Journal of Hydrology: Regional Studies 38 (2021) 100954

Contents lists available at ScienceDirect

Journal of Hydrology: Regional Studies

journal homepage: www.elsevier.com/locate/ejrh

Application of artificial substrate samplers to assess enrichment of metals of concern by river floodwaters to lakes across the Peace-Athabasca Delta

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ARTICLE INFO

Keywords:
Contaminant monitoring
Periphyton
Floodplain
Peace-Athabasca Delta
Athabasca Oil Sands
Wood Buffalo National Park Action Plan

ABSTRACT

Study region: Peace-Athabasca Delta (PAD), northeastern Alberta.
Study focus: Potential for downstream delivery of contaminants via Athabasca River floodwaters to lakes of the PAD has raised local to international concern. Here, we quantify enrichment of eight metals (Be, Cd, Cr, Cu, Ni, Pb, V, Zn) in aquatic biota, relative to sediment-based pre-industrial baselines, via analysis of biofilm-sediment mixtures accrued on artificial substrate samplers deployed during summers of 2017 and 2018 in > 40 lakes. Widespread flooding in the southern portion of the delta in spring 2018 allows for assessment of metal enrichment by Athabasca River floodwaters.
New hydrological insights: River floodwaters are not implicated as a pathway of metal enrichment to biofilm-sediment mixtures in PAD lakes from upstream sources. MANOVA tests revealed no significant difference in residual concentrations of all eight metals in lakes that did not flood versus lakes that flooded during one or both study years. Also, no enrichment was detected for concentrations of biologically inert metals (Be, Cr, Pb) and those related to oil-sands development (Ni, V). Enrichment of Cd, Cu, and Zn at non-flooded lakes, however, suggests uptake of biologically active metals complicates comparisons of organic-rich biofilm-sediment mixtures to sediment-derived baselines for these metals. Results demonstrate that this novel approach could be adopted for lake monitoring within the federal Action Plan.

1. Introduction

Northern freshwater ecosystems provide invaluable natural resources and hold cultural and societal significance but are increasingly threatened by anthropogenic activities (Dudgeon et al., 2006; Schindler, 2010; Schindler and Smol, 2006). To safeguard these ecosystems, evidence-based management decisions need to be guided by monitoring data capable of quantifying the extent of degradation (Roach and Walker, 2017). Ability to conduct informed monitoring for contaminants of concern, however, is often hindered by a lack of knowledge of pre-disturbance concentrations and natural variation because data collection tends to be initiated after onset of anthropogenic disturbance (Blais et al., 2015). Long-term datasets that extend to pre-disturbance periods are critical to

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https://doi.org/10.1016/j.ejrh.2021.100954
Received 21 May 2021; Received in revised form 19 October 2021; Accepted 25 October 2021
Available online 3 November 2021

identify the influence of anthropogenic activities versus natural processes on the environment (Lindenmayer and Likens, 2009, 2010), especially when located downstream of natural sources of substances of concern (Wiklund et al., 2014). It is therefore advantageous, but infrequent, to design monitoring programs that incorporate information about pre-disturbance concentrations to allow for rapid real-time determination of the degree of degradation.

One such at-risk system is the Peace-Athabasca Delta (PAD), located in northeastern Alberta, Canada. As the world's largest inland freshwater boreal delta, the hundreds of productive, shallow lakes in the PAD provide critical habitat to biota and support traditional lifestyles of local Indigenous communities (Timoney, 2013). These ecosystem services merited the PAD's designation as a Ramsar Wetland of International Importance and contributed to Wood Buffalo National Park's (WBNP) recognition as a UNESCO World Heritage Site, which contains ~80% of the PAD. However, ongoing expansion of mining in the Alberta Oil Sands Region (AOSR) has long generated concerns that contaminants are being released into the Athabasca River and delivered downstream to the PAD where they have potential to degrade water quality of rivers and lakes (Dowdeswell et al., 2010; Schindler, 2010; Timoney and Lee, 2009). The urgent need for better monitoring practices in the PAD has been emphasized in a petition by the Mikisew Cree First Nation in 2014 to enlist WBNP as "World Heritage in Danger" (MCFN Mikisew Cree First Nation, 2014), recommendations by the UNESCO World Heritage Committee (WHC/IUCN, 2017), and priorities within the Wood Buffalo National Park Action Plan (Wood Buffalo National Park WBNP, 2019).

An approach that has been proposed for effective monitoring of oil sands pollution is paleolimnological investigation of sediment cores from lakes to establish the range of natural variability of substances of concern prior to industrialization (Dowdeswell et al., 2010; Wrona and di Cenzo, 2011). This approach has been implemented in the PAD and the results have served to develop knowledge of the natural variability of metal concentrations in pre-1920 lake sediment (Kay et al., 2020; Wiklund et al., 2012, 2014). Sediment deposited before 1920 pre-date the earliest paleolimnological evidence of atmospheric deposition of pollutants from industrial emissions, which was followed by a decline in atmospheric deposition after ~1970 as industrial practices improved (Wiklund et al., 2012). These baselines enabled evaluation of contamination of lakes by atmospheric and fluvial pathways using contemporary concentrations of metals of concern in surface sediment of lakes across the PAD, which demonstrated no substantial enrichment above the pre-1920 baselines (Owca et al., 2020). Their study analyzed 1-cm thick surface sediment samples collected in 2017, which captured recent time periods of sediment deposition that are variable among lakes (~2–5 years). Also included was analysis of exclusively flood-supplied surface sediment samples collected in 2018 from lakes that had received river floodwaters two months prior. However, because such flood events are episodic and of varying magnitude, development and testing of additional 'real-time' monitoring approaches of metal concentrations in lakes are warranted.

Among the plethora of complementary approaches for monitoring contaminants, artificial substrate samplers, which accrue a mixture of biofilm, consisting of communities of periphytic algae, fungi, and microbes, and entrapped suspended sediments (Jowett and Biggs, 1997; Szlauer-Łukaszewska, 2007; Wu, 2017), may provide some advantages. They offer careful control of biofilm-sediment accrual time and circumvent potential difficulties associated with locating and collecting appropriate natural substrates (MacDonald et al., 2012; Wiklund et al., 2010). Because of these advantages, artificial substrate samplers have been tested and used for monitoring water quality (Biggs, 1989), including metals content (Holding et al., 2003; Namba et al., 2020; Pederson and Vaultonburg, 1996; Ramelow et al., 1987, 1992; Tang et al., 2014). For several metals of concern, concentrations in biofilm-sediment mixtures accrued on artificial substrate samplers have been shown to correspond well with concentrations in sediment from the same locations (Holding et al., 2003; Ramelow et al., 1987, 1992). This suggests there is potential to combine use of artificial substrate samplers with measurements of pre-industrial metal concentrations in sediment cores to evaluate for evidence of enrichment relative to natural conditions, which, to our knowledge, is a novel approach.

Here, we use concentrations of metals in pre-industrial (pre-1920) lake sediment and in biofilm-sediment mixtures accrued on artificial substrate samplers during the open-water seasons of 2017 and 2018 to quantify enrichment of metals of concern at the base of food webs in lakes of the PAD. Given the novelty of the approach, we first test the assumption that concentrations of metals in biofilm-sediment mixtures and lake surface sediment follow the same relation with the geochemical normalizer (aluminum) using a subset of lakes that received floodwaters in 2018. This is important because it would then suggest that estimates of enrichment derived from analysis of biofilm-sediment mixtures relative to pre-1920 sediment baselines is a reasonable approach and not reflective of differences in analytical substrates. To assess the role of river floodwaters on potential metal enrichment of biofilm-sediment mixtures, our study spans two years, which includes numerous lakes inundated by the large ice-jam flood event in spring 2018 (Remmer et al., 2020a). Most of the flooding in spring of 2018 occurred in the southern Athabasca sector of the delta, which provides opportunity to evaluate the extent of contamination in Athabasca River water and sediment by upstream oil sands development. The suite of metals selected for this study follow those analyzed in prior studies of sediment and water in this region (Kay et al., 2020; Kelly et al., 2010; Owca et al., 2020; Wiklund et al., 2014) and include seven priority pollutants listed under the US Environmental Protection Agency's Clean Water Act (beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn)), as well as vanadium (V), a known indicator for oil sands pollution (Gosselin et al., 2010; Jacobs and Filby, 1982; Reynolds et al., 1989). The study aims to refine current understanding of perceived threats from upstream oil sands development and evaluate an approach that could be adopted for integrated hydrological and contaminants monitoring of lakes of the PAD within the federal WBNP Action Plan (WBNP, 2019).

2. Material and methods

2.1. Site description

Centered at 58.6°N, 111.8°W in northeastern Alberta, Canada, the 6000 km^2 PAD contains hundreds of shallow lakes (Fig. 1). The

