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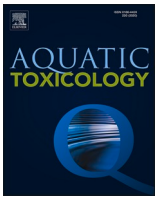

Paleoecotoxicology: Developing methods to assess the toxicity of lake sediment records influenced by legacy gold mining

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ABSTRACT

The contamination of lakes by industrial emissions is an issue of international concern. Traditional paleolimnology examines sedimentary micro-fossils to infer the biological response to natural and anthropogenic stressors over time. Here, we calculate a theoretical biological effect for historic sediment sections using Probable Effect Concentration Quotient (PEC-Q) and arsenic specific quotient methods and develop novel time-constrained sediment toxicity test methods using a cultured *Daphnia* sp. combined with a whole cell microbial biosensor to assess the toxicity of past industrial contamination with modern testing methods. These methods were developed using sediments collected from Pocket Lake (Northwest Territories, Canada), a lake known to have exhibited a significant ecological shift following input from nearby gold smelter emissions during the mid 20th century. We then applied these methods to near-, mid-, and far-field sites to assess the response of *Daphnia* sp. to varying contaminant load. *Daphnia* sp. mortality exposed to dated sediments indicated a strong concordance with the timing of mining activities, and a strong concordance with PEC-Q and arsenic specific toxicity quotients. In contrast, a decrease in *Daphnia* mortality was observed during pre-, and post-mining periods when the contaminant burden was lower. Initial assessments of bioavailability using a microbial biosensor indicated that arsenic in porewater is 72–96% bioavailable, and limited evidence that oxidative stress may contribute to the *Daphnia* sp. toxic response. These results indicate that lake sediment archives can be used to infer missing biomonitoring data in sites of legacy anthropogenic influence, which will be useful for those seeking to conduct cost-effective and efficient preliminary environmental risk assessments.

1. Introduction

Biomonitoring allows scientists to create baseline ecosystem health data, and potentially observe shifts in the health of an ecosystem while they are occurring (Buss et al., 2015; Chapman et al., 1996). These data are essential to track environmental damage or to provide endpoints for remediation following contaminant deposition into an affected area (Lari et al., 2017; Nikinmaa, 2014). Biomonitoring became prevalent in North America following the implementation of the Clean Water Act of 1972 by the U.S. Environmental Protection Agency. However, uncontrolled industrial emissions at many sites of legacy contamination occurred before the 1970s, which has resulted in many contaminated sites with little biomonitoring data available to provide local benchmark information to remediation specialists (Kostarelos et al., 2015).

In an effort to establish regional ecological baselines in areas without biomonitoring data, methods to observe historic aquatic population shifts in modern times have been made through the evolution of paleolimnology (Haworth et al., 1984). Preserved remains of organisms are used to determine shifts in the population structure of dated lake sediments and comparing these shifts to chemical contaminant signatures can provide insight into possible causes of ecosystem population level disturbances. These methods require detailed taxonomic knowledge, and do not provide mechanistic data as to the cause of observed population level shifts. Paleoecotoxciology is emerging in contaminant research to marry classic paleolimnology with standard toxicity exposure tests to simulate reconstructed historic toxicity at sites of legacy contamination (Korosi et al., 2017). Paleoecotoxicology aims to provide a regional baseline of environmental conditions in impacted areas, where biomonitoring data are scarce or unavailable. This technique provides the unique opportunity to develop methods that can determine

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causative mechanisms that result in population level disturbances based on the record of preserved remains.

Sediments are critical to the lake ecosystem. They serve as both a source and a sink for contaminants into the overlying waterbody and provide essential habitat and refuge from predators for many organisms. Assessment of sedimentary health before industrial development is crucial to understanding baseline ecosystem health. Several sedimentary toxicity exposure assessments are regulated, including sedimentary exposure to amphipods such as *Hylella azteca* (Siegler et al., 2015) or chironomids (Allen Burton et al., 1996) (*Chironomus riparius*). These tests use large volumes (>100 g) of surface sediment per treatment and are therefore not suited to the testing of small quantities of dated lake sediments (Environment Canada, 1994). To our knowledge, no standardized solid-phase sediment toxicity test methods currently exist that can evaluate the toxicity of small volumes of dated lake sediments.

