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Toxic Burdens of Freshwater Biofilms and Use as a Source Tracking Tool in Rivers and Streams

William O. Hobbs,*[,†] Scott A. Collyard,[†] Chad Larson,[†] Andrea J. Carey,[‡] and Sandra M. O'Neill[‡]

[†]Washington State Department of Ecology, P.O. Box 47600, Olympia, Washington 98502, United States

[‡]Washington Department of Fish and Wildlife, P.O. Box 43150, Olympia, Washington 98504, United States

Supporting Information

ABSTRACT: Biofilms, composed of periphyton, bacteria, and organic detritus, are the base of the food web in many streams and rivers. This media adsorbs and actively sequesters organic and inorganic contaminants from the water column. Here, we demonstrate the utility of using the contaminant concentrations in the biofilm matrix as an environmental media in source tracking and understanding biological impacts at higher trophic levels. Physical partitioning of polychlorinated biphenyl (PCB) and polybrominated diphenyl ether congeners is the dominant mode of uptake from water to biofilm and bioaccumulation factor: log K_{ow} relationships suggest that PCB uptake is often near equilibrium between log K_{ow} 5−7. We show that the concentrations of metals in biofilms are more effective at delineating and recording spatial and temporal differences in metal inputs than bed sediments and water samples. The burden of metals in the biofilm matrix explained adverse impacts and variability in periphyton metrics and ecological integrity in macroinvertebrates. This work provides new insights into the partitioning of organic chemicals onto biofilms and shows clear linkages between metals in the biofilm matrix and ecological health of invertebrates that depend on biofilms as a food source.

INTRODUCTION

The biofilm matrix is a collection of living and dead algae (periphyton), microbial biomass, and organic detritus, which contribute to the base of the food web in rivers and streams. In waterbodies impacted by organic and inorganic toxic contaminants, biofilms uptake and adsorb contaminants from the water.[1,2] The impacts of toxic contaminants on biofilms and periphyton have focused on both the community structure and health of the organisms[3,4] and on the impacts of contaminant burdens in biofilms on higher trophic levels.[5−7] Indeed, biofilms represent the point of entry for many toxic contaminants into freshwater food webs, leading to the accumulation of toxics in freshwater fishes at concentrations high enough to impact human health, which is common in many large rivers and streams throughout the United States.[8,9]

Periphytic communities, representing the photosynthetic component in biofilms, are often dominated by diatoms (Bacillariophyceae). Diatoms secrete extracellular polymeric substances (EPSs) that are composed of polysaccharides, glycoproteins, and other biopolymers.[10] EPS are used to produce and contribute to a variety of structures, including sheaths around the cell wall, pads, tubes, and stalks.[11] The EPS act as a means to protect the cell and sequester nutrients. This complex diatom EPS matrix also creates microhabitats for microbial growth, which produce further secretions of EPS and become a substrate for the filtering of detrital organics from overlying water. Collectively, the carbon-rich components of biofilms provide the necessary binding sites for halogenated organic chemicals and metals.

The mechanism by which toxics are adsorbed or incorporated into the algal cells or biofilm matrix differs with the availability and complexity of the compound or element. For complex halogenated organics such as polychlorinated biphenyls (PCBs), there has been no work completed documenting the empirical partitioning from water to biofilms. Some work in lakes suggests that PCBs can partition from water into phytoplankton cell lipids at a rate proportional to the octanal/water partition coefficient (log K_{ow}).[12,13] In lakes, the thermodynamic equilibrium between the dissolved phase in water and algae has been observed for compounds with a log K_{ow} in the range of 5−7,[13] and the same has been observed between water and marine phytoplankton[14] and zooplankton.[15] The available surveys of halogenated organics in stream biofilms have focused mainly on documenting bioaccumulation within a food chain or food web.[5,1,7] What is suggested from the previous work is that the carbon-rich matrix of biofilms in streams is an efficient scavenger for hydrophobic compounds in water.

The active uptake and release of dissolved forms of metals by periphyton have received more intensive study than organic chemicals.[2,16] The trace element concentrations in the waters and biofilms of streams and rivers can be influenced by the photosynthetic activity of periphyton, where the biogeochemical cycling is controlled by photosynthetically induced changes

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in pH.[17−19] The organic EPS matrix of biofilms also provides a large surface area that has an anionic charge, which is ideal for binding metals.[20] There have been many studies documenting the presence of metal-impacted waters and biofilms but few documenting the direct ecological impacts and the relationships of metal concentrations among environmental media in streams.[6,16,21] Furthermore, measured adverse impacts on higher biological communities (e.g., macroinvertebrate communities) are sometimes not properly accounted for or appropriately protected when assessing environmental media like bed sediments and water samples and associated regulatory criteria aimed at protecting the aquatic life.