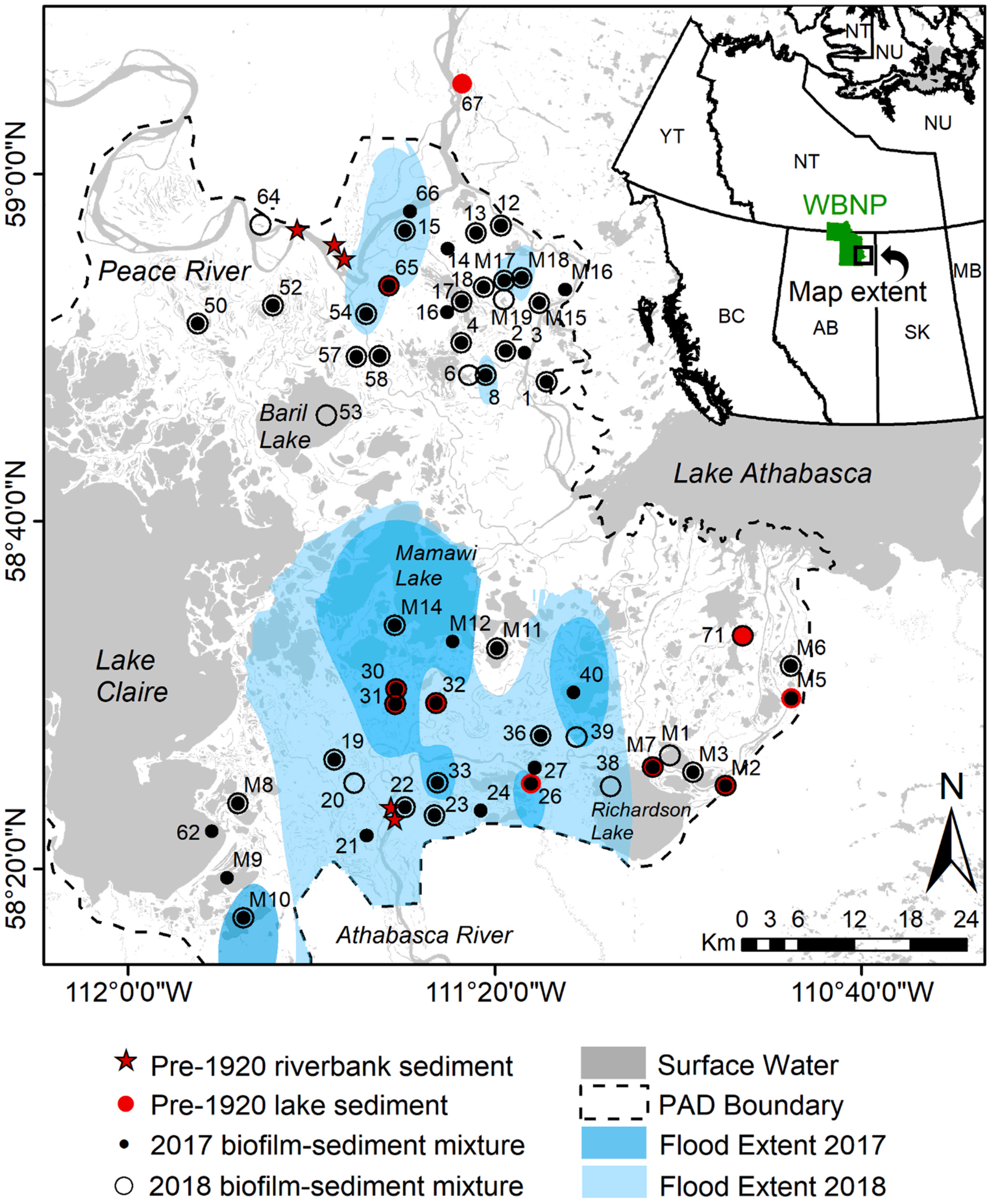

Fig. 1. Map of the Peace-Athabasca Delta (PAD), Alberta, showing the location of lakes from which biofilm-sediment mixtures were collected (n = 49 in 2017; n = 42 in 2018) and locations of lakes and riverbank sites from which pre-1920 sediment was collected. Also shown is the combined extent of spring ice-jam and open-water flooding in 2017 and 2018, adapted from The Peace sector includes PAD 53 and lakes north of Mamawi Lake, whereas the Athabasca sector includes Mamawi Lake and areas to the south.
Adapted from Remmer et al. (2020a, 2020b).

PAD is composed of two hydrologically distinct sectors: the northern Peace sector and the southern Athabasca sector. Lakes in the relic delta of the northern Peace sector experience limited river connectivity (Wolfe et al., 2007). Episodic ice-jam events on the Peace River during the spring freshet are required to generate widespread flooding (Peters et al., 2006). In contrast, lakes in the relic and active delta portions of the southern Athabasca sector experience a spectrum of limited, intermittent, or continuous surface connection with the northward-flowing Athabasca River and its distributaries (Remmer et al., 2020b; Wolfe et al., 2007).

Previous research has documented the extent of ice-jam and open-water flooding with respect to the study lakes in the PAD during 2017 and 2018 (Remmer et al., 2020a, 2020b; Fig. 1). Ice-jam flooding was more widespread in the Athabasca sector in spring 2018 than in spring 2017. Compared to spring ice-jam flooding, a smaller number of lakes in the Athabasca sector also received open-water flooding in summer 2017 and summer and fall 2018. In the Peace sector, no lakes were flooded in 2017, limited ice-jam flooding occurred in spring 2018, and one lake (PAD 54) also received open-water flooding in summer and fall 2018.

2.2. Fieldwork

Artificial substrate samplers were deployed from the pontoon of a helicopter into 25 lakes in the Athabasca sector and 24 lakes in the Peace sector on May 22–24, 2017 and retrieved on September 12–14, 2017 (Fig. 1). Samplers were deployed again the following year on May 21–23, 2018 and retrieved from 20 lakes in the Athabasca sector and 22 lakes in the Peace sector on September 15–17, 2018 (Fig. 1). Samplers were retrieved from the same lake in both years for 15 lakes in the Athabasca sector and 16 lakes in the Peace sector. Samplers consisted of 52.5 cm by 18 cm high-density polypropylene shields attached by rope on one end to a wooden float covered in Styrofoam to maintain buoyancy, and to a rock on the other end to remain anchored to the lake bottom (Fig. 2). Rope lengths were sufficient to ensure that samplers were suspended vertically at approximately 10–15 cm deep in the water column. Polypropylene shields act as an inert substrate that accrue a periphytic biofilm of algae, microbes, and other biota, to which suspended particles may adhere via passive transport. A previous study of lakes in the PAD has shown that differences in algal community composition between artificial substrate samplers and macrophytes were comparable to differences between macrophyte species, providing confidence that samplers accrue representative algal communities (Wiklund et al., 2010). Upon retrieval of the samplers, shields were placed individually into plastic *Ziploc* bags, frozen, and transported to University of Waterloo where they remained frozen until processed for analysis. Ice-jam flooding in the Peace and Athabasca sectors in spring 2018 provided opportunity to acquire recently deposited river-supplied flood sediment from 6 flooded lakes in the Athabasca sector (PAD 19, PAD 22, PAD 30, PAD 31, PAD 33, and PAD 36) and 7 flooded lakes in the Peace sector (PAD 15, PAD 50, PAD 54, PAD 58, PAD 64, M17, and M18) in July 2018 (Owca et al., 2020). These surface sediment 'flood-layer' samples captured a similar timeframe of metals accumulation as biofilm-sediment mixtures collected in 2018, and therefore were used to directly compare metal-aluminum relations for these two substrate types.

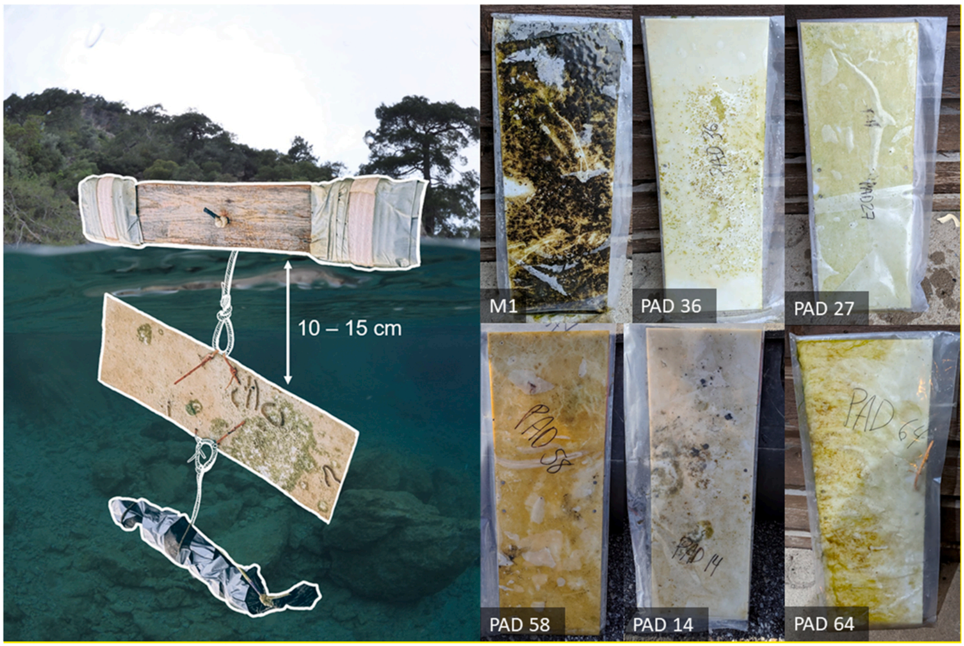

Fig. 2. Schematic showing design of the artificial substrate sampler (left) and photographs showing a range of representative biofilm-sediment mixture compositions (right).

2.3. Laboratory analyses

All processing was performed on the shields after they were removed from the freezer and thawed. The biofilm-sediment mixtures were removed from the shields by scraping and rinsing with a recorded volume of deionized water. The final volume of contents (biofilm-sediment mixture plus rinse water) was also recorded before further subsampling. For loss-on-ignition (LOI) analysis, 20 ml were pipetted into a glass scintillation vial. For metals analysis, the remaining contents were added to a plastic cup and frozen until further processing.

LOI was performed to measure the dry mass and organic matter content (%OM) of biofilm-sediment samples following standard methods (Heiri et al., 2001). For each lake, the 20 ml LOI subsample was added to a pre-weighed porcelain crucible. The crucibles were then dried in an oven at 90 °C for 18 h to determine the dry sample mass. The crucibles were combusted in a muffle furnace at 550 °C for 2 h, cooled and reweighed to determine %OM (relative to dry mass).

For determination of metal concentrations, periphyton subsamples were freeze-dried, weighed, and analyzed by ALS Environmental (Vancouver, BC) following EPA Method 200.3/6020A (United States Environmental Protection Agency US EPA, 1998). This is a hotblock digestion with HNO_3 and HCl, in combination with H_2O_2, which provides a conservative estimate of bioavailable metals in organic tissue. These methods are similar to the analysis of metal concentrations used to establish pre-industrial baselines from lake sediment cores (Kay et al., 2020; Owca et al., 2020). Uncertainties are ± 231.8 mg/kg for Al, ± 0.022 mg/kg for Be, ± 0.013 mg/kg for Cd, ± 0.356 mg/kg for Cr, ± 1.352 mg/kg for Cu, ± 0.678 mg/kg for Ni, ± 0.715 mg/kg for Pb, ± 0.738 mg/kg for V, and ± 2.398 mg/kg for Zn based on mean difference of 4 sample repeats in 2017 and 1 sample repeat in 2018.

2.4. Data handling and analysis

Pre-industrial baselines were established in previous studies from concentrations of metals in river-supplied sediment deposited before 1920 at two flood-prone lakes in the Peace sector ($n = 65$ sediment samples; Owca et al., 2020) and at eight flood-prone lakes in the Athabasca sector ($n = 122$ sediment samples; Kay et al., 2020; Fig. 1). For our study, we added concentrations of metals in sediment from riverbank exposures at sites situated along the lower Peace (3 sites, $n = 33$ samples) and Athabasca (2 sites, $n = 37$ samples for all metals, except $n = 26$ for Be, $n = 29$ for Cd, and $n = 34$ for Cr) rivers to these pre-1920 baselines (Fig. 1). The riverbank samples were collected at locations sampled by Hugenholtz et al. (2009) and from strata older than 1920 based on their chronological data. Pre-1920 baselines are represented as linear relations with 95% prediction intervals (PI) between concentrations of the metals of interest (Be, Cd, Cr, Cu, Ni, Pb, V, Zn) and Al. Normalization to Al accounts for variations in sediment-metal concentrations associated with energy conditions and grain size (Covelli and Fontolan, 1997; Forstner and Müller, 1981; Kersten and Smedes, 2002; Loring, 1991), and has been widely used in the region (AOSR and PAD; Cooke et al., 2017; Kay et al., 2020; Klemt et al., 2020; Owca et al., 2020). Owca et al. (2020) determined that Al is the generally superior normalizing agent for the metals of interest in both sectors of the PAD and that the slopes of all metal-Al relations differ between sectors. Therefore, separate linear relations for each metal were used for each sector.