Here we assessed the toxicity of past contamination from a gold mine to lake sediments using dated sediment cores. Specifically, we used a paleoecotoxicology approach (Korosi et al., 2017) to track historical changes in sediment toxicity. This approach combines chemical and toxicity measurements in dated lake sediment cores to assess how sediment toxicity changed historically in relation to the timing and magnitude of industrial contamination. We chose Pocket Lake as one of our study sites, a lake within 1 km of the Giant Mine roaster stack in Yellowknife, Northwest Territories Canada. We assessed oxidative stress as a possible mechanism for sediment toxicity, and we used a microbial bioreporter to assess the bioavailability of arsenic released from the sedimentary matrix. Arsenic has been a focus for toxicity studies at Giant Mine due to the very high arsenic emissions from the roaster stack (e.g. Jamieson 2014). Due to the potential for human and ecological health risks, the concentration (Galloway et al., 2012; Houben et al., 2016; Palmer et al., 2015), behavior (Andrade et al., 2010; Palmer et al., 2019; Schuh et al., 2019, 2018; Van Den Berghe et al., 2018), and biological effects ((Gavel et al., 2018; Persaud et al., 2020; Sivarajah et al., 2020, 2019; Thienpont et al., 2016) of legacy arsenic contamination in lake surface water and surface sediment has been a research focus in recent years in the Yellowknife region. Further, elevated concentrations of other potentially toxic elements, including lead, cadmium, manganese, mercury and zinc have been observed in sediment and peat records (Cheney et al., 2020; Pelletier et al., 2021; Thienpont et al., 2016). This study develops new tools to assess the history of toxicity from a known contamination source as a way to hindcast the history of toxicity decades after the contamination occurred.

2. Methods

2.1. Sampling location and sediment cores

Lake sediment cores were collected from 4 lakes in the prevailing wind direction (Galloway et al., 2018) within a 40 km radius of Yellowknife (Fig. 1). Arsenic concentrations through time are indicated for each lake (Fig. 1). Initial methods were developed using sediments collected from a lake that has been highly impacted from arsenic-bearing mine emissions, Pocket Lake. Pocket Lake is a small lake ~ 1 km from the Giant Mine roaster stack, and considered to be "ground zero" for emission contaminants in the region due to its severe contamination (Thienpont et al., 2016) (Fig. 1B).

Ore processing procedures at Giant Mine (1948–1999), and to a lesser extent Con Mine (1938–2003), released contaminants into the environment, consistent with the rock formations associated with the mined ore. Much research in the area has focused on arsenic released from Giant Mine, as over 20,000 tonnes of arsenic trioxide was emitted by the roaster, and consequently deposited on the landscape, throughout the lifetime of the mine (Jamieson, 2014). In addition to arsenic, other metal(loid) contaminants (antimony, lead, zinc, copper, chromium) were emitted at a lesser extent and deposited aerially to the lake surface and sequestered in sediments. Resultantly, the sediment archives are reflective of the time-constrained and sediment associated contaminant mixtures from the roaster at Giant Mine (Cheney et al., 2020; Galloway et al., 2012).

The extent of metal(loid) contamination in lakes within 50 km of the historic Giant Mine has been characterized in recent years. Using the known extent of contamination, near-field (YK-42), mid-field (YKC-1), and far-field (YKW-1) sites were selected from cores documented by Cheney et al. (2020) (Fig 1). Full sampling details are provided in Cheney et al. (2020). Briefly, cores were extruded in 0.5 cm intervals, freeze dried, dated using radiometric methods, and analyzed for total metal(loid) concentration. Dated sediments were classified into pre-mining (pre-1948), during mining (1948–1999), or post-mining

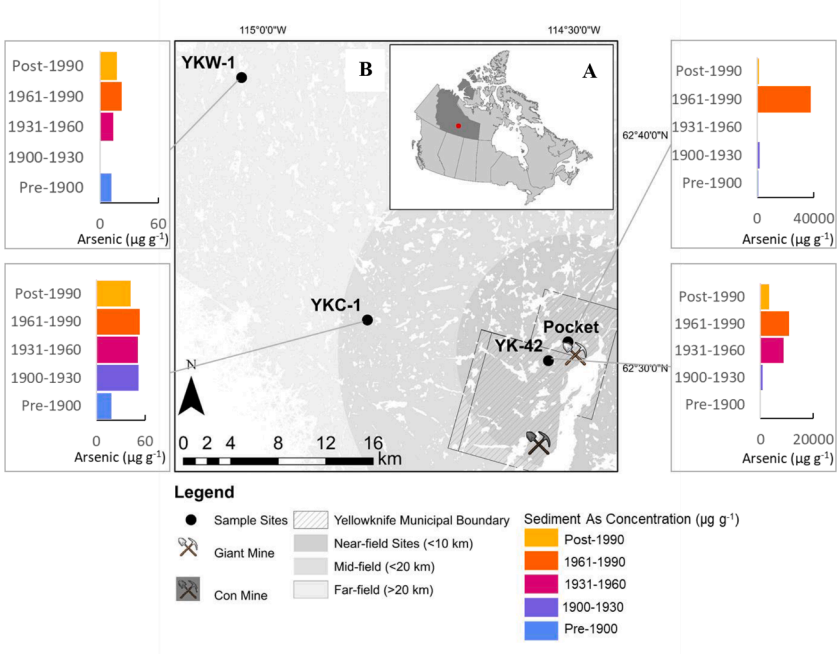

Fig. 1. (A) The location of the study site within Canada. (B) The municipal border of the City of Yellowknife, Giant Mine, and Con Mine. The mean sedimentary arsenic concentration for each lake is plotted on the x-axis, and each time grouping is plotted on the y-axis according to the mean CRS date determined by ^{210}Pb gamma spectrometry. Note the varying scale for arsenic concentrations in each plot. The radius from the Giant Mine roaster stack is indicated by graduated gray colouring indicating near-, mid-, and far-field sites.