Our group has conducted a number of investigations into toxic inputs to streams and rivers in Washington State using biofilms. In this paper, we present findings that address the following questions: (1) do stream and river biofilms effectively sequester and bioconcentrate halogenated organics, such as PCBs, and polybrominated diphenyl ethers (PBDEs), (2) do halogenated organic compounds reach the thermodynamic equilibrium between water and biofilms, (3) do metal concentrations in stream biofilms describe adverse impacts on periphyton and macroinvertebrate communities, and (4) can organics and metal concentrations in the biofilm matrix accurately reflect the location of contaminant sources?

METHODS

Study Sites. Data presented in this paper is drawn from a number of studies in the State of Washington. Most of the data is derived from studying rivers originating in the eastern slopes of the Cascade Mountain Range (Wenatchee River and Railroad Creek) (Figure S1). These rivers and streams are snowmelt-dominated and high-energy systems, discharge usually peaks in May−June and low flow occurs in September. They are, generally, low suspended sediment, low conductivity rivers with a substrate of sand, gravel, and cobble. The Wenatchee River is a major tributary to the Columbia River and flows 85 km (53 miles) from headwater tributaries in the mountains through a number of biogeoclimatic zones. The geology of this region is variable, comprising a number of different landforms ranging from the alpine and sub-alpine peaks of the Cascades to the low-lying Columbia plateau. The climate in this region is continental with hot, dry summers and cold, wet winters.

North of the Wenatchee River is Railroad Creek, which is a major tributary to Lake Chelan, a large freshwater fjord draining through both natural and manmade dams into the Columbia River. The headwater tributaries of Railroad Creek are marked by small glaciers above 1200 m. From 1938 to 1957, a large copper mine operated adjacent to Railroad Creek; the site was declared a USEPA superfund site in the 1980s, and mine cleanup activities have been ongoing since the mid-1990s. However, only recently have resources been invested to restore aquatic life uses at Railroad Creek.

The Nisqually River is located on the western slopes of the Cascade Mountains and originates from the Nisqually Glacier on Mount Rainier, flowing 130 km (81 miles) to the Puget Sound. It traverses montane forested regions in the upper reaches, transitioning to the Puget Sound Lowlands ecoregion where southern Puget prairie landscapes are present. The river is glacially turbid for much of the year and is hydrologically controlled for power generation at approximately river mile 40. Downstream of the dams, a number of tributaries contribute to the river as it flows through more-forested and low-density municipalities. Prior investigations of the contaminant impact to the juvenile anadromous steelhead trout (*Oncorhynchus mykiss*) populations in this basin have led to efforts to identify sources of PBDEs.[22]

Water and Sediment Sampling. Concentrations of halogenated organics in water were measured using semipermeable membrane devices (SPMDs) or passive samplers. SPMDs were manufactured by Environmental Sampling Technologies, St. Joseph, MO and are a thin-walled, layflat polyethylene tube (91.4 cm × 2.5 cm × 70−95 μm thickness) filled with 1 mL of triolein, a neutral lipid compound. SPMDs (five) were deployed at each sampling location for approximately 30 days, an accepted period of deployment for chemicals of interest to integrate into the triolein oil based on sampling rates of the device.[23] Temperature loggers were used to ensure that the device stayed submerged during deployment. Performance reference compounds (PRCs) were used to assist in modeling biofouling and chemical uptake.[24] For PCBs: native congeners PCB-14 (CAS 34883-41-5) and the isotopically labeled congeners PCB-31L, PCB-95L, and PCB 153L; for PBDEs: native congeners BDE-10 (CAS 51930-04-2), BDE-37 (CAS 147217-81-0), and BDE-126 (CAS 366791-32-4) and the isotopically labeled congener BDE-138L were injected into the triolein oil prior to manufacturing the SPMD. The recovery of the PRCs with a measurable loss ranged from 56 to 79% for PCB-14, 71 to 87% for PCB-31L, 41 to 68% for BDE-10, 74 to 92% for BDE-37, and 77 to 96% for BDE-126. SPMDs were shipped on ice directly to the lab from the field and stored in a −20 °C freezer prior to analysis. SPMDs were physically cleaned and extracted twice by dialysis in hexane; the extract was then cleaned using a layered silica column, blown down to 20 μL, and analyzed by high-resolution mass spectrometry using USEPA methods 1668c (PCBs) and 1614 (PBDEs). Samples were analyzed on a SPB-octyl column with a high-resolution mass spectrometer. Water concentrations were calculated using the spreadsheet models of David Alvarez, USGS based on the octanal−water partition coefficients.[23−25] Field replicates had a good precision, as described by a relative percent difference (RPD) ranging from 10 to 23% for total PCBs and 16% for total PBDEs.