As a first step of data exploration, we tested the assumption that metal concentrations in both the biofilm-sediment mixtures and lake surface sediment follow the same relationship with the geochemical normalizer (Al). For this exploration, we used measurements of metal concentrations in biofilm-sediment mixtures and the flood layer of river-supplied surface sediment collected in 2018 at 13 lakes in the PAD. The biofilm-sediment mixture collected from PAD 33 was below the detection limit for Ni concentration, so it was excluded from the analysis of Ni. For each of the 8 metals of interest, an analysis of covariance (ANCOVA) was performed to test if slopes and y-intercepts of the metal-Al regression lines differed between biofilm-sediment mixtures and surface sediment. ANCOVA models were defined by each metal concentration as the dependent variable, Al concentration as the independent variable, substrate type (biofilm-sediment mixture or surface sediment) as the factor, and the interaction between Al concentration and substrate type. ANCOVA tests were performed using R version 3.5.3 and function aov() (R Core Team, 2020).

We then compared concentrations of metals measured in sediment-biofilm mixtures obtained in 2017 and 2018 to the pre-1920 baselines using a series of cross-plots with Al as the independent variable and each metal of interest as the dependent variable. This step was performed on samples from all biofilm-sediment mixtures that were retrieved during either study year, and cross-plots are presented separately for 2017 ($n = 49$) and 2018 ($n = 42$) and by sector.

The subset of lakes where artificial substrate samplers were retrieved in both study years ($n = 31$) provided opportunity for paired-samples comparisons to elucidate the influence of river flooding on concentrations of metals in the biofilm-sediment mixtures. These lakes were assigned to a 'flood-category' based on the number of years, out of the two study years, that the lakes received spring ice-jam and/or open-water river floodwaters. Lakes were placed in the '0′ category if they did not receive river floodwaters in either year (Athabasca sector, $n = 6$; Peace sector, $n = 11$), in the '1′ category if they received river floodwaters in 2018 but not in 2017 (Athabasca sector, $n = 5$; Peace sector, $n = 5$), or in the '2′ category if they received river floodwaters in both years (Athabasca sector, $n = 4$, Peace sector, $n = 0$). No lakes received river floodwaters in 2017 but not 2018. Designation of the flood-category for each lake followed Remmer et al. (2020b).

We assessed enrichment of metals in biofilm-sediment mixtures for the paired-samples analysis of flood influence using residual concentrations. The residual concentrations were calculated as the difference between the measured concentration of the metal in the biofilm-sediment mixtures and the mean pre-1920 concentration of the metal determined from the baseline linear metal-Al relation at the measured concentration of Al in the biofilm-sediment mixtures. For a given metal, X, this calculation is expressed as: $[residual]_X = [measured]_X - [mean\ pre-1920]_X\ at\ [measured]_{Al}$.

To evaluate if residual concentrations exceeded the range of variability before 1920, we calculated an enrichment threshold for each metal in each sector as the upper 95% prediction interval (PI) for residual concentrations at an Al concentration of 1000 mg/kg, which closely approximates the median Al concentration of all biofilm samples obtained in this study. Use of residual concentrations to quantify enrichment is consistent with studies by Wiklund et al. (2014) and MacDonald et al. (2016), and expresses enrichment in units of concentration of the metal of interest. Enrichment factors have also been used to quantify enrichment of metals in lake and river sediment in the PAD and Lower Athabasca River (Cooke et al., 2017; Kay et al., 2020; Klemt et al., 2020; Owca et al., 2020; Wiklund et al., 2014), but were inapplicable across the full range of concentration in our study because baseline concentrations were less well-constrained at the low range of Al concentrations in biofilm-sediment mixtures for some metals and resulted in some illogical EFs (i.e., values below zero).

Using two series of boxplots and statistical tests, distributions of residual metal concentrations in biofilm-sediment mixtures were grouped to compare among the lake flood-categories and study years in the Athabasca sector and in the Peace sector. When the upper whisker of the boxplots for the residual metal concentrations in a flood-category exceeded the enrichment threshold, this was considered enriched above the expected range of pre-1920 variability.

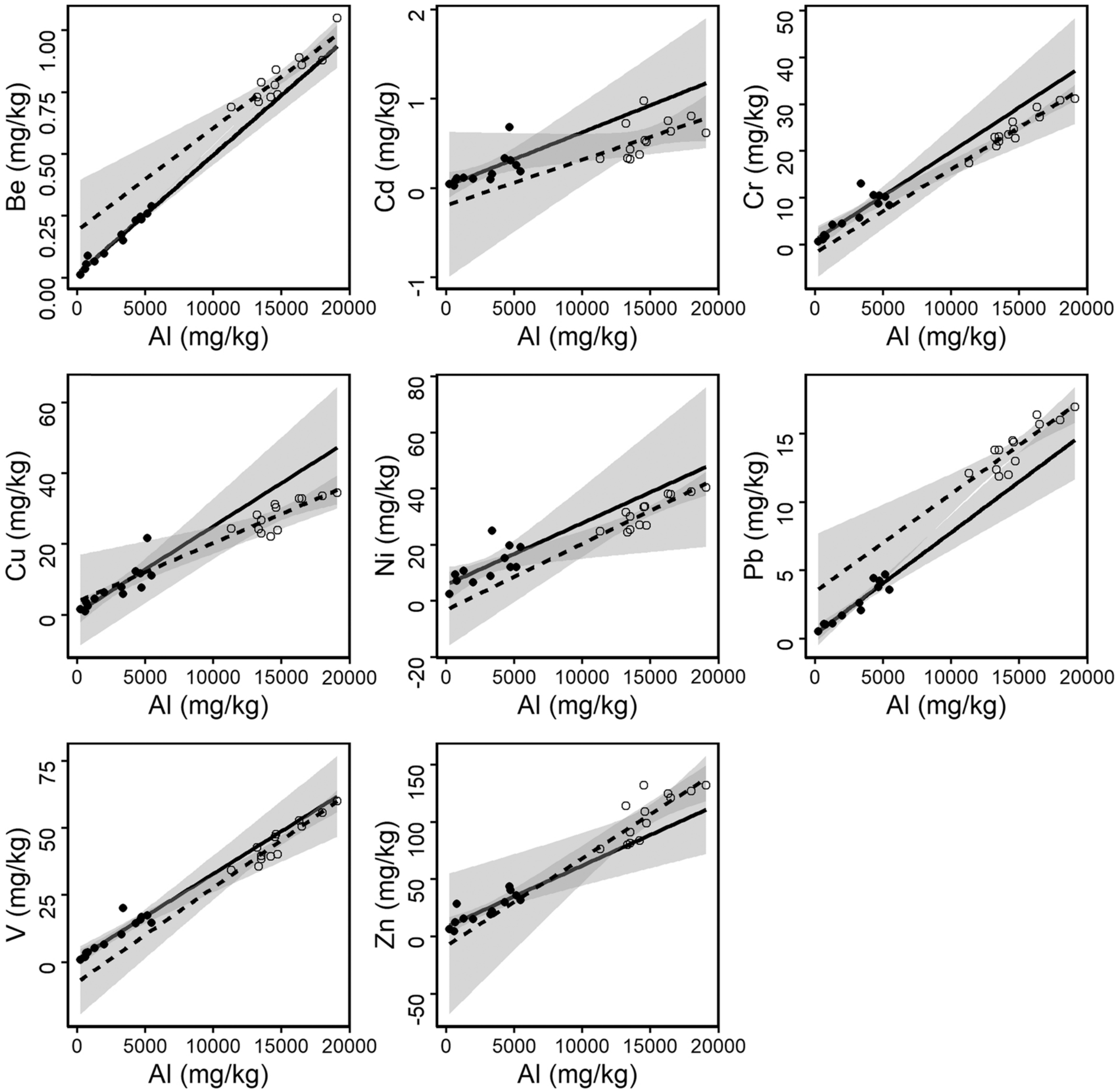

Fig. 3. Cross-plots demonstrating linear relations and 95% CIs between concentrations of metals of concern and the geochemical normalizer (Al) in biofilm-sediment mixtures (solid line; filled circles) and surface sediment ‘flood layers’ (dashed line; empty circles) collected from 13 lakes in the PAD that were flooded in spring 2018.

We used Multivariate Analysis of Variance (MANOVA) tests to determine if the residual concentrations of the suite of all 8 metals differed significantly for the above two comparisons. The use of a single test to assess for differences in residual concentrations of the suite of all 8 metals achieves the pre-determined type-1 error rate, which is not readily achieved by multiple tests on each metal individually. We ran two different repeated-measures two-way MANOVA tests, one on samples from lakes in the Athabasca sector and one on samples from lakes in the Peace sector. These tests allowed us to determine differences in residual metal concentrations between the study years (within-subject factor), among the flood-categories (between-subject factor), and the interaction of these two factors. MANOVA tests were performed using R version 3.5.3 (R Core Team, 2020) and function multRM() in the MANOVA.RM package (Friedrich et al., 2020). We report the MANOVA-type statistic (MATS) because it does not assume multivariate normality, covariance homogeneity, and singularity of covariance matrices (Friedrich and Pauly, 2018; Friedrich et al., 2017, 2019). We also report p-values based on re-sampling approaches (parametric bootstrap with 9999 iterations), which are robust for small sample sizes (Friedrich and Pauly, 2018; Friedrich et al., 2017, 2019). For all tests, alpha was set to 0.05.

3. Results

3.1. Comparisons of metal-Al relations in biofilm-sediment mixtures versus surface sediment collected from flooded lakes in 2018

Comparisons of metal-Al relations in biofilm-sediment mixtures relative to surface sediment collected from flooded lakes in 2018 show no significant interaction term for ANCOVA models for all 8 metals (Fig. 3; Table 1). This indicates that the slopes of the regressions between concentrations of each metal and Al do not differ between biofilm-sediment mixtures and surface sediment. Due to non-significant interaction terms, a new set of eight ANCOVA models were defined without an interaction term. Results show a significant and positive relation between Al and each of the 8 metals, but no significant effect of substrate type for 6 of 8 metals (Cd, Cr, Cu, Ni, V, Zn; Fig. 3; Table 2). This indicates no significant difference in intercepts between the regression lines of biofilm-sediment mixtures and surface sediment for these 6 metals and that the normalized metal concentrations can be readily compared between the substrate types. Although surface sediment has a higher intercept for Be and Pb than biofilm-sediment mixtures, we proceed to include them in the analyses below, but consider their interpretation less well constrained (Fig. 3; Table 2).

Table 1

Results of a series of analysis of covariance (ANCOVA) tests run with the interaction term to determine if the slopes and intercepts differ for relations between concentrations of Al and the 8 metals of concern in biofilm-sediment mixtures versus surface sediment. The table presents the F-test statistic, p-value, and degrees of freedom for each model term (Al concentration, substrate type, interaction between Al and substrate type) for each metal investigated. Degrees of freedom for residuals are also shown. Statistically significant p-values (at alpha = 0.05) are identified in bold text.

