3 of the jars. The minimum water quality criteria were set to at 30% oxygen saturation, a pH range from 6–9 and 10 mg/l ammonia (OECD, 2007, Schubauer-Berigan et al., 1995). The water in all the systems was replaced during the experiment, if the minimum criteria were not met. The experiment was conducted in two batches.

The normality of the samples was tested using the Shapiro-Wilks test and the homogeneity of variance using the Levene's test (IBM SPSS Statistics 21). The reproduction data were not normally distributed, and the difference of the means were analyzed using the nonparametric Kruska-Wallis test (GraphPad Prism 5). The normally distributed growth data were analyzed using the one-way ANOVA, followed by Tukey's post hoc test (GraphPad Prism 5).

The ***Vibrio fischeri*** toxicity tests were conducted using the ISO 21338 standard for the kinetic determination of the inhibitory effects of sediments and other solids and color containing samples on bacteria bioluminescence (ISO, 2010). The sediments were diluted 1:5 (weight) to 2% NaCl and a series of 11 dilutions (1:1 dilution, n=2). The results were validated by conducting part of the tests twice. The bacteria suspension was incubated according to the protocol, and equal volumes of sample and bacteria suspension (300 µl) were used for the measurements (kinetic measurement at 0.2 s intervals during 15 seconds initially and single measurements after 30 min, Berthold Sirius 1, Pforzheim, Germany). In addition to bulk sediments, pore water samples were tested. Pore waters were extracted using Rhizon samplers as described by Väänänen et al. (2016). The toxicity tests were performed as with the sediment samples, but without the initial dilution step. Lakes PJ and Hy were used as a reference. The EC_{50} (effective concentration for 50% of the tested bacterial batch) values were calculated from the inhibition percentage of light production between the two sampling points, corrected by luminescence changes in the solution control sample (2 % NaCl).

The behavior of ***Chironomus riparius*** was observed using a Multispecies Freshwater Biomonitor® - device (MFB), which quantitatively records different behavioral patterns of animals by an impedance conversion technique (Gerhard, 2001, Gerhardt et al., 1998). Changes in the animal locomotion and ventilation activity within the test chamber generate signals specific to different kinds of behavior, allowing measurement of the behavioral responses of sediment-burrowing species (Gerhardt et al., 2002, Sardo and Soares, 2010, Wallin et al. 2018). The MFB device consists of test chambers, a recording unit and a laptop with software. Each MFB test chamber has one pair of electrodes creating an electrical field within a chamber and another pair of electrodes functioning as an impedance sensor. The data are analyzed via a stepwise discrete Fast Fourier Transform (FFT), an algorithm converting the original periodic sinusoidal signal into its component frequencies. The frequency analysis yields a percentage of the time (%) an animal spends on movements with frequencies that can be associated with certain behavioral patterns, such as ventilation or locomotion. The recording system covers frequencies between 0.5 and 8.5 Hz. With *C. riparius*, the lower frequency range (0.5–2.5 Hz) mainly corresponds to locomotion and higher frequencies (> 3 Hz) to ventilation, i.e., dorsoventral, undulating, regular movements. Both patterns may take place simultaneously, and thus, the sum percentage can be higher than 100%.

The four contaminated and two reference sediments (Hy and AFS) were applied in the chambers in a ratio of 1:4 (2 cm sediment:8 cm artificial water). The artificial fresh water used was similar to the one in *L. variegatus* test. The water was aerated for one day before the initiation of the test (The Finnish Standards Association SFS, 1984). First instar larvae were placed individually in the MFB test chambers (2 x 5 cm, n=6), and the chambers were submerged in AFW in separate 2 l glass vessels. Experimental conditions and water quality parameters were the same as in the experiments with *L. variegatus*. The larvae were fed with ground Tetramin fish food (0.25 mg/larva/day). The larvae were collected from six separate egg masses as there were six different exposure series altogether.

The larvae were exposed in chambers for 9 days and the behavioral recordings were started at the beginning of the 10^{th} day, one hour before the lights were turned on and continued for the next 12 h. The locomotion behavior ($\leqslant$ 2.5 Hz) and ventilation ($\geqslant$ 3 Hz) of each larva were measured for four minutes at ten-minute intervals, so that altogether 71 repeated measurements were obtained from each individual during the 12 h exposure. The animals were not fed during the measurements. For the statistical analysis, mean values of the locomotive and ventilation percentages were calculated over the 12 h exposures. The percentages were arcsine-transformed and tested for equality of variances by the Levene's test followed by randomized blocks ANOVA. The design tested fixed treatment (sediment) effect, random block (egg mass) effect and interaction. This methods was chosen since our preliminary study with *C. riparius* showed that the species was not suitable for the standard OECD growth test (OECD, 2004). If the required minimum O_2 saturation level (> 60%) of the standard was reached, it resulted in a distinct fall in pH and high mortality.

Lymnaea stagnalis was cultured in an AFW (Elendt M7, ISO, 2000), with the exception of having lower hardness (Ca 3.7 mg/l and Mg 1.4 mg/l) and trace metal (half of the original for Fe, Mn, Sr and Zn) concentrations than in the standard. The pH was set to 6.5–7. Three reference sediments (PJ, AFS and PaJ) were used, and 10 ml of thoroughly mixed sediment was placed in plastic petri dishes (Ø 5.3 cm, height 13 mm), forming a shallow layer at the bottom of the petri dish. The dishes were filled up with AFW (10 ml) and allowed to settle for 3 days. A large water to air surface area helps with oxygen diffusion.