(post-1999) for analysis purposes. Full dating profiles are provided in (Cheney et al., 2020). We refer to intervals in this paper by their midpoint, so if we refer to the 8.75 cm interval, we are speaking of the 8.5–9.0 cm interval in the sediment core.

2.2. Estimating sedimentary risk

A screening level risk assessment was performed on select time-constrained sediments prior to further analysis using the Probable Effect Concentration Quotient (PEC-Q) method (Macdonald et al., 2000). The PEC-Q estimates sediment toxicity based on concentrations of elements (arsenic, cadmium, chromium, copper, lead, nickel and zinc) relative to their probable effect concentrations (Cheney et al., 2020) using Eq. (1) and data from Table S2:

$$PEC - Q = \sum_{m=1}^{n}\left(\frac{Ms}{PECm}\right) \text{ x } \frac{1}{n} \quad (1)$$

where *Ms* is the metal(loid) concentration in sediment, *PECm* is the probable effect concentration of each metal(loid) *m* (values provided in Cheney et al., 2020 supplemental information), and *n* is the number of metal(loids) in the summation. Interpretation of the PEC-Q follows the guidelines outlined by Rose et al. (2018), with PEC-Q values >0.5 indicating biological effects possible and PEC-Q values >2.0 indicating biological effects probable.

As arsenic is the main contaminant of concern in the region, the risk of arsenic alone to aquatic biota was also calculated as the Probable Effect from arsenic (PE_{As}) using Eq. (2).

$$PE_{As} = \frac{[As]}{PEC_{As}} \quad (2)$$

Here, PE_{As} is a measurement of the probable effect to aquatic biota due to arsenic, [As] is the arsenic concentration, and PEC_{As} is the consensus based probable effect concentration (PEC) of arsenic (33.0 mg/kg dw) presented by Macdonald et al. (2000).

2.3. Daphnia cultures

Four *Daphnia* were collected from BC-36, a lake located 22 km east of Giant Mine, in July 2016 using a 63 μm mech size plankton net from a helicopter pontoon, and transported to the University of Ottawa (See S1 for taxonomic details). Chemical parameters of BC-36 are included in supplemental table S1. The *Daphnia* were identified to genus level using a dissecting microscope at the University of Ottawa within 48 h of collection, and were cultured in separate 1000 mL jars for two weeks. One of the jars was chosen at random to continue the *Daphnia* line, and the other three jars were discarded. The selected line of *Daphnia* was cultured in a 16:8 h light cycle at 20ºC at the University of Ottawa in 1 L glass containers. Culturing protocols were adapted from the Ontario Ministry of the Environment and Climate Change's (OMECC) Standard Operating Procedure (SOP) for *Daphnia magna* culturing (Ministry of the Environment and Climate Change, 2014a). Animals were fed a mixture of *Raphidocelis subcaptata* (formerly *Pseudokirchneriella*) and *Chlorella fusca* initially obtained from Environment and Climate Change Canada. Algae was cultured in accordance with OMECC Standard Operating Procedures (Ministry of the Environment and Climate Change, 2014b). Mixed Algae Culture (MAC) water was changed daily with aerated dechloraminated municipal water. Genetic information for the mono-culture of *Daphnia* collected from BC-36 was obtained from the Canadian center for DNA Barcoding (CCDB) at the University of Guelph. In brief, a single specimen collected at random from the *Daphnia* culture was photographed in the CCDB imaging center prior to analysis (Fig. S1). The DNA was then isolated from the provided specimen, and specific sections of the mitochondrial DNA was amplified using a Polymerase Chain Reaction (PCR) performed with full and short length barcode primer cocktails. Sequencing reactions were analyzed by high-voltage capillary electrophoresis, and the resulting DNA sequences were compared to species in the Barcode of Life Data System (BOLD).