Metal	Factor	F-statistic	p-value	Degrees of freedom
Be	Al	2664.235	**$<2.2 \times 10^{-16}$**	1
	Substrate type	8.163	**0.009**	1
	Al x Substrate type	1.027	0.322	1
	Residuals			22
Cd	Al	44.207	**1.1×10^{-6}**	1
	Substrate type	2.037	0.168	1
	Al x Substrate type	0.080	0.780	1
	Residuals			22
Cr	Al	852.420	**$<2 \times 10^{-16}$**	1
	Substrate type	2.675	0.116	1
	Al x Substrate type	0.083	0.776	1
	Residuals			22
Cu	Al	339.582	**7.32×10^{-15}**	1
	Substrate type	0.762	0.392	1
	Al x Substrate type	1.654	0.212	1
	Residuals			22
Pb	Al	1501.601	**$<2 \times 10^{-16}$**	1
	Substrate type	8.763	**0.007**	1
	Al x Substrate type	0.032	0.859	1
	Residuals			22
Ni	Al	168.792	**1.66×10^{-11}**	1
	Substrate type	2.209	0.152	1
	Al x Substrate type	0.037	0.849	1
	Residuals			22
V	Al	1098.742	**$<2 \times 10^{-16}$**	1
	Substrate type	2.531	0.126	1
	Al x Substrate type	0.396	0.536	1
	Residuals			22
Zn	Al	387.090	**1.88×10^{-15}**	1
	Substrate type	0.031	0.863	1
	Al x Substrate type	1.056	0.315	1
	Residuals			22

Table 2
Results of a series of analysis of covariance (ANCOVA) tests run without the interaction term to determine if intercepts differ for relations between concentrations of Al and the 8 metals of concern in biofilm-sediment mixtures versus surface sediment. The table presents the F-test statistic, p-value, and degrees of freedom for each model term (Al concentration, substrate type) for each metal investigated. Degrees of freedom for residuals are also shown. Statistically significant p-values (at alpha = 0.05) are identified in bold text.

Metal	Factor	F	p	Degrees of freedom
Be	Al	2661.077	**$<2 \times 10^{-16}$**	1
	Substrate type	8.154	**0.009**	1
	Residuals			23
Cd	Al	46.050	**6.39×10^{-7}**	1
	Substrate type	2.122	0.159	1
	Residuals			23
Cr	Al	887.806	**$<2 \times 10^{-16}$**	1
	Substrate type	2.786	0.109	1
	Residuals			23
Cu	Al	330.189	**3.87×10^{-15}**	1
	Substrate type	0.741	0.398	1
	Residuals			23
Pb	Al	1570.691	**$<2 \times 10^{-16}$**	1
	Substrate type	9.166	**0.006**	1
	Residuals			23
Ni	Al	176.520	**5.46×10^{-12}**	1
	Substrate type	2.310	0.143	1
	Residuals			22
V	Al	1128.400	**$<2 \times 10^{-16}$**	1
	Substrate type	2.600	0.121	1
	Residuals			23
Zn	Al	386.150	**7.1×10^{-16}**	1
	Substrate type	0.030	0.863	1
	Residuals			23

3.2. Assessment of metal concentrations in biofilm-sediment mixtures relative to pre-1920 baselines

The dataset used to establish pre-1920 baseline linear relations between the 8 metals of interest (Be, Cd, Cr, Cu, Ni, Pb, V, Zn) and Al was derived from previous studies (Kay et al., 2020; Owca et al., 2020), plus coarser-grained riverbank sediment deposited before 1920 that captured Al concentrations comparable to the low values in the biofilm-sediment mixtures (Fig. 4). Statistically significant (at alpha = 0.05) positive linear relationships between all metals in both sectors and Al enabled development of Al-normalized baselines (Table 3). High R^2 values of the linear regressions support the inclusion of both lake sediments and riverbank sediments to develop the pre-1920 baselines. Slopes between concentrations of all metals, except Cr, and concentrations of Al are higher in lakes in the Peace sector than in the Athabasca sector. We note that Al-normalized baselines for Cd and Cu in the Athabasca sector were not possible with former datasets that did not include the riverbank sediment samples (Kay et al., 2020; Owca et al., 2020).

The organic content of biofilm-sediment mixtures varied among lakes (Figs. 5 and 6). This is consistent with observation of different textures and amounts of homogenous material, ranging in colour from brown to bright green, accrued on the shields (Fig. 2). Nonetheless, most metal concentrations in biofilm-sediment mixtures that accrued on artificial substrate samplers in 2017 and 2018 are captured within the pre-1920 baseline metal concentrations for the Peace and Athabasca sectors (Figs. 5 and 6). In 2017, concentrations of Be, Cr, Ni, Pb, and V are primarily along the baseline linear relations with Al concentration in both sectors, although concentrations of Cr, Ni, and V in the Peace sector and Be in the Athabasca sector more closely follow the lower 95% PI (Fig. 5). In contrast, concentrations of Cd, Cu, and Zn are well above the upper 95% PI for several OM-rich (>60% OM) biofilm-sediment mixtures at the low end of the Al concentration range, and this is more apparent in the Peace sector than the Athabasca sector. The relations between metal concentrations in biofilm-sediment mixtures and pre-1920 baselines are similar for samples obtained in 2018 (Fig. 6). Concentrations of metals are within pre-1920 baselines for both sectors, apart from some OM-rich exceptions for Cd, Cu, and Zn. In contrast to 2017, a small number of biofilm-sediment mixtures in 2018 also have concentrations of Cr, Ni, and Pb that exceed the upper 95% PIs.

Overall, metal concentrations in biofilm-sediment mixtures from both sectors in 2017 and 2018 follow similar Al-normalized concentrations as the pre-1920 baselines. The exceptional cases include primarily concentrations of Cd, Cu, and Zn in biofilms with high OM content and low Al concentration, which are above the upper 95% PI. These results indicate that the sediment-derived baselines permit calculation of residual metal concentrations (as the difference between the metal concentration in the biofilm and the baseline linear relation) and comparison to enrichment thresholds (i.e., the upper 95% PI) to quantify and interpret the extent of enrichment above the range of natural variability.

3.3. Assessment of residual concentrations of metals for enrichment

For lakes from which biofilm-sediment mixtures were collected in both study years, concentrations of metals in the mixtures were converted to residual concentrations relative to baseline concentrations, and are displayed as a series of boxplots in Figs. 7 and 8 for

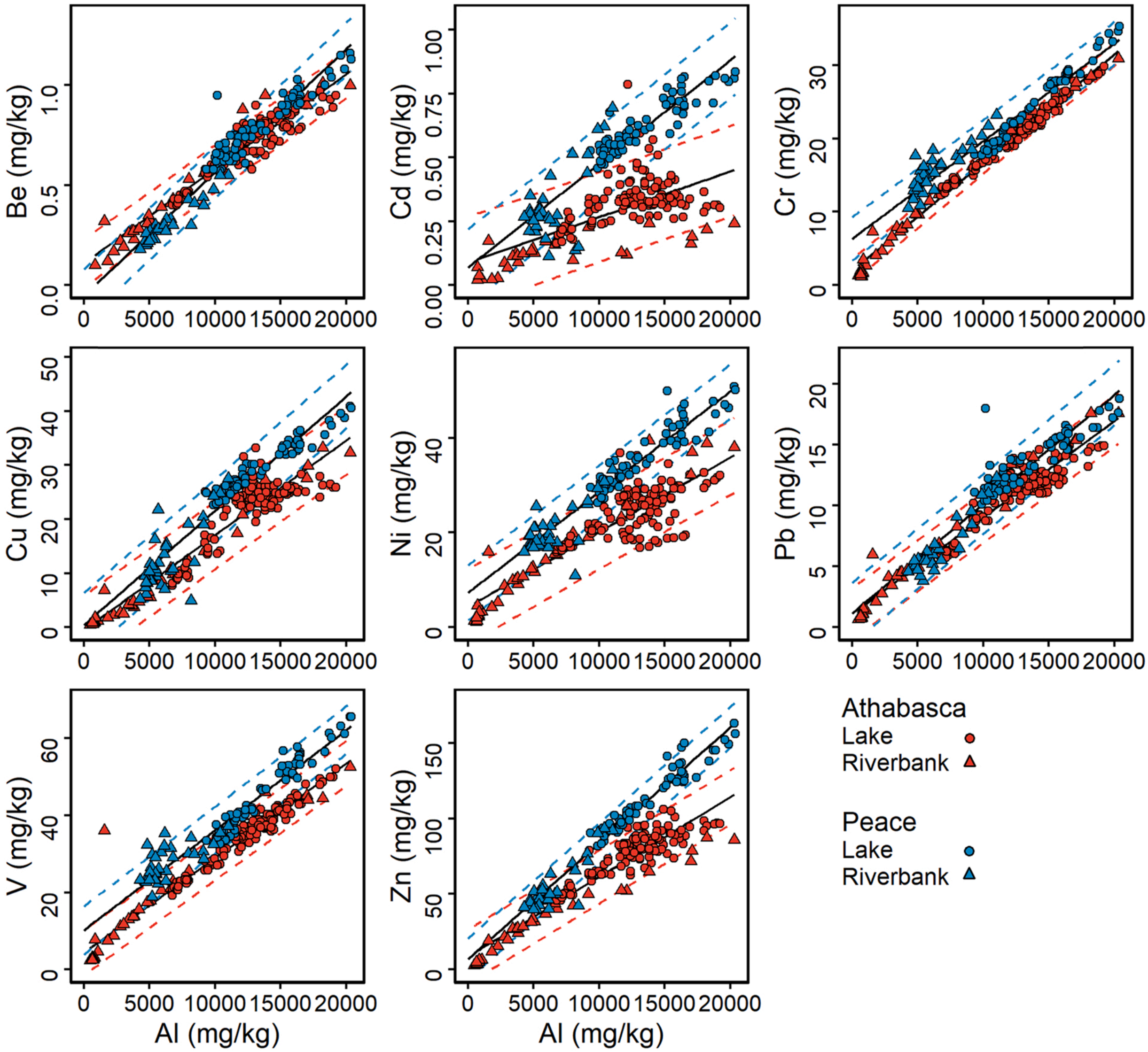

Fig. 4. Cross-plots demonstrating linear relations between pre-1920 metal concentrations and the normalizer (Al) in lake and riverbank sediment samples. The Peace River 95% PI (blue dashed line) and regression lines are based on pre-1920 measurements of metals from lake sediment (PAD 65, PAD 67; blue circles; n = 65) and riverbank sediment (blue triangles; n = 33). The Athabasca River 95% PI (red dashed lines) and regression lines are based on pre-1920 measurements of metal concentrations from lake sediment (PAD 26, PAD 30, PAD 31, PAD 32, M2, M5, M7, and PAD 71; red circles; n = 122) and riverbank sediment (red triangles; n = 37 for all metals, except n = 26 for Be, n = 29 for Cd, and n = 34 for Cr).

lakes in the Athabasca and Peace sectors, respectively. Lakes are categorized by flood-category ('0′ = not flooded in 2017 nor in 2018, '1′ = flooded in 2018 only, '2′ = flooded in both 2017 and 2018) and measurements are colour-coded by study year (dark = 2017, light = 2018), generating 6 groups for each of 8 metals, and a total of 48 groups in the Athabasca sector, and 4 groups for each of 8 metals, for a total of 32 groups in the Peace sector. There were fewer groups for the Peace sector because none of the lakes received floodwaters in both years.