Three similar age egg clutches were allowed to hatch in culture water (2 weeks). Upon hatching, less than 24 h aged juveniles were placed randomly in five replicates per treatment. The snails were fed twice a week with ground Tetramin (1.44 mg). Evaporated water was replaced with MilliQ water. Most of the overlying water was replaced after one week of exposure. The exposure was terminated after 14 days and the dw of surviving snails were obtained after drying them for overnight at 105 °C. Snail dw were ln transformed, and the normality (Shapiro-Wilk) and the variance (Levene's) assumptions of the parametric tests were checked prior to the one-way analysis of variance (ANOVA) and the Tukey HSD post hot tests (IBM SPSS Statistics 23).

2.3 Field data from the organisms

Monitoring data from the mining companies and local authorities were used, together with our own sampling data. The data gathered from authorities included information on metal concentrations (C_{metals}) in fish muscle tissue for pike (*Esox lucius*) and perch (*Perca fluviatilis*), and the community structure of benthic invertebrates. Since there were no existing community structure data for our reference lake (PJ), data were gathered during an additional sampling campaign. We sampled the profundal lake sediment with an Ekman grab sampler (6 replicates) and preserved the collected benthic organisms in 70% ethanol. The species identification was conducted by a private company (Probenthos, Finland). No benthic community structure data were available for Lake JS.

We collected benthic invertebrate (Chironomidae) samples from our study lakes KS, JS and SJ (October 2013) and from our reference lake PJ (October 2016) in order to analyze the metal body residues. From the Lake LJ, the collected chironomid biomass was not sufficient for the metal analysis. The chironomid samples were rinsed with MilliQ water, weighed and dried in an oven (+40 °C) overnight, after which they were analyzed with ICP-MS (Inductively coupled plasma mass spectrometry, SGS Inspector Services, Finland). As, Cu, Ni and Zn were included for this study, since they were earlier identified as the metal species most likely to induce adverse effects in these lakes (Väänänen et al., 2016). Correlations between metal concentrations in biota, pore water, overlying water and sediment were tested with the Pearson's correlation (GraphPad Prism 5).

The reports from environmental monitoring programs were gathered from the consulting companies Ahma Environment Ltd (Lakes KS and JS, data from year 2012), Savo-Carelian Environmental Monitoring (Lake SJ, year 2012) and Pöyry Ltd (Lake LJ, year 2013) (Ahma Group, 2012, Pöyry Ltd., 2014, Savo-Carelian Environmental Monitoring, 2013). For the metal body residuals in fish, the multi-metal analyses (ICP-OES or ICP-MS) were analyzed for fresh muscle tissue. Benthic community structure data were taken from the *Hertta* database, which is a national monitoring database for ecological status assessment of water bodies (www.ymparisto.fi/oiva, 2016), managed by The Finnish Environmental Institute SYKE. Benthic community structures in the *Hertta* database were analyzed by collecting profundal lake sediment samples (5–6 replicates, using Ekman grab samplers, stored in 70% ethanol), from which the species' abundancy was determined.

The ecological status in the sediments based on benthic community structures was calculated using the Profundal Invertebrate Community Metric index (PICM, equation 1) and by calculating the ratio of oligochaete and chironomids (normalized to sampling depth), as instructed by the Swedish EPA (Swedish Environmental Protection Agency, 2000). The classification of oligochaete/chironomid index is based on the Swedish sediment quality guideline, since Finland does not have its classifications for this metrics. The natural conditions in Finland and Sweden are similar, but the minor changes may bring uncertainty to the results. For PICM, Finnish criteria is used, whichassess the sediment quality based on the abundance of the pre-selected 46 benthic invertebrate species living in boreal lake basins and the total number of samples, as well as sampling depth. The observed values were compared to the calculated expected values, this ratio describing the state of the benthic population (The Finnish

Environment Institute, 2012). The PICM is an improved method from benthic quality index. The amount of species is increased from seven to 46, which enables more detailed evaluation of lake profundal.

$$PICM\ (observed) = \frac{\sum_{i=0}^{46} Indicator\ point * log10(species\ abundancy\ per\ m2)}{log10(species\ abundancy\ per\ m2)} \tag{1}$$

$$PICM\ (expected\ 1) = 0.935 + 0.099 * average\ depth\ (m) + 0.292 * \sqrt{sampling\ depth\ (m) - 0.579 * log10(color\,\frac{mgPt}{l})}$$

$$PICM\ (expected\ 2) = 1.001 + 0.459 * \sqrt{sampling\ depth\ (m) - 0.699 * log10(color\,\frac{mgPt}{l})}$$

where PICM observed/PICM expected explains the ecological status. PICM (expected 1) is chosen when the average depth of the lake is known and PICM (expected 2) is used when there is no information about the average depth. Classification for the grouping is explained in the Table 3.

2.4 The effects of metal bioavailability and environmental factors on observed toxicity

The effects of metal bioavailability on toxicity were tested with linear regression (IBM SPSS Statistics 21). Metal bioavailability in the sediment compartment was evaluated in our earlier study with the SEM-AVS approach. Bioavailability in pore water and overlaying water was evaluated with a user-friendly BLM (biotic ligand model) Bio-met, version 2.3 (Väänänen et al., 2016). This BLM evaluates the bioavailability of Cu, Ni and Zn. The raw data of the bioavailability analyses are represented in the Supplementary material (S1). Normality of the data were tested by the Shapiro-Wilk test, and the correlation between toxicity responses and bioavailability by Spearman’s (non-parametric) correlation, since the normality requirements of the data were not met (IBM SPSS Statistics 21). The tested toxicity endpoints were growth of *L. variegatus,* locomotion and ventilation of *C. riparius,* growth of *L. stagnalis* and EC_{50} of *V. fischeri*.

Besides metal bioavailability, the effects of several environmental factors on observed toxicity were tested using correlation matrices. The data were not normally distributed and the non-parametric Spearman's correlation was used (IBM SPSS Statistics 21). The measured environmental parameters in the sediment phase were: ΣMetals, Fe/ΣMetals –ratio, Fe, pH, sediment dw% and the level of small particles in the sediment (clay content from laser diffraction, SGS Inspection services, Finland). For the pore waters, the tested environmental parameters were ΣMetals, Fe/ΣMetals –ratio, Fe, SO_4, pH, conductivity (Orp, YSI sonde), redox-potential, Mn and dissolved organic carbon (DOC).