2.4. Daphnia exposure

The cultured *Daphnia* sp. were exposed to radiometrically dated (time constrained) lake sediments at the University of Ottawa in sediment-water co-existence systems (Li et al., 2017). For exposure, time-constrained sediment was added to a falcon tube to a final sediment mass of 2.5 g and 10 mL of dechloroaminated municipal water. This results in a 4:1 water to sediment ratio, and follows established standard protocols for sedimentary toxicity tests (Environment Canada, 1994). Three replicate falcon tubes were used for each sediment exposure interval. Tubes were agitated and centrifuged at 2000 rpm for 2 min. This step ensured the sediment was fully settled at the bottom of the tube prior to the exposure. The prepared exposure tubes rested at 20 ºC for 24 h to allow time for the sediment and water to equilibrate. Following the equilibration, 10 *Daphnia* were added to each exposure tube to create a *Daphnia* exposure ratio of 1 mL of water per daphnid. Less than 24hr old neonates were used for testing, to ensure molting had not yet occurred (Barata et al., 2005). The Daphnia were exposed in the sediment-water co-existence system (Li et al., 2017) for 24 h. Following the exposure, the overlying water was removed to a separate falcon tube to be processed within 8 h after isolation and subsequently analyzed for arsenic concentration by inductively coupled plasms-mass spectrometry (ICP-MS) analysis, and by a microbial biosensor. The *Daphnids* extracted from the sediment-water coexistence system were assessed for mortality and were stored in 2.0 mL centrifuge tubes on ice until prepared for Thiobarbituric acid reactive substances (TBARS) analysis. A control sediment consisting of Ottawa Sand (Fisher Chemical) was prepared and processed in the same manner as the time-constrained sediments and was used as an exposure control to assess Daphnia mortality. If more than 10% of the Daphnia were deceased in the exposure control, that exposure was considered invalid, although this did not occur during the experiments.

2.5. TBARS assay

Daphnids were kept on ice throughout the TBARS preparation phase, which occurred as quickly as possible, usually within 30 min of removal from the sediment-water coexistence system. After being extracted from the sediment-water coexistence system, *Daphnids* were washed three times in PBS solution, and preserved in 400μL of protease inhibitor solution. Daphnia were lyophilized in the protease solution using a probe sonicator. The resulting solution was aliquoted into two independent vials and stored at −20ºC until transported on ice to the National Wildlife Research Centre in Ottawa for analysis with a TBARS assay kit (Cedarlane Labs). The TBARS assay determines the degree of lipid peroxidation using the biomarker malondialdehyde (MDA) (Barata et al., 2005). This assay quantifies the fluorescence produced when thiobarbituric acid reacts with MDA (Tang et al., 2011). Manufacturer's instructions were followed for the preparation of cell lysates for the TBARS assay, and optical density was determined as instructed by the manufacturer. Optical densities were corrected using lipid correction, determined by bicinchoninic acid (BCA) analysis. Significant differences were determined between sedimentary exposure depths using a Kruskal-Wallis test followed by Dunn's post-hoc test performed using the *dunn.test* package in R (Dinno, 2017).

2.6. ICP-MS and biosensor analysis

To determine the bioavailability of arsenic in the overlying exposure water, we used a whole cell biosensor using a previously described method (Pothier et al., 2018, 2020). The biosensor chassis is an *E. coli* strain transformed with a genetic construct hosting a sequence coding for the fluorescent mCherry protein, which expression is controlled by

the presence of arsenic within the cell. Fluorescence emitted by the biosensor was compared to the total arsenic concentration available in the overlying exposure water matrix obtained with ICP-MS analysis using a linear regression analysis. The overlying exposure water was extracted from the sediment-water coexistence system exposure tubes and was prepared for ICP-MS and biosensor analysis. The overlying exposure water was filtered for ICP-MS analysis using a 0.45 μm PES filter syringe and preserved with omni-trace nitric acid to a final concentration of 0.5 M and stored in the fridge at 4°C until analysis could be completed. Within 1 week following the Daphnia exposure, the overlying exposure water samples were exposed to the microbial biosensor following a previously described assay (Pothier et al., 2018, 2020). Samples were diluted to within the linear working range of the cellular sensors (25 to 800 nM) using ultra-pure water and a phosphate free growth medium. All fluorescent outputs were corrected for autofluorescence of the cells, background noise of the samples, and to the culture health using previously described methods (Pothier et al., 2020).

3. Results

3.1. Pocket Lake

3.1.1. Sediment risk assessment

The concentration of metals used in the PEC-Q calculation from Pocket Lake are indicated in Table S2. At Pocket Lake, the PEC-Q for the sediment analyzed in each section is indicated in Fig. 2A and Table S2. Prior to the onset of mining (pre-1850), the minimum PEC-Q was 3.5, the peak PEC-Q of 273.2 occurred during mining in ~1983 (12.25 cm). In the most recent sediments, deposited in 2017, the PEC-Q was 3.7 (0.25 cm) (Fig. 2A, Table S2).

The 48-h acute toxicity of Pocket Lake surface water to *Daphnia sp*. is indicated in Fig. S2. Daphnia began experiencing some toxicity (mortality >10%) at 15% of the exposure water sourced from Pocket Lake. The Daphnia began experiencing 50% mortality when 50% of the exposure water was from Pocket Lake, and experienced 90 ± 14.1% mortality when exposed to 100% Pocket Lake water.

3.1.2. Daphnia mortality, TBARS analysis, and arsenic bioavailability

In Pocket Lake, *Daphnia* mortality increased as sediments approached those deposited during the period of mining (Fig. 2B). Prior to the onset of mining, Daphnia mortality ranged from 3.33 ± 5.77% to 41.48 ± 20.16%. Daphnia mortality peaked (100 ± 0.00%) in sediment deposited in 1986 ± 3.5 (10.75 cm). Daphnia mortality in Pocket Lake decreased in sediments deposited after this peak with the post-mining mortality ranging from 0.0 ± 0.0% to 23.03 ± 12.07%.