Water and sediment samples for metal analysis were collected from Railroad Creek as grab samples from the study reach. Water samples were field-filtered and preserved, while bulk sediment samples were collected from depositional areas within sampling reaches and homogenized. All samples were shipped on ice to Washington State Department of Ecology's Manchester Environmental Laboratory within analytical hold times. Metal analysis was done using inductively coupled plasma mass spectrometry (ICP-MS) under the EPA method 200.8. Field replicates had a good precision, as described by a RPD ranging from 0 to 6% for dissolved metals in surface waters and 1 to 4% for total metals in sediments.

Biofilm Sampling. In the Wenatchee and Nisqually rivers, biofilms sampled for PCBs and PBDEs, respectively, were collected directly from epilithic habitats in the river or stream bed. Opportunistic scrapes of biofilms from 5 to 30 rocks at a sample location were taken using stainless steel tools and composited in a stainless steel bowl. Efforts were made to remove silt and sand particles in the water prior to sampling the biofilm. Approximately, 12 g of the wet weight was required for the analysis of halogenated organics and sampled into certified glass jars. Composite biofilm samples were shipped directly to the lab on ice, where they were allowed to

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gravity settle and overlying site water was siphoned off. Samples were spiked with ^{13}C-labeled surrogate standards and Soxhlet-extracted with dichloromethane. Canola oil was used for laboratory method blanks and ongoing precision, recovery samples. Biofilms were analyzed using the same EPA analytical methods as the SPMDs as well as organic carbon (OC) and nitrogen abundance and stable isotope ratios, lipid content, and areal biomass (g DW/cm^2). C and N isotopes were analyzed using a ThermoFinnigan MAT 253/Costech EA and had an instrument precision of 0.005−0.08‰ for $\delta^{15}N$ and 0.06−0.13‰ for $\delta^{13}C$ during the analytical runs. Sample replicates had a RPD of 6−13% for total PCBs and 71% for total PBDEs. The poor RPD for PBDEs is possibly due to variability in the lipid content and low measurable PBDE concentrations in the replicates, near background levels for the river. Laboratory limits of quantitation ranged from 0.032 to 2.11 pg/g (median = 0.081 pg/g) for PCBs and 0.12 to 1.35 pg/g (median = 0.12 pg/g) for PBDEs. All analytical results were censored against laboratory method blanks at a threshold of 5 times positively identified compounds. Additional analytical quality control data are summarized in Tables S1−S3.

In Railroad Creek, composite samples of biofilms along each stream reach were collected by scrubbing a minimum area of 1.7 m^2 of the surface area (approximately 16 cobbles) across eight randomly selected pool/riffle transects at each stream reach. Prior to scrubbing, cobbles were agitated in the stream to remove silt and sand particles. Biofilms were composited in acid-washed containers and kept on ice in the dark for a maximum of 96 h prior to processing for analysis. Composite biofilms were centrifuged at 11 000 rpm for 10 min, and the supernatant water was poured off. The biofilm plug was then sent to Manchester Environmental Laboratory where it was processed and analyzed for total metals with an ICP-MS. Laboratory quality control data can be found in the Supporting Information (Tables S4 and S5). In 2013, the Cd and Cu recoveries of the laboratory-fortified blank BS1 were elevated at 171 and 202%, respectively; however, both SRMs and all matrix spikes recovery were within the acceptance criteria and no further corrective action were warranted. Prior to centrifuging, the composite was subsampled for taxonomic composition and preserved. Samples were sent to Rithron Associates (Missoula, Montana) for identification and enumeration of the periphyton community. Sample replicates had a RPD of 0.8−6% for total metals.

Invertebrate Sampling. In the Wenatchee River, invertebrates were sampled for the analysis of halogenated organics in studies where there was a direct link to the fish species of interest.[26,27] Trichoptera (caddisfly) and Ephemeroptera (may fly) larvae were picked from the underside of cobbles, removed from the casings, and transported back to the lab where the tissue was homogenized. Analytical methods followed those used for PCBs in the biofilm matrix and SPMDs. Field replicates had a precision of 5% RPD for total PCBs.