3 RESULTS

3.1 Toxicity tests

3.1.1 ***Lumbriculus variegatus*** There was no effect on toxicity based on reproduction, but the growth was statistically significantly lower in natural sediments compared to the artificial reference sediment (p. < 0.001). The growth was lower in the natural reference sediment PJ than in polluted lakes KS, JS and SJ (*p*. <0.01). In addition, growth was lower in the least-polluted lake LJ than in the lakes KS and JS (*p*. <0.01). (Fig. 1). The toxicity in this species was not connected to metal concentrations in sediment or pore water. The fastest growth was seen in the most polluted Lake SJ and the slowest in the least-polluted Lake LJ. The mean number of organisms increased by 15–145% during the experiments. In the AFS (reference sediment), the number of worms increased 1.5-fold, which is slightly less than recommended in the guideline (1.8-fold). Oxygen saturation levels stayed within the required levels throughout the experiments. Overall, the lowest growth and reproduction rates were observed on the least-polluted Lake LJ, where the sediment pH was the lowest (4.53) at the end of the experiment.

3.1.2 ***Vibrio fischeri*** There was clear toxicity on the all the studied lakes in the bulk sediment test, but no toxicity was observed in the reference lakes PJ and Hy. The inhibition of bioluminescence was highest in Lake SJ (EC_{50} 0.07 % of the sediment), followed by LJ (0.17%), JS (0.64 %), and KS (0.98 %). Pore waters did not inhibit bioluminescence in any of the treatments. EC_{50} values are presented in the supplementary material (S2).

3.1.3 ***Chironomus riparius*** The locomotion was more common than the ventilation (Fig. 2). However, the larvae were most of the time inactive. The treatments (sediment) or origin of the larvae did not induce significant changes in larval locomotion (sediment *p*. 0.89; egg mass p. 0.49; interaction *p*. 0.36) or in ventilation (sediment *p*. 0.98; egg mass *p*. 0.68, interaction *p*. 0.31). No changes in temperature, pH or oxygen content were seen during the 5-day exposure (e.g., in the sediment KS; 19.5 °, O_2 90% on average).

3.1.4 ***Lymnaea stagnalis*** All the individuals in KS, JS and LJ sediments were found dead between the second and the fifth day of the experiment (Fig. 3). In addition, loss of snails were observed in random experimental units either due to organisms escaping from the open experimental dishes or they being degraded into the sediment. All the snails stayed alive in the SJ sediment. There was a clearly significant difference between the sediments (ANOVA*; p*. 0.000). The mean dw were significantly higher in the reference PaJ than in the reference PJ (Tukey HSD; *p*. 0.000) and in the SJ (*p*. 0.002). The snails in the reference sediments PaJ and AFS did not differ from one another (*p*. 0.285), and they were larger than the ones in the metal-contaminated sediment SJ. However, in the third reference sediment (PJ), the snails were lighter than in the metal-polluted SJ sediment (*p*. 0.014).

3.2 Metal body residues and benthic organism community structure analysis

The metal concentrations in the field-collected chironomids were in the same range as in the fish muscle tissue (Fig. 4). The body residues in chironomids were the highest for Zn (4.5–27 mg/kg fw) and the lowest for Ni (0–1 mg/kg fw) and As (0.1–0.7 mg/kg fw) (Fig. 4, Supplementary material S3). The metal concentrations in the biota were larger in the lakes with highest metal sediment concentrations. However the variation in the chironomid body residues was small (less than five-fold), despite the large differences of the sediment meal concentrations at different sites.

The correlations of metal concentrations between different environmental phases were the clearest for Ni. Ni concentrations in sediment correlated with concentrations in pore water (*r*. 0.93, *p*. 0.02) and in overlying water (*r*.

0.98, *p*. 0.005), and there was a positive correlation between Ni concentration in pore water and overlying water (*r*. 0.98, *p*. 0.002). A correlation between environmental phases and biota body residues were found for Ni (overlying water vs. chironomus *r*. 1, *p*. 0.007) and Zn (overlying water vs. chironomus *r*. 0.954, *p*. 0.05, and overlying water vs. perch *r*. 0.99, *p*. 0.01). Pore water concentrations did not correlate with metal body residues in biota for any of the metal species (Supplementary material S4).

In the benthic invertebrate community structure analysis, the total number of organisms and the abundancy of specimen were the smallest in Lake SJ, which had the highest metal contamination (Table 3). In general, the ecological quality based on PICM followed the metal contamination level. For oligochaete/chironomid –ratio, the indices were low in all of the lakes, even in the reference Lake PJ. When deviation from the reference lake was included for the evaluation, there was a clear difference between the lakes, following the metal contamination levels.

3.3 The effects of bioavailability and environmental factors on toxicity

The toxicity of metals was not dependent on metal bioavailability in the sediment (SEM-AVS), measured by linear regression (Table 3). In pore waters, the bioavailable metal concentrations did not generally affect the observed toxicity (Table 4). However, the bioavailable Zn increased the growth of *L. variegatus* (R^2 0.93, β-coefficient 38.87, *p*. 0.01), which could be due to the role of Zn as a micronutrient. Cu and Ni bioavailability were not normally distributed (Shapiro-Wilk *p*. 0.07 and 0.013) and the linear regression could not be performed. The $\Sigma Ni,Zn,Cu_{bioavailable}$ was normally distributed and statistically significant positive effects were found for *L. variegatus* growth (R^2 0.87, β-coefficient 31.96, *p*. 0.02).