We observed no significant changes in malondialdehyde (MDA) concentrations throughout Pocket Lake sediments, nor a significant difference between any treatments relative to the Ottawa Sand control (Fig. 2C). Regression analysis of the overlying exposure water indicated that arsenic was 96% bioavailable to the microbial biosensor when compared to ICP-MS analyzed arsenic concentrations (p-value <0.001) (Figs. 2D, S3).

3.2. Near, mid, and far-field sites

3.2.1. Sediment risk assessment

The PEC-Q of both the far- (YKW-1) and mid-field (YKC-1) sites was below the threshold of biological effects possible (<0.5) in all samples analyzed (Fig. 3A, 3F). The toxicity quotient of arsenic alone exceeded the threshold of biological effects possible in 56% of analyzed sediment sections in YKW-1, and 80% of sediment sections analyzed in YKC-1 (Fig. 3B, 3G). The near-field site (YK-42) exceeded the PEC-Q threshold at which biological effects are probable (>2.0) in all sections analyzed, except for the deepest interval (32.25 cm), which had a PEC-Q value of 0.9 (Fig. 3 K). The minimum toxicity quotient of arsenic was 4.5, well above the 2.0 biological effects probable threshold (Fig. 3 L).

3.2.2. Daphnia mortality, TBARS analysis, and arsenic bioavailability

Mean Daphnia mortality in far- (YKW-1) and mid-field (YKC-1) sites was relatively consistent throughout both sediment cores (5.1 ± 5.3% and 9.9 ± 6.4% respectively) (Fig. 3C, 3H). At the near-field (YK-42) site, baseline Daphnia mortality (pre-1900) was 3.2 ± 5.6%. Daphnia mortality markedly increased in sediments deposited between ~1926 and 1993 (98.8 ± 2.1%). In recent sediments (~2016), there is a marked decrease in Daphnia mortality (0 ± 0%) (Fig. 3 M).

During the time of mining in the far-field site (YKW-1), the MDA concentration for Daphnia exposed to the 5.75, 6.75, 7.75, and 8.75 cm (1967–1993) sediment intervals was 2.04 ± 0.28, 0.79 ± 0.27, 2.76 ± 0.42, and 2.89 ± 0.72 μM respectively. This represents a 2.1, 0.8, 2.9,

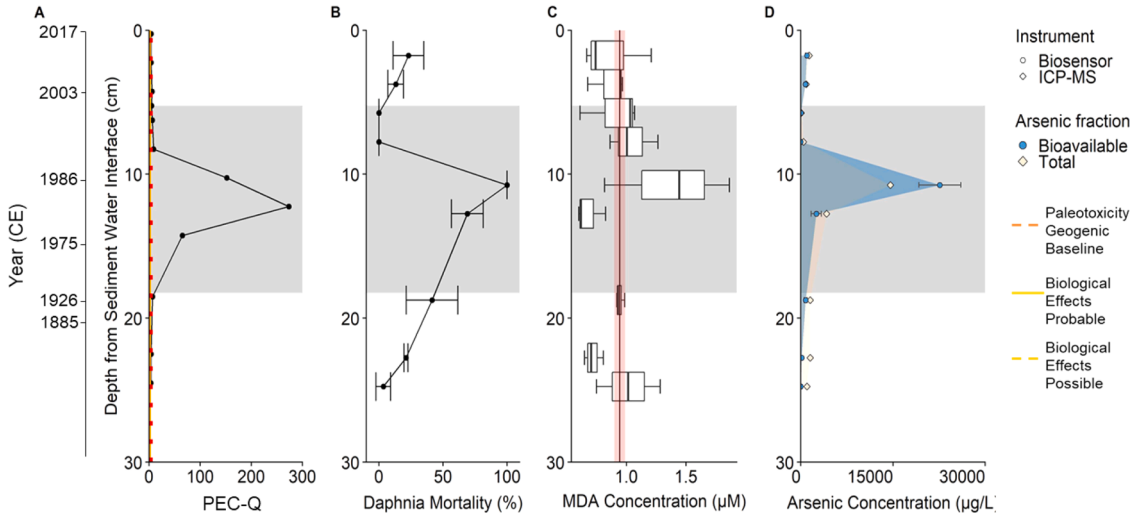

Fig. 2. The PEC-Q calculated from metal(loid) concentrations in dated sediments at Pocket Lake (A), the mortality of *Daphnia* spp. exposed to time-constrained Pocket Lake sediment (B), the MDA concentration, with control mean ± SD indicated with solid black line and red shading, of whole *Daphnia* spp. following 24 h of sediment exposure (C), and the bioavailability of arsenic in the overlying exposure water used in the sedimentary *Daphnia* spp. exposures to a microbial biosensor (D). Biosensor measurements are depicted by blue shading and circles and the ICP-MS As concentrations are depicted by yellow shading and diamonds. The dashed red line represents the geogenic PEC-Q value in the region. The orange solid and dashed lines indicate biological effects probable and possible, respectively. The time of active mining (~1948–1999) is represented by the gray shaded region.