We assess for enrichment of each metal above the range of natural variability by comparison of the upper whisker of the boxplot for each group against the enrichment thresholds for each metal. The upper whiskers of residual metal concentrations are below the enrichment thresholds for most groups, indicating concentrations in the biofilm-sediment mixtures are within the range of natural variation that existed before 1920 (Figs. 7 and 8; Table 4). In the Athabasca sector, there are six exceptions to this general pattern among the 48 groups: 1) enrichment of Cr above the threshold is observed for flood-category '2′ lakes in 2018; 2–4) enrichment of Cu above the threshold is observed for all three flood-categories in 2017; 5) enrichment of Ni above the threshold is observed for flood-category '2′ lakes in 2018, and, 6) enrichment of Zn above the threshold is observed for flood-category '0′ lakes in 2017 (Fig. 7). In the Peace sector, there are nine exceptions to this general pattern among the 32 groups: 1–2) enrichment of Cd above the threshold is observed for flood-category '0′ and flood-category '1′ lakes in 2017; 3–6) enrichment of Cu above the threshold is observed for lakes in

Table 3
Regression equations and adjusted R-squared values for pre-1920 baseline metal-Al linear regressions for the Peace and Athabasca sectors. All p-values are $< 2.2 \times 10^{-16}$.

Sector	Metal	Regression equation	Adjusted R-squared
Peace	Be	$y = 6.167\times10^{-5}$- 0.05342	0.9445
	Cd	$y = 4.056\times10^{-5} + 0.06952$	0.8539
	Cr	$y = 1.332 \times 10^{-3} + 6.320$	0.9422
	Cu	$y = 2.113\times10^{-3} + 0.4144$	0.9094
	Ni	$y = 2.126\times10^{-3} + 7.301$	0.9155
	Pb	$y = 8.973\times10^{-4} + 1.148$	0.9106
	V	$y = 2.593\times10^{-3} + 10.21$	0.9328
	Zn	$y = 7.666 \times 10^{-3} + 6.874$	0.9620
Athabasca	Be	$y = 4.730\times10^{-5} + 0.1092$	0.9145
	Cd	$y = 1.787 \times 10^{-5} + 0.08797$	0.4304
	Cr	$y = 1.478 \times 10^{-3} + 1.934$	0.9882
	Cu	$y = 1.756 \times 10^{-3} + 0.5241$	0.8706
	Ni	$y = 1.577 \times 10^{-4} + 4.323$	0.7895
	Pb	$y = 7.935 \times 10^{-4} + 1.086$	0.9302
	V	$y = 2.458 \times 10^{-3} + 4.328$	0.9438
	Zn	$y = 5.257 \times 10^{-3} + 8.4858$	0.8918

flood-categories ‘0′ and ‘1′ in 2017 and in 2018; 7) enrichment of Ni above the threshold is observed for flood-category ‘0′ lakes in 2018; and, 8–9) enrichment of Zn above the threshold is observed for lakes in flood-categories ‘0′ and ‘1′ in 2017 (Fig. 8). The combined total of these 15 exceptions in both sectors identifies that most occurrences of enrichment (11 of 15) are associated with measurements of Cd, Cu, Ni, and Zn at lakes that did not flood. Two of the remaining occurrences of enrichment (2 of 15) are for Cu at lakes that received floodwaters: in 2017 at flood-category ‘2′ lakes in the Athabasca sector, and in 2018 at flood-category ‘1′ lakes in the Peace sector. But, enrichment of Cu occurred at all flood-categories in 2017 in the Athabasca sector and at all flood-categories in 2018 in the Peace sector. The last two occurrences (2 of 15) are for Cr and Ni in 2018 at flood-category ‘2′ lakes in the Athabasca sector that received floodwaters that year. However, these flood-category ‘2′ lakes do not exhibit enrichment of Cr and Ni in 2017 when they also flooded, nor do flood-category ‘1′ lakes that flooded in 2018. Thus, there is no consistent association between enrichment of any metal and lakes that received floodwaters from the Athabasca or Peace rivers.

3.4. Statistical tests of residual concentrations of metals among flood-categories and between study years within each sector

Repeated-measures two-way MANOVA tests were conducted on the subset of 31 lakes where artificial substrate samplers were retrieved in both study years to provide a paired comparison, by lake, of residual metal concentrations among flood-categories. Two tests were run to assess if residual concentrations of the suite of 8 metals differs among 1) the 6 groups in the Athabasca sector (2 years x 3 flood-categories (0, 1, 2)), and 2) the 4 groups in the Peace sector (2 years x 2 flood-categories (0, 1)). The interaction terms (Athabasca, MATS = 12.268, P = 0.553, d.f. = 2, 12, 14; Peace, MATS = 9.246, P = 0.298, d.f. = 2, 3, 15) were not significant (Table 5), thus the interaction term was removed from the model and each factor was tested individually. Residual concentrations of the 8 metals did not differ significantly among the flood-categories in either sector (Athabasca, P = 0.124; Peace, P = 0.167), suggesting occurrence of flooding in one or both study years does not alter concentrations relative to non-flooded lakes (Table 5). Consistent with this result, the pooled boxplots for both study years illustrate broad overlap of interquartile ranges for most metals in all flood-categories for both sectors (i.e., first 8 panels of Figs. 7 and 8). The MANOVA tests also determined that residual concentrations of all 8 metals do not differ significantly between 2017 and 2018 in the Athabasca sector (P = 0.171; Table 5), but differ significantly between 2017 and 2018 in the Peace sector (P = 0.007; Table 5). A non-significant inter-annual difference in the Athabasca sector is depicted by differences in residual concentrations (2018 residual – 2017 residual) which have interquartile ranges that intersect zero for all metals (last panel of Fig. 9). In contrast, a significant inter-annual difference in the Peace sector is depicted by interquartile ranges of differences in residual concentrations that are substantially below zero for Zn, and marginally below zero for V (last panel of Fig. 10). Thus, residual concentrations of metals remained relatively unchanged from 2017 to 2018, except for Zn and, to a lesser extent, V in the Peace sector, which decreased from 2017, when there was no lake flooding, to 2018, when a few lakes received floodwaters that spring.

4. Discussion

Biofilm-sediment mixtures have long been explored as monitors for metal pollution (Pederson and Vaultonburg, 1996; Ramelow et al., 1987, 1992), and here we demonstrate a novel application of these media with reference to baseline metal concentrations measured in pre-industrial (pre-1920) lake sediments. Comparison of the two media is supported by statistically equivalent relations between Al and 6 metals of concern (Cd, Cr, Cu, Ni, V, Zn) in biofilm-sediment mixtures and surface sediments that capture similar timeframes of metal accumulation. Statistically higher normalized concentrations of Be and Pb in surface sediment compared to biofilm-sediment mixtures weakens ability to detect enrichment of these metals in biofilm-sediment mixtures relative to the sediment-derived pre-1920 baselines. However, the low concentrations and tight linear clustering of Be and Pb relative to Al in the

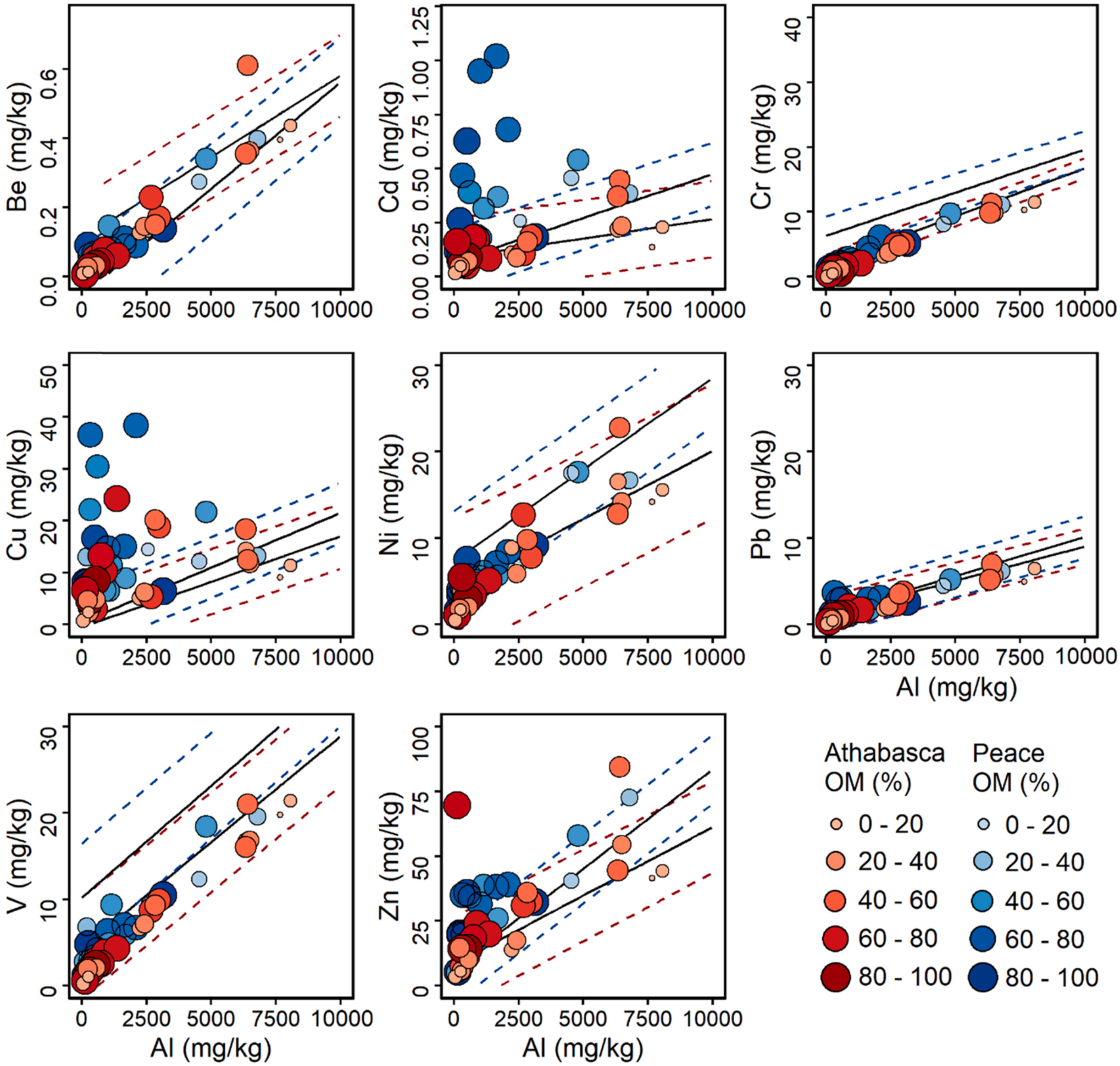

Fig. 5. Metal concentrations in biofilm-sediment mixtures accrued from May to September 2017 from Peace sector lakes (blue circles) and Athabasca sector lakes (red circles) plotted on the pre-1920 linear regressions and 95% PIs. Circles are sized according to organic matter (OM) content.

biofilm-sediment mixtures are unlikely to reflect enrichment (Figs. 5 and 6).