L. variegatus growth increased with increasing Fe and DOC concentrations in pore water, (ρ 0.9, *p*. 0.037 for both) (Supplementary material S5). Redox potential had a negative correlation with *V. fischeri* bioluminescence (ρ -0.9, *p*. 0.037). No correlation with other toxicity tests were found. In the sediment phase, ΣMetals and Fe correlated positively with *C. riparius* locomotion and ventilation (ρ 1, *p*. < 0.01 and ρ 0.9, *p*. 0.037, respectively) and negatively with *V. fischeri* bioluminescence (ρ -0.9, *p*. 0.037).

4 DISCUSSION

The observed ecological risk in these mining-impacted lakes was dependent of the used method (Table 5). Our earlier study (Väänänen et al., 2016) gave us reason to expect hazardous impacts of metal mining on the recipient waterbodies. In this study, the results from toxicity tests, chemical analyses, biota analyses and bioavailability assessment were not consistent. The selection of toxicity tests within this study showed only minor toxicity in these lakes. There was no clear connection between metal concentrations and toxicity to benthic invertebrates. The Zn and Ni concentrations in overlying water correlated with body residues in biota, but no correlations were found for As and Cu. The correlations for Ni can be explained by its exposure route, which is mainly pore water, whereas majority of Zn is ingested from sediment particles (Camusso et al. 2012). Pore water concentrations alone could not predict the bioaccumulation of metals and, therefore, extra precautions should be taken, if pore waters are used instead of whole sediment in metal toxicity tests. This disadvantage of lacking all the exposure routes, when using pore water to test sediment toxicity, is also stated in a review article by Chapman et al. (2002). Another disadvantage is the lack of environmental relevance, since the pore water extraction methods can alter metal speciation and bioavailability. Because of these problems, Chapman et al. (2002) encourage the use of pore water testing in ecological risk assessment only in the cases, when a conceptual diagram of the conditions can be built. As in other waters, DOC and cations are the main components controlling metal bioavailability and hence, the chemical information about the lakes should be analyzed thoroughly, if pore waters are used.

The observed toxicity in the ***L. variegatus*** experiments was low, but overall, reduced growth was observed in all of the natural sediments, even in the reference sediments, when compared to artificial sediment. Interestingly, Lake LJ sediment, with the lowest total concentrations of metals, seemed to be the most harmful sediment to *L. variegatus*. Lake LJ had the lowest Fe/total metal ratio of the studied lakes, which has previously been associated with toxicity (Sardo and Soares, 2010). Fe and Mn oxide precipitates tend to adsorb on other metals and reduce their bioavailability (Dong et al., 2000). In addition, Lake LJ has the lowest DOC content and the highest redox-potential of the studied lakes (Väänänen et al., 2016), and the high redox-potential has seen to be related with the release of metals into the surrounding solutions (Miao et al., 2006). The reference lake PJ was among the most unsuitable sediments for *L. variegatus*. Lake PJ had few distinct characteristics: It has the lowest percentage of small particles, which is connected to increased metal bioavailability in the sediments. Small particles (clay and silt) can absorb more metals as they have a high surface-to-volume ratio (Bentivegna et al., 2004). Thus, several environmental factors affect toxicity along with metal concentrations.

The great difference between the observed pore water and whole sediment toxicity in the ***V. fischeri*** test leave ample room for speculation. Adjusting the pH to an optimal range of the luminescence bacteria (6–7.5) affects metal speciation. In addition, the filtration of pore water from sediment could lead to a situation where a part of the bioavailable metal fraction would remain loosely bound to sediment particles and, therefore, would not end up in the water sample. In addition, metal bioavailability is altered by changing the sodium concentrations in the extracts according to the requirements of *V. fischeri* (2% NaCl), which also hampers the test usability of the test for assessing metal toxicity. This study confirms the existing concerns about using the luminescence bacteria *V. fischeri* in metal toxicity assessment (Besser et al., 2015). In the earlier binary metal toxicity studies with luminescence bacteria, Fulladosa et al. (2005) observed EC_{50} values for Cu to be 0.35 mg/l and for Zn 0.86 mg/l. The pore water concentrations of Cu and Zn in our study are several magnitudes lower from those. The disadvantages in using bioluminescence bacteria for metal toxicity assessment could be overcome by using metalloid-specific microbial sensors or lux-marked bioassays (Ritchie et al., 2001, Tauriainen et al., 2000).

The sediments were not toxic to ***C. riparius*** and the organisms were rather inactive throughout the experiment in all the treatments. However, we do not have water quality information from the sediment-water interface, because we wanted to rule out disturbance-caused avoidance or escapes in the test system. Similar test setup (MFB) has been recently tested for metal toxicity on *L. variegatus*, with promising results (Wallin et al. 2018). In future, these novel toxicity tests, potentially more sensitive indicators of environmental stress, can be a valuable addition to the traditional ecotoxicological testing for the purposes of the risk assessment. In the ***L. stagnalis*** test, mortality occurred in the test vessels with the lowest pH, and it was probably the major stress factor in the sediments. *L. stagnalis* is a common species in Holarctic, temperate waters Berrie 1965). In Finland, it inhabits circumneutral fresh- and brackish waters. The acidity of the overlying water and the associated metal contaminated sediment was most likely a detrimental combination for juvenile snails in the KS, JS and LJ treatments. The pH of the reference sediment PJ was also outside the normal tolerable conditions for the snails. Growth of *L. stagnalis* seems to be a suitable endpoint for sediment toxicity testing, but it should be ensured that the water quality conforms to the requirements of the species.