A commonly used indicator of ecological health in streams is the benthic index of biotic integrity (B-IBI) for macroinvertebrates.[28,29] This index is a quantitative method for determining and comparing the health of stream communities, based on the tolerance and presence of certain taxa. Invertebrates from Railroad Creek, where metal concentrations of biofilms are known, were analyzed for community composition and used to calculate a B-IBI.[29] Adjacent to biofilm sampling locations, composite samples of macroinvertebrate communities were collected from 0.75 m^2 of surface area, across eight randomly sampled pool/riffle transects at each stream reach using a D-frame kick net with a 500 μm net. Samples were preserved in ethanol and sent to Rhithron Associates, Inc. (Missoula, Montana) for sorting, identification, and enumeration.

Numerical Analysis. All statistical analyses were carried out using R.[30] To explore the changes in bioaccumulation factor (BAF) along a gradient of log K_{ow}, we used segmented or piecewise regression with breakpoint analysis to define ranges or thresholds of log K_{ow} where the linear relationship with BAF is either stronger or has a change in the slope.[31] This analysis defines points in the relationship when a statistically relevant change has occurred (the breakpoint).[32] The breakpoint is estimated using bootstrap methods to give confidence intervals.[33] In addition, we completed a Davies' test to assess whether there was a significant change in the slope of the linear relationship. To explore any important covariates with halogenated organic concentrations on biofilms, we used a stepwise akaike information criterion for multiple linear regression.[34] The variables, % organic carbon, % lipids, contaminant source area (categorical), and hydrologic flow regime (categorical) were tested for covariance. The only significant variable was the contaminant source area (i.e., samples located near a contaminant source; $p = 5.17 \times 10^{-8}$).

For Railroad Creek, principal component analysis (PCA) was used to evaluate metals in the periphyton matrix from samples collected in 2013 and 2015. Scores for the first principal component axis were highly correlated with total metals ($r = 0.95$) and used to evaluate the relationship between the metal concentration and the number of acidophilous diatoms (those with optimum requirements for pH < 7). We implemented variance partitioning for the relationship between B-IBI and tissue metal concentrations to calculate the pure and covariance effects for the three most abundant metals (i.e., iron, zinc, and copper) in the predictor set.[35]

RESULTS AND DISCUSSION

Bioaccumulation Factors and Partitioning of Organics. During investigations of selected rivers in Washington State, we have observed a strong linear relationship, which describes the bioconcentration of halogenated organics from the freshwater onto biofilms ($r^2 = 0.81$; $p < 0.001$; Figure 1). This relationship is based on samples, which represent high- and low-flow hydrologic regimes over multiple years, meaning it is broadly applicable. Biofilms sampled over the course of several studies had concentrations of organic carbon (%OC) ranging from 0.9 to 19.6 % (median = 6.9%) and lipid content ranging from 0.02 to 1.27% (median = 0.11%) (Table S6). Generally, the periphyton communities were dominated by diatoms (e.g., *Achnanthidium* sp. and cymbelloid forms), but the algal composition was not quantified at all locations. Biofilm growth on the bed of the rivers we sampled occurs during the spring-fall, with scouring of the river bed occurring with high flows (>10 000 cfs) associated with snowmelt discharge. Late summer, when river flows are at their lowest (~300−700 cfs), is the period of the highest observed growth.

The relationship between the biofilm and water concentrations is also based on both chlorinated (PCBs) and brominated compounds (PBDEs). There are more points present at lower concentrations, where the relationship still holds but greater variability is evident (Figure 1). This

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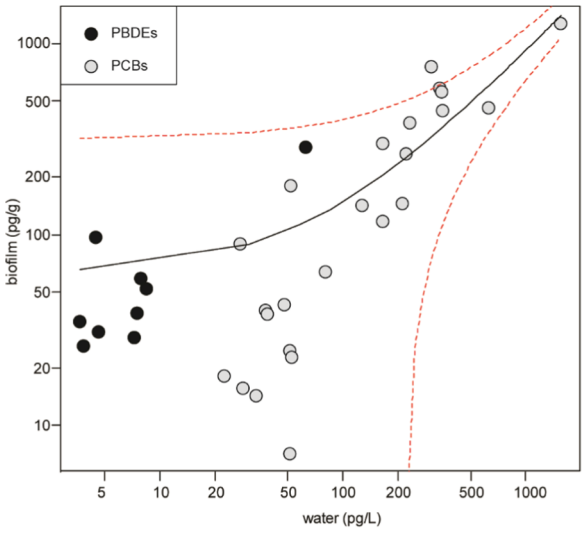

Figure 1. Linear relationship between contaminant concentrations in biofilms vs water; axes are logarithmic. A version of the relationship plotted on arithmetic axes can be found as Figure S2. Dashed lines are the 95% confidence interval of the regression line.

variability is likely attributable to both the sample media and our ability to accurately measure such low concentrations. Examining possible relationships with the %OC and lipid content of the biofilms does not provide a defendable explanatory variable for the contaminant concentrations (Table S7). Because lipid content and %OC are not significantly correlated with the concentrations of halogenated organics in biofilms, we choose not to normalize or correct the contaminant concentrations to these variables. To our knowledge, this is the first explicit field examination and comparison of the concentrations of halogenated organics in water and biofilms.