Analysis of biofilm-sediment mixtures accrued in lakes of the PAD shows no evidence to support that river floodwaters are a pathway of contemporary metal enrichment above the baseline concentrations. This finding aligns with results from previous studies based on analyses of lake sediment cores and surface sediments of lakes and rivers in the PAD (Kay et al., 2020; Owca et al., 2020; Wiklund et al., 2014). The few occurrences of metal enrichment above the range of pre-1920 variability (15/80 groups) are primarily associated with lakes that did not receive floodwaters (11/15 occurrences). Furthermore, the occurrences of metal enrichment are about two-fold more common in the Peace sector (9/32 groups or 28.1%) than the Athabasca sector (6/48 groups or 12.5%) despite that the Athabasca River, not the Peace River, traverses through a region of oil sands surface mining.

Our assessment of enrichment is contingent on well-defined enrichment thresholds. Strong baseline linear relationships, demonstrated by adjusted R-squared values greater than 0.85 for all baselines except Cr and Ni in the Athabasca sector, indicate that the natural range of variability is well characterized. Due to minimal flare in 95% PIs, even at low Al concentrations, the Al concentration selected for enrichment threshold calculations was of no consequence on assessment of enrichment. For example, the same groups of lakes were designated as enriched when an Al concentration of 5000 mg/kg versus 1000 mg/kg was used to calculate enrichment thresholds. Furthermore, a group of lakes was designated as enriched even if only one lake in the group, excluding outliers, had a

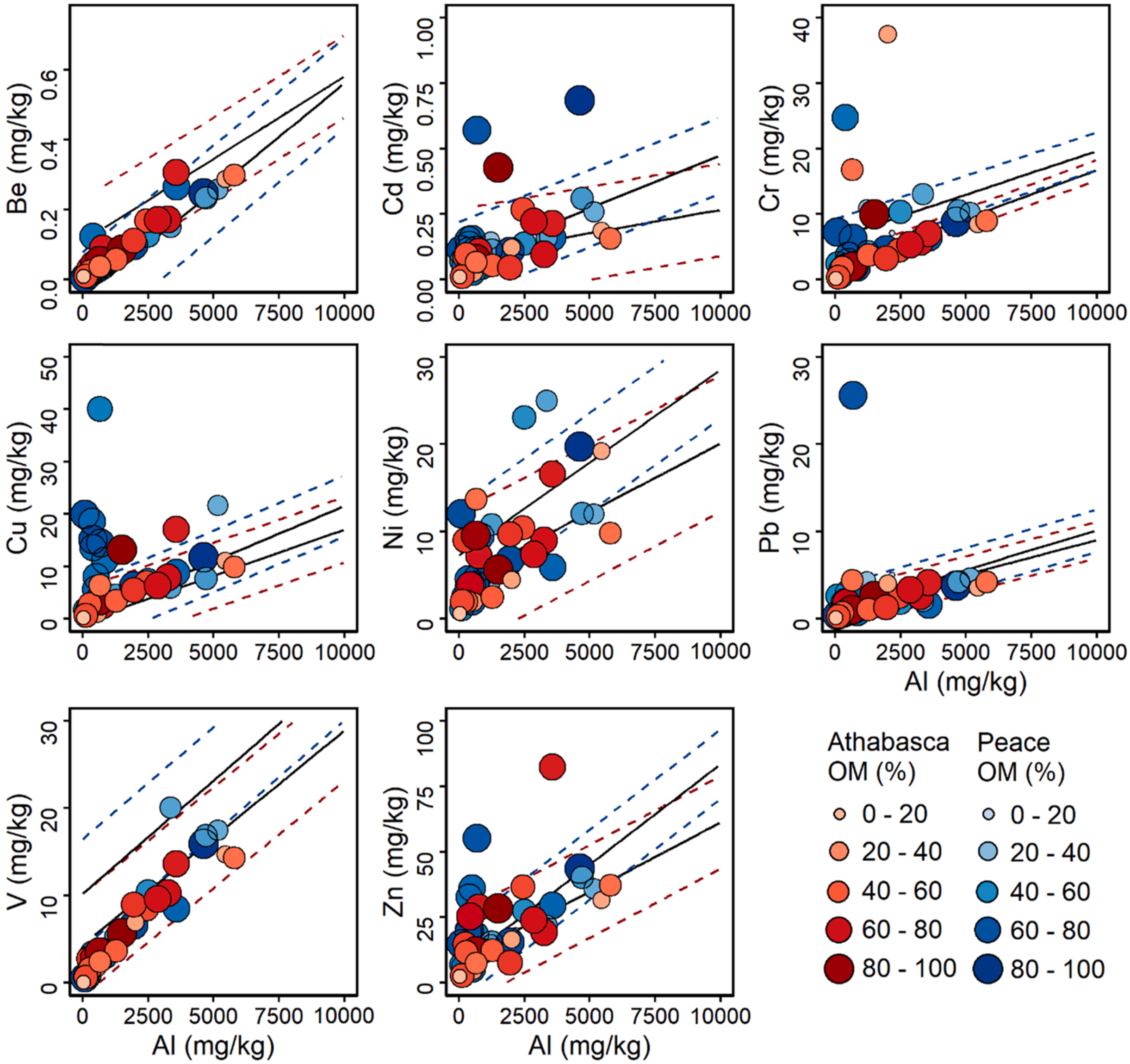

Fig. 6. Metal concentrations from biofilm-sediment mixtures accrued from May to September 2018 from Peace sector lakes (blue circles) and Athabasca sector lakes (red circles) plotted on the pre-1920 linear regressions and 95% PIs. Circles are sized according to organic matter (OM) content.

residual metal concentration greater than the enrichment threshold. These features lend confidence to the sensitivity of our calculated enrichment thresholds and approach for assessing enrichment.

The study design was able to discern the role of river floodwaters on metal enrichment because it incorporated measurements from a year with minimal flooding (2017) and a year with a spring ice-jam flood event that inundated many lakes in the Athabasca sector (2018; Remmer et al. 2020a). This provided opportunity to compare inter-annual differences in residual concentrations of metals at lakes that did not flood in either year ("negative control"), lakes that flooded in 2018 but not 2017 ("treatment"), and lakes that flooded in both years ("positive control"). Statistical outcomes, namely non-significant interaction terms between the two factors (study year and flood-category), indicate the inter-annual differences in residual metal concentrations are similar among the control groups and the treatment groups. Statistical test outcomes also demonstrate that residual metal concentrations do not differ significantly among lakes that flooded in none, one or both of the study years, as illustrated by the substantial overlap of values in flooded versus non-flooded lakes (i.e., flood-category '0′ vs. flood-category '2′, Fig. 9; flood-category '0′ vs. flood-category '1′ in 2018, Fig. 8), and values in lakes that flooded in 2018 with previous measurements in 2017 when they were not flooded (i.e., 2017 vs. 2018 in flood-category '1′, Figs. 7 and 8). An additional strength of the study design was inclusion of some spring-flooded lakes that also flooded in summer of 2017 and 2018, so supply of metals via summer river flow was captured in this study.

More than one occurrence of enrichment is discernable only for metals Cd, Cu and Zn, and primarily in non-flooded lakes where the

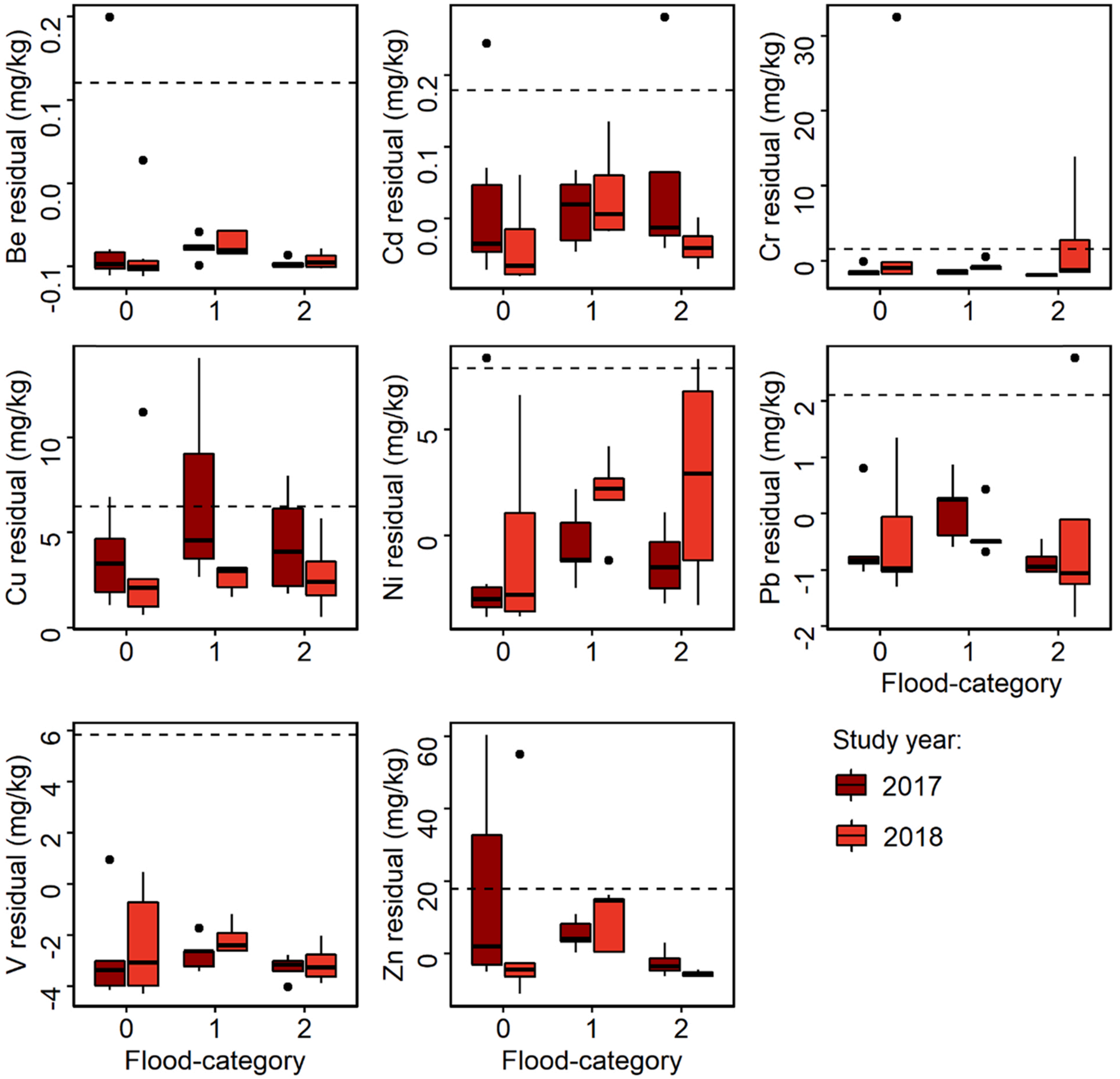

Fig. 7. Clustered boxplots showing distribution of residual concentrations of metals from pre-1920 baselines in biofilm-sediment mixtures collected from lakes in the Athabasca sector. Lakes are categorized by flood year, where "0" represents lakes that did not flood in 2017 nor in 2018 (n = 6) and "1" represents lakes that flooded in 2018 but not in 2017 (n = 5), and "2" represents lakes that flooded in both 2017 and 2018 (n = 4). Dark red boxes represent the 2017 study year and light red boxes represent the 2018 study year. Dashed lines indicate the enrichment threshold, which represents the residual concentration metal corresponding to the upper 95% prediction interval for a metal residual at an Al value of 1000 mg/kg based on pre-1920 baselines for the Athabasca sector.