and 2.9-fold change from the Ottawa Sand control (0.97 ± 0.35 μM) in sediments deposited during mining. The Kruskal-Wallis test indicated a significant difference between the sediment intervals and the Ottawa Sand Control (p-value<0.005), and further analysis with Dunn's post-hoc test indicated that sediment intervals 7.75 cm and 8.75 cm were significantly higher than the Ottawa sand control (adj. p-value <0.05). Additionally, the MDA concentration in the 8.75 cm interval was significantly higher than both the 3.75 cm and 6.75 cm sediment intervals (Fig. 3D). The mean MDA concentrations at 7.75 cm and 8.75 cm are 1.19- and 1.24-fold greater, respectively, than the background MDA concentration (2.33 ± 0.36 and 2.31 ± 0.32 μM) at 10.75 cm and 12.75 cm (1932 and pre-1900), respectively. The MDA concentration in recently deposited sediments (2.17 ± 0.51 μM and 0.62 ± 0.07 μM) was 2.2 and 0.64-fold different than the control at 1.75 cm (2014) and 3.75 cm (2006) respectively. The Dunn's post-hoc test indicated no significant difference between sediment MDA concentrations pre- and post-mining.

At the mid-field site (Fig. 3I), the Kruskal-Wallis test indicated no significant relationship between the MDA concentration in Daphnia exposed to mid-field sediments relative to the control Ottawa Sand at the 95% confidence level (p-value=0.107). We observed minimal changes in MDA concentrations in Daphnia exposed to sediments deposited pre-, during, and post-mining. Further analysis of the MDA concentration with the Dunn's post-hoc test indicated that at the mid-field site, pre-mining sediments in 1945 (8.75 cm) were significantly greater (adj. p-value<0.05) than during-mining sediments deposited in 1962 (6.75 cm). The Kruskal-Wallis test indicated no significant difference between the MDA concentration in Daphnia exposed to the control Ottawa Sand relative to near-field sediments (adj. p-value=0.78). Dunn's post-hoc analysis indicated no significant relationship between sediment intervals at the near-field site.

Arsenic concentrations in the overlying Daphnia exposure water at the far-, mid-, and near-field sites ranged from 1.5 μg L^{-1} to 2.6 μg L^{-1}, 2.1 μg L^{-1} to 9.6 μg L^{-1}, and 4.0 μg L^{-1} to 2766.6 μg L^{-1}. The maximum arsenic concentration in the far-, mid-, and near-field sites overlying water was at 6.75 cm (1981), 4.75 cm (1982), and 9.25 cm (1970) respectively (Fig. 3E, 3 J, and 3O). Arsenic bioavailability at the far-field site was not obtained because the arsenic concentration in the overlying exposure water of YKW-1 was below the limit of detection for the arsenic biosensor (<25 nM). The bioavailability of arsenic in the overlying exposure water determined by linear regression was 72% at the mid-field site (p-value<0.05) and 76% at the far field site (p-value<0.05) (Fig. S4).

4. Discussion

4.1. Daphnia as a sediment biomonitoring tool

We observed significant temporal overlap between the predicted sedimentary toxicity (PEC-Q), and *Daphnia* mortality in Pocket Lake (Fig. 2), YKW-1, YKC-1 and YK-42 (Fig. 3) indicating that Daphnia can be a fast and efficient indicator of environmental contamination in time-constrained sediments. Low *Daphnia* mortality in the oldest sediment interval (pre-1900) tested was recorded (3.3 ± 6%) in Pocket Lake. However, coincident with increasing sedimentary predicted toxicity (PEC-Q) due to mining activities, *Daphnia* mortality increased to 100%. Following the cessation of mining, and a decrease in sedimentary predicted toxicity, *Daphnia* mortality decreased to (0 ± 0%) in the most recent sediments (Fig. 2B). This finding suggests *Daphnia* sp. can survive in current sediment conditions, although Cladocera were extirpated in the fossil record of Pocket Lake, and remain absent (Thienpont et al., 2016).

We observed a similar pattern at the near-field site (YK-42) with pre-mining mean Daphnia mortality of 3.2 ± 5.6%, which then increased to 100% mortality coeval with mining operations (Fig. 3 K & 3 L). More recent (post-mining) sediments returned to near baseline mortality of 0 ± 0% post-mining (Fig. 3M). Similarly, where the predicted toxicity was lower in YKC-1 and YKW-1 (mid- and far-field sites), Daphnia mortality did not exhibit substantial change throughout the core with mean mortality at YKW1 at 5.1 ± 5.3% (Fig. 3B) and YKC1 at 9.9 ± 6.4% (Fig. 3G).