The bioconcentration of halogenated organics from water to biofilms appears to preserve the original congener distribution present in the water (Figures 2 and 3). Source identification studies for PCBs in the Wenatchee River basin in Washington State have led to the observation that two chemically distinct sources to the river exist.[27] One of the sources is dominated by congeners from the tetra-, penta-, and hexa-chlorobiphenyl (CB) homologue groups and resembles the technical mixture Aroclor 1254. The second source is dominated by congeners in the di-, tri, and tetra-CB homologue groups and resembles the technical mixture Aroclor 1242/48 (Figure 2). Also evident in the two samples from the Wenatchee River is that some of the more lipophilic congeners in the penta-CB range measured in the upstream sample are captured in both the water and biofilm sample at the downstream location. In the Nisqually

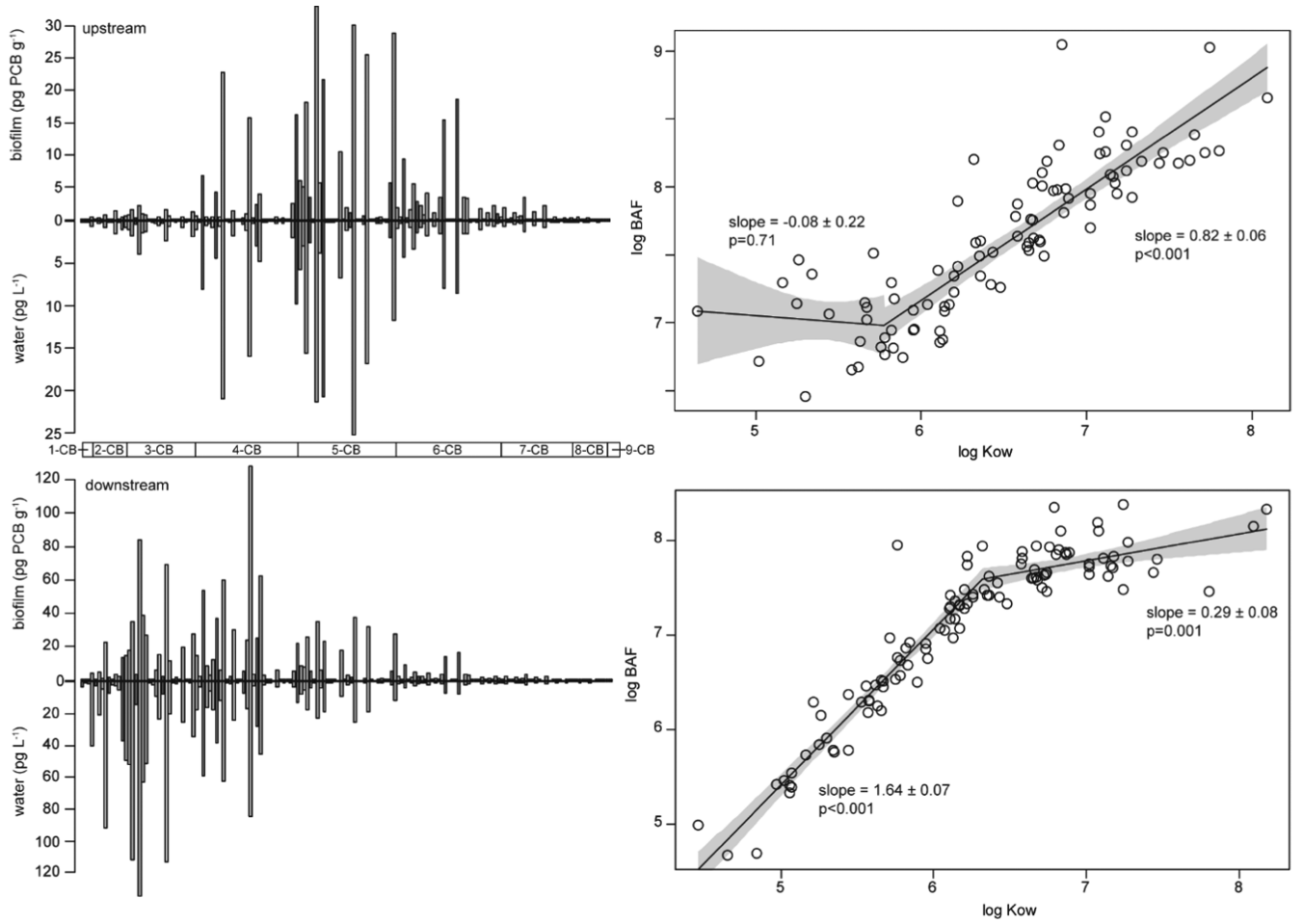

Figure 2. (Left) PCB congener concentrations in the biofilm and water samples from upstream (upper) and downstream (lower) locations collected in 2015. Congeners increase in chlorination from left to right on the *x* axis. (Right) Scatterplots of log BAF vs log K_{ow} with segmented regression and 95% confidence intervals around the regressions.

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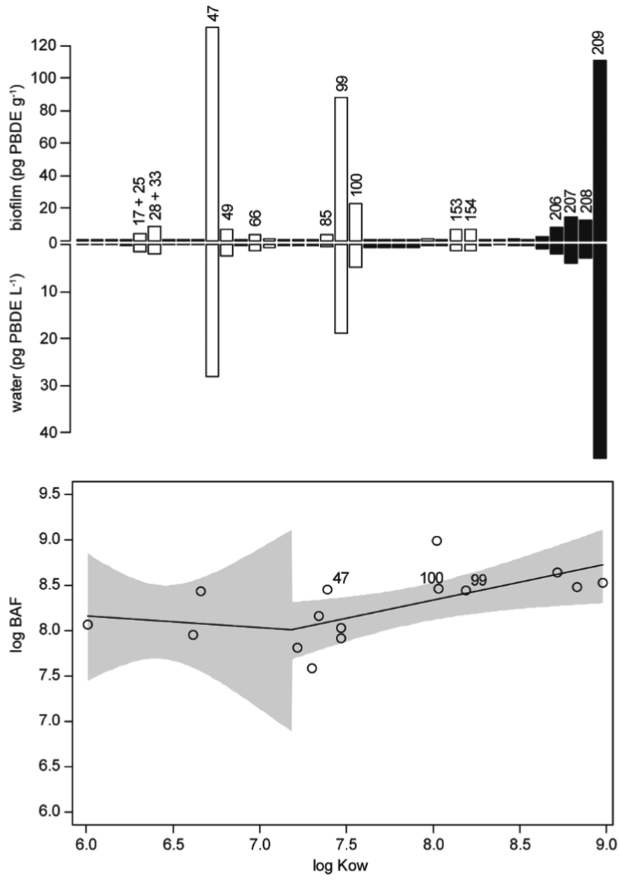

Figure 3. (Upper) PBDE congener concentrations in the biofilm matrix and water from a location sampled in 2017. Solid black bars are analytical results qualified as nondetected, due to laboratory blank contamination. (Lower) Scatterplots of log BAF vs log K_{ow} with segmented regression and 95% confidence intervals around the regressions. The dominant congeners BDE-47, -99, and -100 are labeled on the plot.