artificial substrate samplers accrued organic-rich biofilm-sediment mixtures. Thus, enrichment of these metals is not caused by river floodwaters, as is also demonstrated by equivalent metal-Al relations among the biofilm-sediment mixture and surface sediment datasets for spring-flooded lakes in 2018 (Fig. 3) and the pre-1920 baselines (Fig. 6), and more likely reflects active intracellular uptake by periphytic biota in the absence of flooding. In support of this inference, it is known that photosynthetic organisms concentrate Cu and Zn within organelles to a greater degree than all other metals measured in this study (Blaby-Haas and Merchant, 2012; Epstein, 1965; Rodriguez and Ho, 2018). Evidence from other studies have shown biofilm-sediment mixtures accumulate Cu and Zn to a greater degree than sediment (Kumari and Maiti, 2020; Ramelow et al., 1992). Photosynthetic organisms transport non-essential Cd using non-specific divalent metal transporters, which results in Cd uptake during biomass accrual (Blaby-Haas and Merchant, 2012; Rodriguez and Ho, 2018; Hill et al., 2000). Owca et al. (2020) demonstrated that surface sediments collected from the same lakes in the same years did not exhibit enrichment in Cd, Cu, and Zn. Hence, active biological uptake by periphytic biota appears to complicate comparisons of these three metals between organic-rich biofilm-sediment mixtures accrued in non-flooded lakes and baselines

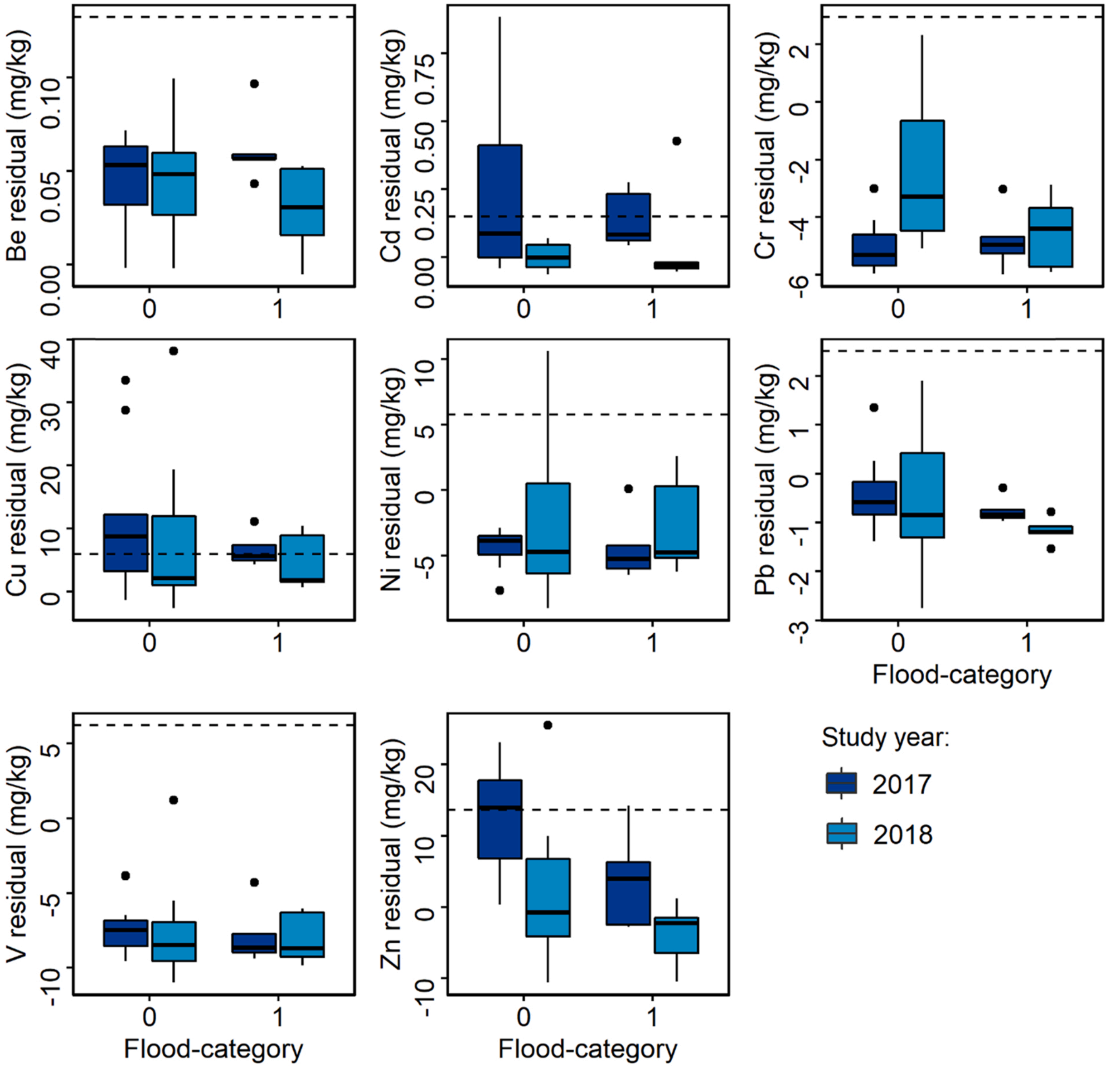

Fig. 8. Clustered boxplots showing distribution of residual concentrations of metals from pre-1920 baselines in biofilm-sediment mixtures collected from lakes in the Peace sector. Lakes are categorized by flood year, where "0" represents lakes that did not flood in 2017 nor in 2018 (n = 11) and "1" represents lakes that flooded in 2018 but not in 2017 (n = 5). Dark blue boxes represent the 2017 study year and light blue boxes represent the 2018 study year. Dashed lines indicate the enrichment threshold, which represents the residual concentration of metal corresponding to the upper 95% prediction interval at an Al value of 1000 mg/kg based on pre-1920 baselines for the Peace sector.

constructed using sediment deposited in flood-prone lakes. However, accumulation of Cu, Zn, and Cd in biota at the base of aquatic food webs is an important finding to inform potential risks to ecosystem health. Fortunately, for Cd and Zn, these results do not compromise ability to assess metal enrichment in biofilm-sediment mixtures in flooded lakes.

Biofilm-sediment mixtures are a viable approach for 'real-time' monitoring of enrichment of metals in flooded lakes (Be, Cd, Cr, Ni, Pb, V, Zn) and non-flooded lakes (Be, Cr, Ni, Pb, V) of the PAD given the alignment of their metal-Al concentrations with pre-1920 baselines. Comparison to sediment-derived pre-industrial baselines is a novel application of biofilm-sediment mixtures, and provides critical context for assessment of pollution in a landscape where substances of concern are supplied by natural processes as well as potentially by industrial releases to the environment. Ability to inform about enrichment in biota at the base of aquatic food webs and to control the duration of biofilm accrual, by setting the time of deployment and retrieval of the artificial substrate samplers, complements assessments based on systematic analysis of surficial sediment from lakes with inherent variation and uncertainty of their sediment accumulation rates (Owca et al., 2020). This research directly addresses a recommendation for strategic environmental assessment included in the WBNP Action Plan by providing information about metals supplied by river floodwaters after they flow

Table 4
Summary of enrichment thresholds for metals (Be, Cd, Cr, Cu, Ni, Pb, V, Zn) for lakes in each sector of the Peace-Athabasca Delta. Enrichment thresholds represents the residual concentration of metal corresponding to the upper 95% prediction interval at an Al value of 1000 mg/kg based on the pre-1920 baselines.

Metal	Peace sector enrichment threshold (mg/kg)	Athabasca sector enrichment threshold (mg/kg)
Be	0.1323	0.1204
Cd	0.1498	0.1794
Cr	2.9465	1.5697
Cu	5.9568	6.3578
Ni	5.7715	7.9111
Pb	2.5114	2.1127
V	6.2172	5.8320
Zn	13.6122	17.8708

Table 5
Summary of results of two repeated-measures two-way MANOVA tests used to assess differences in residual concentrations of metals between study years (within-subject factor), and among flood-categories (between-subjects factor).

	Factor	Modified ANOVA-type statistic	p-values paramBS (MATS)	$d.f._{Groups}$, $d.f._{Error}$, $d.f._{Total}$
Athabasca sector	Flood-category	53.077	0.124	2, 12, 14
	Study year	11.980	0.171	1, 13, 14
Peace sector	Flood-category	16.026	0.167	2, 13, 15
	Study year	31.259	0.007	1, 14, 15

through a heavily industrialized area (Wood Buffalo National Park WBNP, 2019), and provides a monitoring framework to consider for future implementation across lakes of the PAD.

To optimize a monitoring program for lakes in the PAD, we recommend deploying artificial substrate samplers at the beginning of the ice-free season (i.e., mid-May) to capture potential effects of spring ice-jam floods. Dual sampling of surface sediment and biofilm-sediment mixtures is ideal for reliable monitoring of both biologically active (Cd, Cu, Zn) and biologically inert (Be, Cr, Ni, Pb, V) metals. Preference for monitoring may be given to flood-prone lakes in the Athabasca sector as they are most vulnerable to potential pollution from upstream oil sands mining. However, we recommend that monitoring also include non-flooded lakes in the Athabasca sector, and flooded and non-flooded lakes in the Peace sector, which are unlikely to be affected by AOSR oil sands mining, to serve as informative benchmarks for determination of metal enrichment by oil sands mining and processing activities. The approach developed here may be adapted for implementation at other lake-rich and floodplain landscapes but will require firm understanding of baseline metal concentrations against which metal concentrations in biofilm-sediment mixtures can be compared. Among the many options for characterizing baseline concentrations, we advocate for analysis of metal concentrations in samples from cores of lake sediment deposited prior to possible pollution by anthropogenic activities of concern.

5. Conclusions

This research explored roles of river flooding on enrichment of metals of concern at the base of food webs in lakes of the PAD. Assessment of contemporary metal concentrations in biofilm-sediment mixtures in lakes of the PAD relative to pre-industrial (pre-1920) concentrations of metals in lake sediment demonstrate no substantial enrichment of oil sands-derived metal concentrations supplied by Athabasca River floodwaters. The absence of marked metal enrichment in flooded lakes, as well as the lack of difference in enrichment among lakes of different flood status, provide evidence that oil sands industrial metal pollution is not detectable at the base of food webs in lakes of the PAD. Results presented here demonstrate that biofilm-sediment mixtures are useful media for monitoring contemporary concentrations of metals and complement a proposed monitoring framework relying on contemporary concentrations of metals in surface sediment in the PAD (Owca et al., 2020).