Overall, achieving the optimal conditions required by the toxicity tests guidelines (e.g., water salinity, pH, oxygen saturation, and ammonium) is a complicated process when conducting metal toxicity studies in natural sediments. In our studies, drastically decreasing pH proved to be a problem in most of the test methods. The optimal conditions for the standard test species are circumneutral (ISO 2000, ISO 2010, OECD 2004, OECD 2007). Similar, decreasing pH in metal-contaminated sediment toxicity tests *(L. variegatus*) has been observed by Wallin et al. (2018). They concluded that low pH is partly a reason for the adverse effects in the metal-contaminated sediments. It has been also observed that snails have more severe responses to metal pollution, if the pH in the test systems varies from their natural pH level (Lefcort et al. 2015). For *C. rparius*, one study shows pH to be the main parameter explaining variation in chironomid larval development in an *in situ* -research of metal contamination (Faria et al. 2006). In our study, choosing the appropriate toxicity testing methods was a challenge: some of the organisms are sensitive to changes in pH (*C. riparius, L. stagnalis*) or salinity (*V. fischeri*). In addition, some of the species are relatively tolerant of metal pollution [*L. variegatus, C. riparius* (Leonard and Wood, 2013)]. However, these challenges partly represent the real situation in the nature. Mining-affected aquatic ecosystems are often acidic and hence, one developmental challenge is to create and standardize toxicity test methods that are suitable for toxicity testing in these kinds of conditions. In addition, high sulfide concentrations are common in mining-affected sediments. Since acid-volatile sulfides (AVS) are the main components binding metals in anaerobic sediments (De Jonge et al. 2012), oxidation will lead to a situation, where sulfide oxidizes to sulfate releasing metals and hydrochloric acid. This will not only increase metal bioavailability, but it will also lower the pH. The studies have shown benthic invertebrates acclimating to pH changes, which have been connected to changes in metal uptake rates (Andre et al., 2010, Bervoets and Blust, 2000). Therefore, the use of laboratory-cultured organisms in metal toxicity tests may be more sensitive to low pH than the local species living in naturally acid environments.

The **metal body residues** in benthic organisms and fishes collected from the studied lakes were at similar levels between the lakes. There was a correlation between metal concentrations in the overlaying water (Ni, Zn) and the concentrations in field-collected chironomids. Even then, the metal body residues were low even in the lakes with the highest metal loading. Tania et al. (2012) have connected whole-body Cu residues in *L. variegatus* to its mortality. In their study, the critical Cu whole-body residue (CBR) associated with 50% mortality (CBR_{50}) was 38.2—55.6 µg/g fw (fresh weight) and it was not dependent on water hardness or exposure time. They were also able to use CBR_{50} in *L. variegatus* for predicting mortality of *C. riparius* (29.1-45.7 µg/g fw in hard waters).

In our study, the body concentrations in *C. riparius* remained well below that level, being 1.3-4.1 µg/g fw. Leonard and Wood (2013) observed Ni CBR_{50} for *L. variegatus* to be 117.5 µmol/kg fw (6.9 mgkg) in soft waters. Again, the body residues in our chironomid samples stayed below that (0.09–1.11 mg/kg fw). The Ni concentrations in fish muscle tissues (up to 0.03 mg/kg) seem not to be high enough to cause negative effects in higher trophic levels, based on the regional predicted effect of dietary concentration threshold for freshwater birds eating fishes (PEC_{oral}) is 0.2 mg/kg (European Commission, 2009). For Zn, the population level effects (decreased carrying capacity) in chironomids are observed with tissue concentrations above 350 mg/kg dw in natural sediments (Péry et al., 2007). This level is two-fold higher when compared to the levels measured in the field-collected chironomids in this study (100–180 mg/kg dw). For As, Bervoets et al. (2016) have recently achieved critical body burdens to Chironomus sp., which were 65–130 µg Zn/g dw, which are more than ten times higher than in our samples (1.2–6.4 µg/g dw). In addition, the maximum allowable Zn concentrations in water are set to150 µg/l, when the aim is to protect freshwater life. (U.S. EPA, 2006). The pore water concentrations in this study were less than 10 µg/l (Väänänen et al., 2016). There has also been discussion, if the pooling of different chironomus species is advisable when analyzing trace metal in the chironomids. Prolux et al. (2019) studied, if the pooling of chironomid species gives reason to worries. In their study, the body residues of As, Ba, Co,Cu, Mn and Ni were constant between the species, but some differences were observed with Cd, Se and Zn. Even still, the differences for Se and Zn were minor, being generally less than 2-fold. Based on that study, our chosen method for analyzing pooled chironomid samples for metal body residues is appropriate.

The ecological status of the study lakes was poor, especially in the most polluted lakes SJ and KS. The profundal invertebrate community metrics evaluated the ecological status to be higher than the oligochaete-chironomus ratio. In both methods, the ecological status followed the metal concentrations in the lakes. Since the community diversity is already at a poor level, any additional stress, such as metal loading could cause severe deterioration of these lake ecosystems. According to a report by The Finnish Environment Institute (2012). The benthic organism structure is traditionally assessed based on the status of chironomids populations. We used a wider range of macroinvertebrate species (46) living in lake basins, which is expected to increase the sensitivity of the study. In general, the reliability of profundal benthic organism structure analysis as a biomarker for metal contamination is better in deeper lakes and that small, shallow lakes may naturally have low species diversities (The Finnish Environment Institute, 2012). Benthic community structure analysis is an essential part for evaluating, if the studied sediments are polluted, resulting in real-life impacts to populations (Chapman 2007).

Analysis of benthic invertebrates adds environmental relevance to the study, when conducted parallel with the chemical analyses and ecotoxicological tests. Chapman (2007) suggests that when there is chemical contamination and benthos alteration – but not laboratory toxicity – a possible conclusion is that contaminants in the environment are not bioavailable. In our previous study (Väänänen et al. 2016) metals were more bioavailable in water than in sediment. If natural water from the sampling site would be used in toxicity tests instead of artificial fresh water, they would give results that are more reliable. Now it is possible that toxic responses are not observed, since they would have not ben induced only from the sediment, but also from the overlying water. This conclusion is supported by our earlier study (Väänänen et al.), where the metals in these lakes were more bioavailable in the overlying waters than in the sediments.