River, where PBDEs are of interest and the suspected source is the wastewater effluent, the congener profiles in water and biofilms are dominated by BDE-47, -99, and -100, with measurable concentrations of BDE-28 + 33, -49, -66, -153, and -154 (Figure 3). In both matrices, the concentrations of heavier nona- and deca-BDEs were not resolvable due to their presence in laboratory blanks. The uptake of PCB and PBDE congeners by biofilms has not been studied in detail. We show here that there is a strong fidelity between the presence of dissolved PCBs and PBDEs in the water and those bound to biofilms.

The bioaccumulation of PCB congeners by phytoplankton has been modeled and measured in fresh[12,36] and marine waters.[14,15] In the previous work, the bioaccumulation of PCB congeners on a dry weight basis follows a fairly consistent pattern with log K_{ow}. Generally, there is a linear relationship between log BAF and log K_{ow} within the log K_{ow} range of 5−7, where phytoplankton is interpreted to be at equilibrium with the water. The equilibrium between the water and organism suggests that uptake is being governed by physical or thermodynamic partitioning of the congeners and not growth rates of the organism and active uptake into internal lipids.[13,37] Beyond a log K_{ow} of approximately 7, the more lipophilic congeners no longer reach equilibrium and the relationship between BAFs and log K_{ow} plateaus. The main hypothesis as to why this occurs is that the uptake of the more highly chlorinated congeners is too slow to reach equilibrium within or on the organism. Additionally, growth dilution of the organism can impact the calculated BAF for higher chlorinated congeners.[12,15]