Linking timing of flooding with timing of metals accumulation is a distinct advantage of artificial substrate samplers but is contingent on routine isotope monitoring of lake hydrological conditions, as was performed throughout the study years of this research (Remmer et al., 2020b). Alternately, oxygen isotope composition of aquatic cellulose synthesized by periphytic algae present in biofilm-sediment mixtures may also be used to track lake water balance and hydrological processes during the period of accrual (Savage et al., 2021). This possibility, along with other possible metrics indicative of ecological integrity that can be gleaned from biofilm-sediment mixtures (e.g., algal community composition, biomarkers related to metal-stress, deformed diatom cells; Lavoie et al., 2012; MacDonald et al., 2012; Millie et al., 1993), may provide additional opportunities for long-term aquatic ecosystem monitoring.

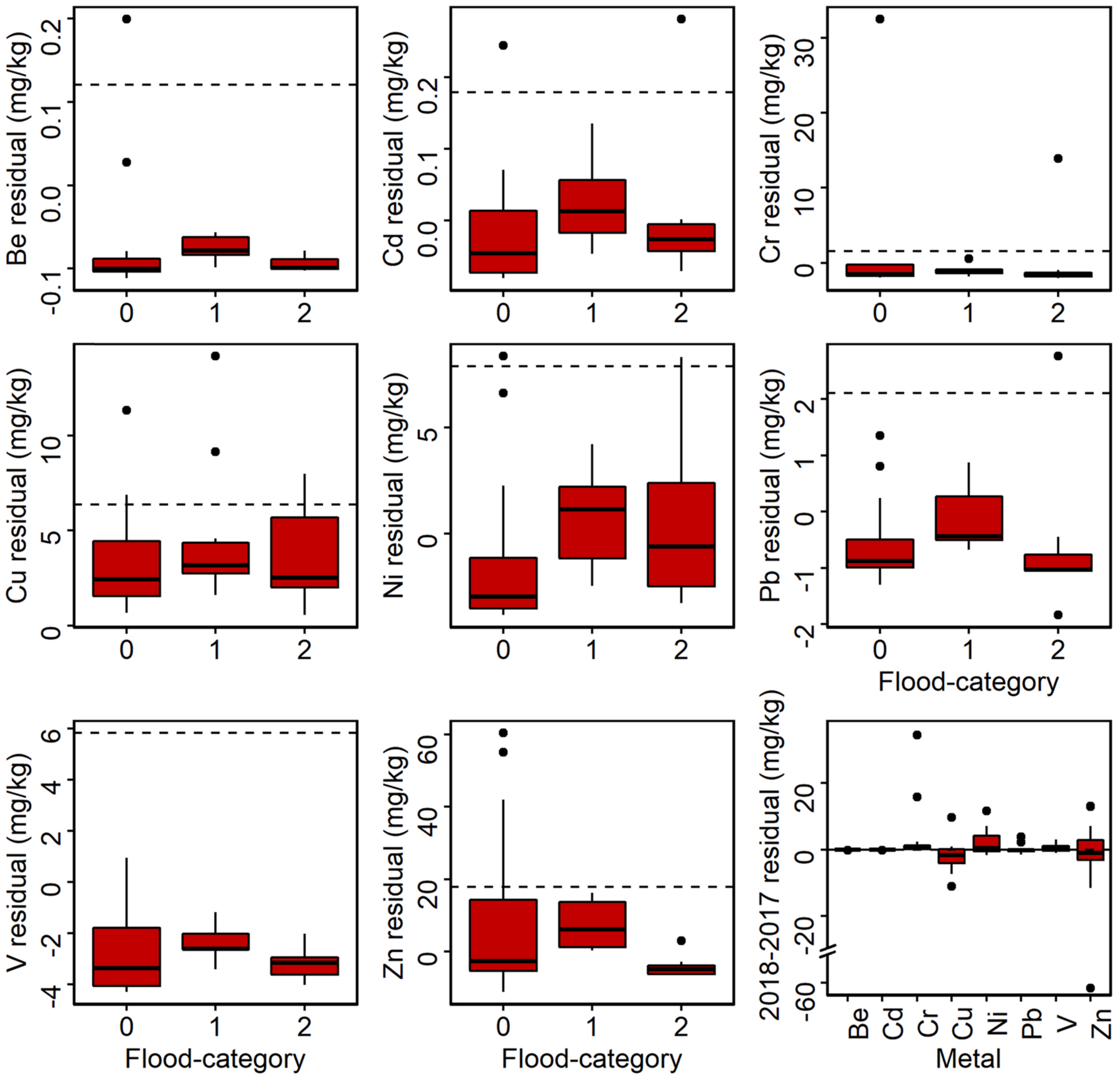

Fig. 9. First 8 panels of boxplots show distribution of residual concentrations of metals in biofilm-sediment mixtures collected from lakes in the Athabasca sector in 2017 and 2018 relative to pre-1920 baselines. Lakes are categorized by flood-category, where “0” represents lakes that did not flood in 2017 nor in 2018 (n = 6), “1” represents lakes that flooded in 2018 but not in 2017 (n = 5), and “2” represents lakes that flooded in both 2017 and 2018 (n = 4). Dashed lines indicate the enrichment threshold, which represents the residual concentration metal corresponding to the upper 95% prediction interval for a metal residual at an Al value of 1000 mg/kg based on pre-1920 baselines for the Athabasca sector. Last panel of boxplots shows the residual differences between study years, calculated by subtracting the 2017 concentration from the 2018 concentration for each lake.

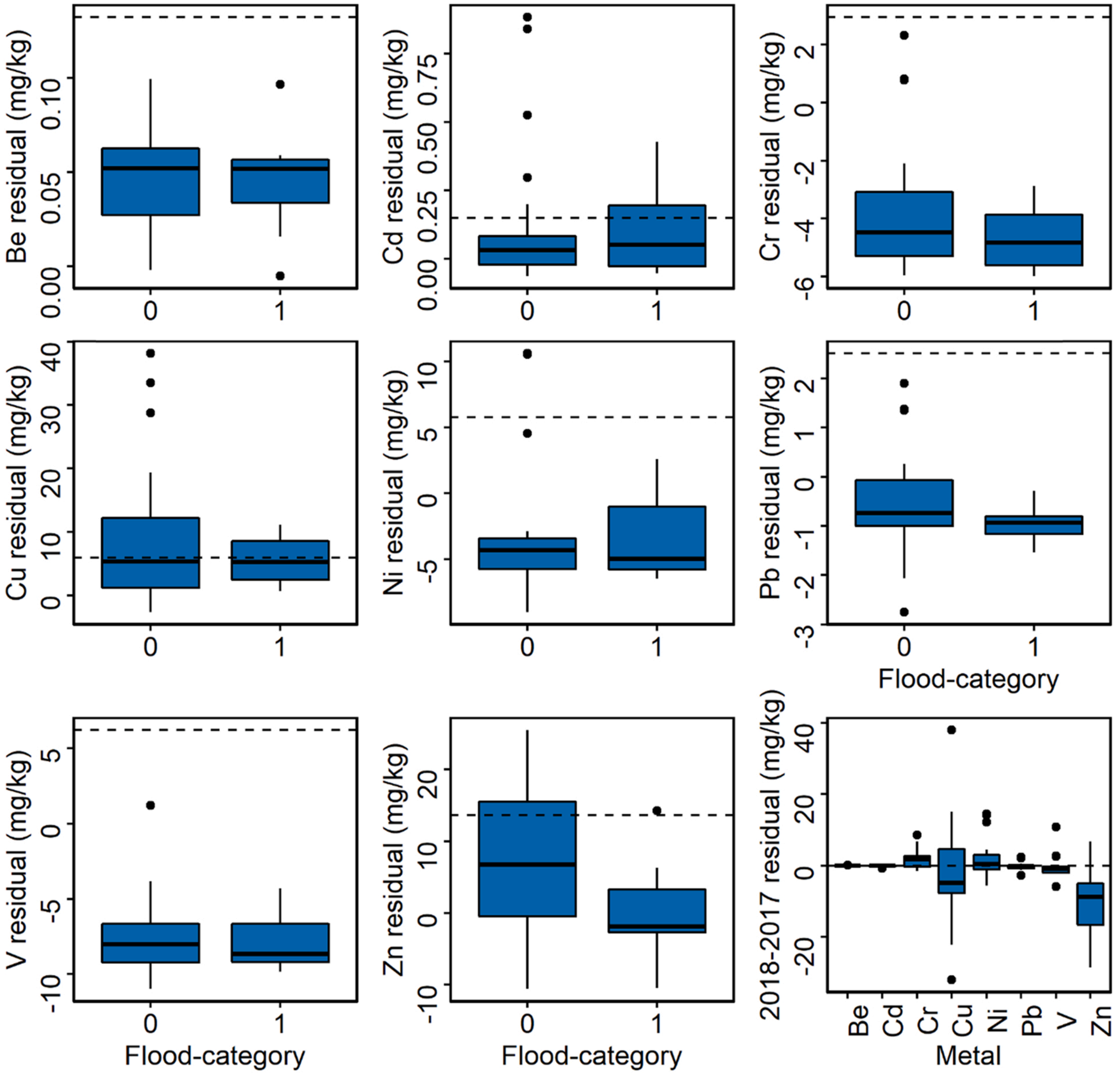

Fig. 10. First 8 panels of boxplots show distribution of residual concentrations of metals in biofilm-sediment mixtures collected from lakes in the Peace sector in 2017 and 2018 relative to pre-1920 baselines. Lakes are categorized by flood year, where "0" represents lakes that did not flood in 2017 nor in 2018 (n = 11) and "1" represents lakes that flooded in 2018 but not in 2017 (n = 5). Dashed lines indicate the enrichment threshold, which represents the residual concentration of metal corresponding to the upper 95% prediction interval at an Al value of 1000 mg/kg based on pre-1920 baselines for the Peace sector. Last panel of boxplots shows the residual differences between study years, calculated by subtracting the 2017 concentration from the 2018 concentration for each lake.

CRediT authorship contribution statement

C. A. M. Savage: Conceptualization, Formal analysis, Investigation, Data collection, Writing – original draft; **T. Owca**: Data collection, Investigation; **M. L. Kay**: Data collection, Formal analysis, Investigation; **J. Faber**: Data collection, Investigation; **B. B. Wolfe**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition; **R. I. Hall**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by funding to RIH and BBW from Natural Sciences and Engineering Research Council of Canada Discovery [2016-03630 to RIH, 2017-05462 to BBW] and Northern Research Supplement grants [2016-305405 to RIH, 2017-391558 to BBW], NSERC Collaborative Research and Development grant [CRDPJ47399-14 to RIH and BBW], the Polar Continental Shelf Program of Natural Resources Canada [62417, 64018 to BBW], Global Water Futures [Northern Water Futures, to BBW and RIH], and Polar Knowledge Canada [Northern Scientific Training Program, to CAMS]. Funding sources had no role in the study design or decision to publish this article. CAMS was supported by an NSERC Alexander Graham Bell Canada Graduate Scholarship (Master's) and a University of Waterloo President's Scholarship. We are grateful to Wood Buffalo National Park for logistical support of the fieldwork.

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