Although typically employed to assess the toxicity of aquatic media, there is a growing body of evidence to support sedimentary *Daphnia* exposures. Historically considered a pelagic, or non-benthic species, *Daphnia* have been shown to graze on sediments, and spend part of their lifecycle in and near sediments (Dodson et al., 2010). This behavior increases their exposure to sediment-bound contaminants and makes *Daphnia* a candidate for sediment exposure studies using small amounts of fresh sediment (Allen Burton et al., 1996; Terra et al., 2010). Suedel et al. (1996) concluded that due to their sediment grazing behavior, *Daphnia* were an appropriate species to use in sediment toxicity exposures. These authors then exposed *Daphnia* to copper-spiked sediments, concluding that sedimentary exposed *Daphnia* did exhibit a response following the exposure (Suedel et al., 1996). Since that time, several studies have employed *Daphnia* to assess whole sediment toxicity. In 2010, Terra and associates used *Daphnia magna* to assess the toxicity of Cai River sediment; Rossi and Beltrami (1998) performed *in situ* experiments with caged *Daphnia* to assess the toxicity of the sediment in Lake Orta; and Li et al. (2017) used *Daphnia* as a test organism to assess the toxicity of cadmium in spiked sediment assays. Li and associates concluded that mortality, cadmium accumulation, and metallothionein (MT) increased due to the ingestion of cadmium contaminated sediments during the exposure (Li et al., 2017). To date, *Daphnia* sediment exposures have been performed on surface sediments, and spiked sediment samples. This work is, to the best of our knowledge, the first known application of *Daphnia* sediment exposure assays using time-constrained lake sediments.

4.2. Determining historic causal mechanisms for population level microfossil changes

Pocket Lake was used to develop methods to elucidate the causal mechanisms of changes observed in population level fossil records. There is a known history of Cladoceran disturbance at Pocket Lake determined by sedimentary fossil analysis (Thienpont et al., 2016). Due to the induction of the oxidative stress response system following elevated metal exposure reported in the literature (Barata et al., 2005; Fan et al., 2009, 2015; Lari et al., 2017; Tang et al., 2011, 2015; Vandegehuchte et al., 2010), oxidative stress has been hypothesized to be a possible mechanistic pathway for the population level extirpation event observed at the site by Thienpont et al. (2016). Oxidative stress is caused by a disruption in the balance of free radical production and extinction within the cell. Disruptions to this balance can lead to adverse outcomes including DNA damage, protein degradation, and lipid peroxidation (Barata et al., 2005). A proposed adverse outcome pathway for how the production of reactive oxygen species (ROS) could lead to increased Daphnia mortality is presented in Fig. S5. In the present study, this pathway was assessed using the thiobarbituric reactive species (TBARS) assay, which measures the concentration of malondialdehyde (MDA), a breakdown product of cellular membranes induced by lipid peroxidation (Barata et al., 2005). This assay measures the concentration of MDA, relative to the protein content in the sample, by quantifying the fluorescence produced when thiobarbituric acid reacts with MDA (Tang et al., 2011).

The oxidative stress response of the Daphnia exposed to the time-constrained sediment is unclear at the lakes examined in this study. In YKW-1, exposure to the slightly elevated concentrations of metals associated with the time of mining (7.75 cm and 8.75 cm) was significantly different from the control. However, the directionality of that difference is inconsistent, suggesting that the Daphnia response is not clearly associated with increased sedimentary metal(loid) burden