In the samples collected from the Wenatchee River, the uptake of PCB congeners appears to be governed by physical partitioning between water and biofilms (Figure 2). This is demonstrated by the statistically significant linear relationships of log BAF and log K_{ow} at different log K_{ow} ranges, depending on the presence of the congeners in the water (Table S8). Equilibrium of the log BAF − log K_{ow} relationship is inferred at a slope of 1.0. The upstream sample from the Wenatchee River is dominated by congeners with a log K_{ow} above 5.75. Breakpoint analysis of the log BAF − log K_{ow} relationship finds that there is a significant change in the slope of the linear relationship at log K_{ow} of 5.78 ± 0.13 [standard error (SE)] (Table S8). At a log K_{ow} > 5.78, the partitioning between water and biofilms is slightly below equilibrium; the slope of this relationship is 0.82 ± 0.06 (p < 0.001). At the downstream sample location, where lower-molecular-weight congeners dominate the PCB composition, there is a significant linear log BAF − log K_{ow} relationship at log K_{ow} 5−6.3 with a slope of 1.64 ± 0.07 (p < 0.001) and partitioning is above equilibrium. At a log K_{ow} 6.3 ± 0.06 (SE), the breakpoint analysis detects a significant decrease in the slope of the linear log BAF − log K_{ow} relationship (slope = 0.29 ± 0.08; p = 0.001) (Figure 2). Given the significant linear relationships for the downstream sample location, it appears that uptake of PCBs by biofilms at this site is at steady state, and we interpret the lower slope at log K_{ow} > 6.3 to represent slower uptake of the higher weight compounds. It is interesting that physical uptake and bioaccumulation appear to continue for the more lipophilic congeners, contrary to previous findings on predicted BAFs where the heavier congeners appear to plateau. Field collections of phytoplankton from the Great Lakes have also demonstrated that under conditions where physical partitioning is occurring, the more lipophilic congeners (log K_{ow} > 7) continue to bioaccumulate with a linear relationship.[12] Both samples from the Wenatchee River suggest that continued bioaccumulation is occurring at log K_{ow} > 7, albeit at a slower rate based on the slope of the downstream sample. This finding possibly reflects the period of deployment and integration that both the SPMDs and biofilms represent (1 month for SPMDs and 2−3 months for biofilms). In contrast, previous studies rely largely on instantaneous grab samples of water.[12,15]

In the Nisqually River, the uptake of PBDE congeners appears at the steady state based on the significant linear relationship for log K_{ow} > 7.2, as defined by the breakpoint analysis (Figure 3 and Table S8). Below a log K_{ow} of 7.2, there is no significant log BAF − log K_{ow} relationship for PBDEs in our samples. A log K_{ow} of ∼7.2 corresponds to the presence of BDE-47 in the samples. A linear relationship of log BAF − log K_{ow} for PBDEs is evident over the range of log K_{ow} (∼7.2−9.0). However, compared to the PCB analysis, there are much fewer congeners present, much greater variability, and there is no significant change in the slope of the log BAF − log K_{ow} relationship. The rate of uptake for the measured PBDEs (slope = 0.4 ± 0.15; p = 0.02) appears comparable to PCB uptake above log K_{ow} ∼ 7.0, based on the slopes of the log BAF − log K_{ow} relationship. The uptake of the more hydrophobic compounds between water and biofilm does not occur fast enough to reach complete equilibrium (i.e., slope = 1.0). In

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Table 1. Bioaccumulation Factors (BAFs) and Biomagnification Factors (BMFs) for PCBs in the Wenatchee River Food Chain, Based on Total PCB Concentrations over Multiple Years of Samples[a]

trophic level	location	BAF	BMF
mountain whitefish	downstream	1.02×10^6 (7.76×10^5)	6.5 (2.3)
invertebrate (caddisfly larvae)	downstream	4.76×10^5 (7.61×10^5)	176.5 (302)
biofilm	downstream	941 (300)	
biofilm	upstream	1751 (136)	NA

[a]Standard deviations are shown in parentheses.

addition, it is possible that with the slower uptake rate, a growth dilution of the BAF is taking place. There is a little previous work where the uptake of both PCBs and PBDEs has been compared for primary producers. In a laboratory dosing study, Magnusson et al.[38] found that PCB and PBDE uptake in marine phytoplankton was similar and strongly influenced by the hydrophobicity of the compound. Frouin et al.[15] found different uptake relationships between PCBs and PBDEs in marine zooplankton that they related to variability in the introduction and diffusion of the contaminant sources to and from the aquatic environment; PCBs being more susceptible to the atmosphere, ocean exchange. In the work presented here, both sources of PCBs and PBDEs are introduced into the aquatic environment largely in the dissolved phase from fairly discrete locations; therefore, exposure of the biofilms to the contaminants is proximal to the source and is not impacted by the atmosphere−water exchange.