Based on this study, metal bioavailability is not a determining factor for metal toxicity in these lakes. Nevertheless, there was a strong correlation between various water quality parameters. For routine monitoring, some of the parameters could maybe be omittedto save resources. For example, SO_4 correlated with redox-potential (+), Mn (-), pH (-) and Fe/ΣMetals (+) and it would be worth of studying, if only one of these parameters could be used in standard monitoring. The implementation of Fe concentrations in ecological risk assessment has been discussed by Hope et al. (2015), since high Fe concentrations are abundant in Fennoscandian freshwaters and they have been seen to effect on the bioavailability of metals. These Fennoscandian waters also share other characteristics: they often suffer from the lack of oxygen and low pH during the late winter (Väänänen et al., 2016), and they are remarkably softer than the average waters in Europe (Hooppe et al. 2015, Väänänen et al. 2016). These conditions may induce adverse effects in the ecosystem level that are not observed in the laboratory studies, conducted under controlled conditions.

5 CONCLUSIONS

Based on microbenthic communities, the ecological status was worse in the lakes with higher metal contamination. Similar results were seen in the evaluation of metal body residues, and in our earlier study based on metal concentrations and bioavailability (Väänänen et al. 2016). However, toxicity tests gave mixed results, sometimes even contradictory to other methods. Based on toxicity tests, the most harmful sediments were those, where pH was the lowest – even if the metal concentrations were low. Metal bioavailability in the sediments or in the pore waters did not itself correlate with metal toxicity. However, several environmental quality parameters behind metal bioavailability (DOC, clay content and Fe/Σmetal –ratio, pH during the toxicity tests) were connected to the toxicity.

One of the important finding of this study is that standard sediment toxicity tests were not suitable for evaluating the toxicity of these mining-affected freshwater sediments. Drastically decreasing pH was problematic with *L. stagnalis* and *C. riparius* standard tests. In these kinds of situations, we believe chemical methods and benthic community structure analyses to give results that are more reliable. There are also other challenges with toxicity tests; some species (*L. variegatus*), are tolerant to metal toxicity and in others (*V. fischeri*), the salinity adjustment may affect the metal speciation. The locally relevant snail species (*L. stagnalis*) could be used in the future for sediment toxicity testing in the cases, where pH is circumneutral. However, the low pH is typical for mining-affected lake ecosystems and these problems could also arise in other studies

It is advisable to use several methods when evaluating toxicity of the sediments affected by metal mining. In addition to traditional SQT approach, metal bioavailability studies reveal important information on the contamination. For metal body residues, more studies are needed before they can be routinely used in ecological risk assessment.

Competing interests

The authors declare no competing interests.

Contributions

KV carried out the tests with *V. fischeri* and partly with *L. variegatus.* KV also conducted all the analyses and had the main responsibility of the manuscript writing. KV and JA planned the study. HA, AK and MTL conducted the tests and analyses with *C. riparius.* MR and MTL conducted the *L. stagnalis* tests. MTL was responsible for the interpretation of the *C. riparius* and *L. stagnalis* experiments and wrote the relevant parts of the manuscript. SA and TO ran the experiments with *L. variegatus*. IN participated in data interpretation, statistical analyses and creating the figures. All the authors reviewed the manuscript and approved the final article.

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LIST OF FIGURES

Fig. 1 a) Increase of *L. variegatus* individual biomass (%) during the toxicity test with metal-contaminated natural sediments and **b)** reproduction of *L. variegatus*, expressed as increase of worms (%). The mean pH at the end of the experiment is expressed as a horizontal dash line in the right-hand y-axis.

Fig 2 Percentage of time (+ standard error) *C. riparius* larvae spent in locomotion and ventilation during a 12-h measurement period in mining-influenced sediments (KS, JS, LJ, SJ) and in the reference sediments (Hy, AFS). n = 3, 3, 3, 4, 5, and 5, respectively.

Fig. 3 Effects of the metal-contaminated natural sediments (KS, JS, LJ, SJ) and the non-contaminated reference sediments (PJ, PaJ and AFS) to the growth of *Lymnaea stagnalis* (dry weight in µg). The mean pH values at the end of the experiment are expressed as a horizontal dash line in the right-hand y-axis. Mortality was 100% in the KS, JS and LJ sediments.

Fig. 4 Concentrations of Cu, Ni, Zn and As in water (mg/l), sediment (mg/kg dw), chironomids (mg/kg fw) and fish (*Esox Lucius*, pike and *Perca fluviatilis*, perch, mg/kg fw). Logarithmic scale is used.

Table 1. Basic information of the study lakes. Detailed water characteristics data, including concentrations for all the elements in different phases (sediment, pore water, overlying water) is presented earlier by Väänänen et al. (2016).

Lake	**Abbreviation**	**Location** (WGS84)	**Size** (km^2)	**Max. Depth** (m)	**As**[1] mg/kg	**Cu**[1] mg/kg	**Ni**[1] mg/kg	**Zn**[1] mg/kg
Kirkkoselkä	KS	63 38.769, 26 00.170	32.2	13	20.9	264	30	885
Junttiselkä	JS	63 42.636, 25 58.019	5.7	8	12.3	167	24	369
Laakajärvi	LJ	63 49.891, 27 55.882	34.7	25	8.4	27	42	168
Sysmäjärvi	SJ	62 41.539, 29 02.519	6.87	6.1	52.6	77.9	204	863
Parkkimanjärvi	PJ (ref.)	63 42.636, 25 58.019	10	4.7	13.2	39	28	187
Höytiäinen	Hy (ref.)	62 41.769, 29 41.098	283	59	na	na	na	na
Palosjärvi	Paj (ref.)	62 03.533, 26 08.167	2.6	na	na	na	na	na

[1] Metal concentrations in the sediment, analyzed by IPC-MS (Väänänen et al. 2016).