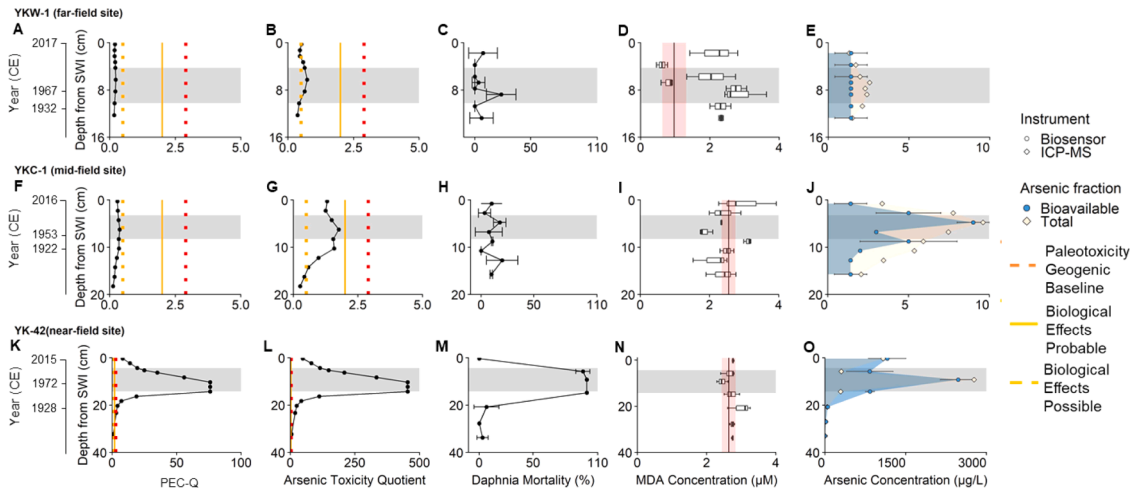

Fig. 3. The PEC-Q (Cheney et al. 2020), Arsenic toxicity quotient, Daphnia Mortality, MDA concentration, biosensor, and ICP-MS concentrations for far- (A-E), mid- (F-J), and near-field (K-O) sites are provided as a function of sediment core depth. Biological Effects Possible is displayed as a dotted orange line, Biological Effects Probable is displayed as a solid orange line, and the PEC-Q for the region derived from Geogenic metal(loid) concentrations indicated with a red dashed line. The time of Giant Mine's operation, derived from CRS dating models, is indicated by the gray shaded region. In the TBARS plots, the solid black line framed by the red shaded region indicates the mean Daphnia MDA concentration and standard deviation, respectively, in the Ottawa Sand control.

(Fig. 3D). The application of the Kruskal-Wallis and Dunn's post-hoc tests was used to determine significance of the change from control as it was the most appropriate non-parametric method available.

In our study, 100% Daphnia mortality was experienced during mining in both Pocket Lake and YK-42, which prevented sub-lethal causal mechanisms from being examined accurately. Often the products of lipid peroxidation, such as MDA, are unstable compounds that readily degrade (Lushchak, 2011) Therefore, in exposures with Daphnia that were deceased at the time of sample collection, the MDA protein may have already degraded prior to sample collection. The absence of MDA in samples where we expected to observe an oxidative stress response may then represent a false negative result. Our results would then indicate that oxidative stress was not the causative mechanism of Daphnia mortality. To mitigate this confounding factor in the future, and establish the true influence of oxidative stress on Daphnia mortality, variable length exposures should be performed to ensure Daphnia samples are collected following a sub-lethal exposure length. Further, future studies could explore heart rate, mobility, feeding, and reproductive rates as potential endpoints for toxicity assessment.

4.3. *Assessing the bioavailability of arsenic to Daphnia*

For a toxic response to have been observed by Thienpont et al. (2016), contaminants must have been bioavailable to the aquatic species within Pocket Lake, however little work relating to the bioavailability of arsenic has been documented in lakes affected by gold mines near Yellowknife. Pothier et al. (2018) assessed the ability of a whole cell biosensor to detect inorganic arsenic in 17 surface water samples collected near Yellowknife. They found that legacy arsenic contamination presented high bioavailability (~96%) and could be accurately quantified, regardless of the surface water matrix. Our study is the first to use microbial biosensors to determine the bioavailability of arsenic in overlying sediment exposure water to live test organisms. The arsenic in the overlying water was found to be 72–96% bioavailable to the microbes. Hence, the arsenic that is present in these sediments is also likely to be highly bioavailable to other aquatic organisms under these laboratory conditions, though we note that bioavailability of arsenic to microbes may not translate directly to other organisms.

5. Conclusion

This study represents what the authors believe to be a novel exposure of an environmentally relevant species of *Daphnia* sp. and of an As-sensitive whole cell biosensor to time-constrained lake sediments. Our approach provides a novel opportunity for scientists to track the toxicity of past industrial emissions to lake sediments and provides a means to develop and test causal relationships between observed population-level shifts in the microfossil record of lake sediments and cellular mechanisms that may have led to these observed changes.

The methods proposed in this study also provide policy makers with a relatively fast, easy, and inexpensive method to screen historic sediments for potential toxicity to aquatic biota without performing more elaborate analyses of specific compounds, which often ignores the impacts of chemical mixtures. In cases of legacy contamination, mixtures of contaminants are often paramount. To perform a screening level risk assessment, scientists must screen for mixture components, and calculate a PEC-Q from those concentrations which can be quite expensive and time consuming. Our proposed method of exposing Daphnia to sediments to assess acute toxicity would be a more efficient protocol and allow for a pre-screening process to a Tier-1 risk assessment that could identify areas of greater concern. This method provides more a targeted approach for policy makers and remediators to use their resources.

Finally, these methods can be used to elucidate effects from historic and current or future industrial processes in areas of re-development. This will be especially useful in areas rich in natural resources where new emission and environmental protection technologies can be independently assessed without the confounding factor of historic contaminant influences.

CRediT authorship contribution statement

Cynthia Cheney: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Martin Pothier:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Philippe J. Thomas:** Methodology, Writing – review & editing. **Sailendra Nath Sarma:** Methodology, Writing – review & editing. **Alexandre J. Poulain:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing. **Jules M. Blais:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2022.106248.

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