Table 2. Summary of the used toxicity test methods and endpoinds, The methods include Sediment Quality Triad approach (toxicity tests, chemical analyses and community structure analyses), studies on metal body residues in field-collected biota and the evaluation of metal bioavailability.

Test method	Studied endpoint	
Toxicity tests		Sediment Quality Triad
Lumbriculus variegatus	Growth	
	Reproduction	
Vibrio fischeri	Bioluminescence (EC_{50})	
Chironomus riparius	Locomotion	
	Ventilation	
Lymnaea stagnalis	Growth	
	Mortality	
Chemical analyses (Väänänen et al. 2016)		
Metal concentrations (sediment, water)		
Environmental parameters (DOC, clay, redox)		
Community structure analyses		
Benthic invertebrate community structure	PICM[1]	
	Oligochaete/Chironomus -ratio	
Metal body residue		Supplementary studies
Cu, Ni, Zn in fish and chironomids	$C_{chironomid\ (ICP\text{-}MS)}$	
	$C_{fish(perch,\ pike,\ ICP\text{-}MS/ICP\text{-}OES)}$	
Bioavailability (Väänänen et al. 2016)		
Bioavailability vs. toxicity in sediment	SEM-AVS	
Bioavailability vs. toxicity in pore water	Biotic ligand model	

[1] Profundal invertebrate community metric index (The Finnish Environment Institute, 2012).

Table 3. The assessment of ecological status based on benthic invertebrate community in the lakes. There is no benthic organism community structure data available for Lake JS.

	LAKE			
	KS	LJ	SJ	PJ (ref.)
Lake type	LLh[1]	Hh[2]	SHh[3]	SHh[3]
Samples (n)	6	6	5	6
Specimen/m^2, mean	608	421	286	197
Taxons	5	7	5	4
Total number or organisms in samples	81	73	33	34
Oligochaete/Chironomids ratio[4]	0	0.01	0	0.01
O/C bottom fauna index	*very low*	*very low*	*very low*	*very low*
Deviation from the control (Lake PJ)	*very high*	*very low*	*very high*	
Profundal Invertebrate Community Metrics (PICM)				
PICM[5] (Observed)	1.42	2.38	0.75	2.38
PICM (Expected)	2.19	2.35	1.67	1.73
Ecological quality ratio	0.65	1.01	0.45	1.38
PICM Ecologcal quality[6]	Passable	Good	Poor	Good

[1] Large lake with low humic acid content

[2] Lake with high humic acid content

[3] Shallow, humidic lake

[4] O/C ratio, normalized to sampling depth. For derivation calculations, zeros are replaced with 0.1x10^{-5} (Swedish Environmental Protection Agency 2000)

[5] Profundal Invertebrate Community Metric

[6] Ecological quality expressed as PICM_observed/PICM_expected ratio. > 0.8 good; 0.6-0.8 satisfactory; 0.4-0.6 passable; <4.0 poor.

Table 4. The effects of bioavailability in sediment and sediment pore water to observed metal toxicity. Linear regression is used for analysis (IBM SPSS Statistics 21). Metal concentrations, SEM-AVS data and bioavailable metal fractions based on biotic ligand model (BLM) are gathered from Väänänen et al. (2016).

Metal bioavailability in sediment and observed toxicity

SEM-AVS_foc[1] (metal bioavailability in anaerobic sediment)

Response	R^2	Coefficient	Sig.
L. variegatus growth	0.194	0.129	0.46
C. riparius locomotion	0.056	-0.019	0.70
C. riparius ventilation	0.377	-0.13	0.27
L. stagnalis growth	0.206	0.044	0.44
V. fischeri bioluminescence	0.016	0.028	0.84

Metal bioavailability in sediment pore water and observed toxicity

Σ(Zn, Ni, Cu), bioavailable[2]

Response	R^2	Coefficient	Sig.
***L. variegatus* growth**	**0.87**	**31.96**	**0.02**
C. riparius locomotion	0.61	7.29	0.12
C. riparius ventilation	0.19	1.05	0.46
L. stagnalis growth	0.25	5.66	0.39
V. fischeri bioluminescence	0.43	-17.52	0.23

Zn bioavailability[2]

Response	R^2	Coefficient	Sig.
***L. variegatus* growth**	**0.93**	**38.87**	**0.01**
C. riparius locomotion	0.47	7.57	0.20
C. riparius ventilation	0.10	0.89	0.61
L. stagnalis growth	0.49	6.51	0.40
V. fischeri bioluminescence	0.44	-20.78	0.22

[1] From Väänänen et al. (2016). SEM-AVS_foc calculations are found in Online Resource (1).
[2] Bioavailability data from Väänänen et al. (2016), calculated using Bio-met Biotic Ligand Model. The BLM calculations are found in Online Resource (2).

Table 5. Summary of the results in this study.

		Study lakes				Reference sediments			
		KS	JS	LJ	SJ	PJ[1]	PaJ[2]	Hy[2]	AFS[3]
Toxicity tests	*Lumbriculus variegatus (later on L.v.)*								
	Vibrio fischeri (sediment)								
	Vibrio fischeri (pore water)								
	Chironomus riparius								
	Lymnaea stagnalis								
Chemical analyses[4]	Metal concentrations (sediment, water)								
	Environmental parameters (DOC, clay, redox)								
Benthic community	PICM								
	Oligochaete/Chironomus -ratio								
Metal body residue	Metals in chironomid, pike, perch		**Cu**		**Ni**				
	Correlation environment and biota	Correlation $C_{overlying\ water}$ with Ni and Zn							
Metal bioavailability	Sediment	No effects to toxicity							
	Pore water	*L.v.* growth correlates to: DOC, Fe and Zn concentrations							

[1] Main natural refrence sediment for the studies

[2] Laboratory-specific additional reference sediments

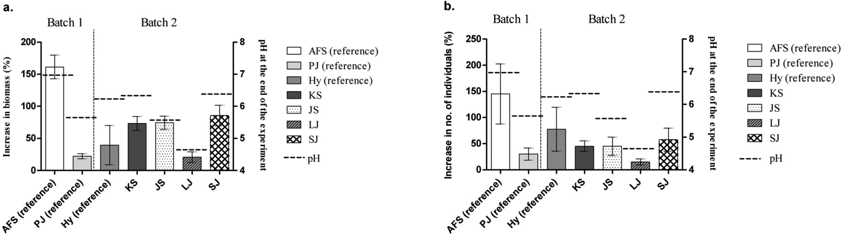
[3] Artificial freshwater sediment reference for toxicity tests

[4] Väänänen et al. (2016)

- No observed adverse effects
- Moderate adverse effects
- Observed adverse effects
- No data available

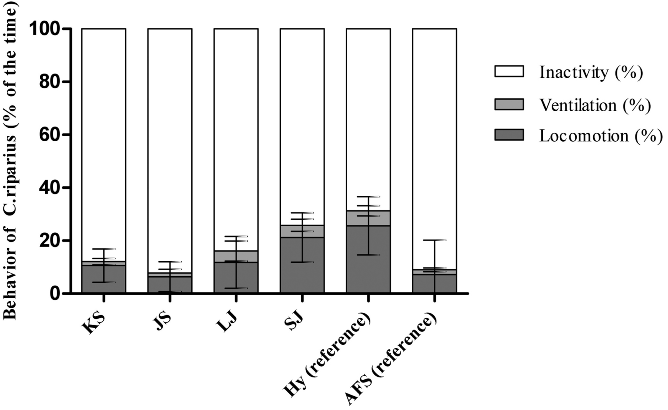
Highlights

- Ecological risk assessment of metal-contaminated boreal lakes and sediments.
- Sediment Quality Triad complemented with bioavailability and body residue studies.
- Toxicity studies for natural sediments with several organisms and endpoints
- Adverse effects observed. High variation on results between different methods.
- Standard toxicity tests not suitable for testing, too low pH hampers the results.



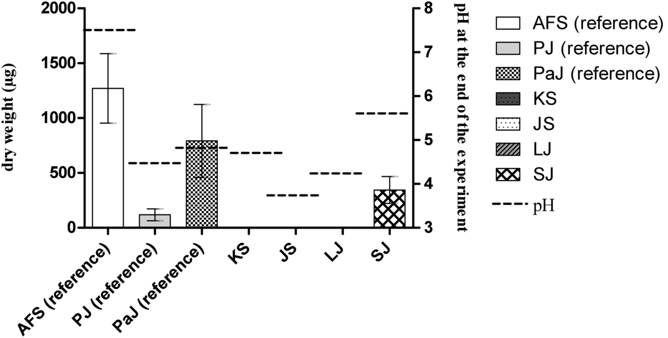
a.
Batch 1
Batch 2
Increase in biomass (%)
pH at the end of the experiment
AFS (reference)
PJ (reference)
Hy (reference)
KS
JS
LJ
SJ
pH
b.
Batch 1
Batch 2
Increase in no. of individuals (%)
pH at the end of the experiment
AFS (reference)
PJ (reference)
Hy (reference)
KS
JS
LJ
SJ
pH

Figure 1



Behavior of C.riparius (% of the time)
100
80
60
40
20
0
KS
JS
LJ
SJ
Hy (reference)
AFS (reference)
Inactivity (%)
Ventilation (%)
Locomotion (%)

Figure 2



dry weight (µg)
2000
1500
1000
500
0
pH at the end of the experiment
8
7
6
5
4
3
AFS (reference)
PJ (reference)
PaJ (reference)
KS
JS
LJ
SJ
pH
AFS (reference)
PJ (reference)
PaJ (reference)
KS
JS
LJ
SJ

Figure 3

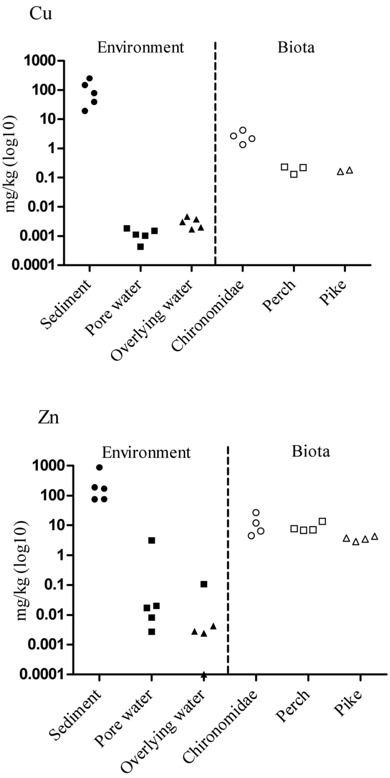

Cu
Environment
Biota
1000
100
10
1
0.1
0.01
0.001
0.0001
mg/kg (log10)
Sediment
Pore water
Overlying water
Chironomidae
Perch
Pike
Ni
Environment
Biota
1000
100
10
1
0.1
0.01
0.001
0.0001
mg/kg (log10)
Sediment
Pore water
Overlying water
Chironomidae
Perch
Pike
Zn
Environment
Biota
1000
100
10
1
0.1
0.01
0.001
0.0001
mg/kg (log10)
Sediment
Pore water
Overlying water
Chironomidae
Perch
Pike
As
Environment
Biota
1000
100
10
1
0.1
0.01
0.001
0.0001
mg/kg (log10)
Sediment
Pore water
Overlying water
Chironomidae
Perch
Pike

Figure 4