Collectively, our observations indicate that the uptake of PBDEs and PCBs is similar and occurring through physical partitioning. We found significant linear $\log \mathrm{BAF} - \log K_{ow}$ relationships, with a slope greater than 1 for samples dominated by lighter congeners ($\log K_{ow}$ 5−7). PBDE and PCB congeners with higher partition coefficients ($\log K_{ow} > 7$) are below equilibrium (slope < 1.0) suggesting that they are slower to be taken up.

Using Biofilms to Track Halogenated Organics. The utility of measuring halogenated organics in biofilms is twofold: (1) it allows for an empirical assessment of the toxic burdens present at the base of a river or stream food web and (2) it is an effective passive sampler of toxics, reflecting spatial variability of toxic inputs. The bioaccumulation of halogenated organics in food webs of freshwaters we have investigated generally follows a predictable pattern (Figure S3). In samples collected from the Wenatchee River in Washington State, the resident food web is relatively simple and short. Periphyton (biofilms) are grazed by invertebrates and large-scale suckers (*Catostomus macrocheilus*), and invertebrates are consumed by predatory invertebrate life stages (e.g., stonefly naiads) and mountain whitefish (*Prosopium williamsoni*).[26,27] The bioaccumulation of PCBs in this river yields median tissue concentrations in the top consumer, the resident mountain whitefish, of 190.6 μg/kg of total PCBs (range of 8.9−3580.0 μg/kg).[27] Of relevance to this paper is the biomagnification of PCBs through the food web of the Wenatchee River is the greatest from water to the biofilm (Table 1). Furthermore, the bioaccumulation factor (BAF) varies with the composition of the PCB source, where a greater BAF was observed in the upstream location with more lipophilic congeners and lower absolute concentrations, an observation which was described earlier by the empirical $\log \mathrm{BAF} - \log K_{ow}$ relationships. In a laboratory dosing study,[13] two phytoplankton species were observed to accumulate PCBs at different rates and greater BAFs were observed for the more lipophilic compounds; however, the log BAFs were generally lower than those observed in this study.

The bioconcentration of PBDEs in the Nisqually River is based on limited samples where PBDEs were detected above an upstream background concentration. In a small tributary of the Nisqually, we calculated a median BAF of 4600 (range 1961−8034) across detectable PBDE congeners. The higher biomagnification of PBDEs compared to PCBs is a function of the more lipophilic congeners, as described by the $\log K_{ow}$, present in the sample media. There is scant data available from water and algae samples to compare this BAF to. In one study from an urban bay in the South China Sea, a marine phytoplankton had a measured BAF on the order of 10^5.[39] Bioaccumulation of BDE-99 in a dosed marine phytoplankton study was found to be ∼5500,[38] which is on the order of our measured BAF for BDE-99 of ∼4600. There is a clear lack of data on the bioaccumulation of PBDEs at the primary trophic levels of freshwater and marine ecosystems.

In this study, the effectiveness of biofilms as passive samplers has been tested empirically against manufactured passive samplers (Figure 1) and has proven to be a valuable tool in assessing the potential source locations of halogenated organics in rivers and streams. Furthermore, the use of biofilms alleviates the necessity to measure concentrations of halogenated organics in water that are often near the limits of analytical detection. Using the Wenatchee River as an example, sampling over a four year period during high- and low-low hydrologic regimes has allowed us to isolate possible locations where PCBs are entering the river. The isolation of sections of the river bank consists of sampling biofilms over multiple sampling events along the river at decreasing distances from the nearest sample. In one location, we have isolated ∼30 m of river bank that appears to be the location of PCB-contaminated groundwater entering the mainstem of the river (Figure S4). It is suspected that PCBs are being contributed via groundwater because the concentrations in biofilms remain elevated during both high and low flow and the PCB congener distribution has remained consistent over the 4 years of sampling (Figure S5),[27] suggesting an ongoing and consistent source. The success of using biofilms as a contaminant source tracking tool then allows us to pursue upland source control activities.

Identifying Sources of Metals Using Biofilms. The impacts of metal contamination from mining activities have been well studied.[40,41] Much of this previous work has focused on the impacts of acid-mine drainage on the structure of periphyton communities.[42−44] In Railroad Creek, WA, we investigated the utility of using water, sediment, and biofilm samples to document the area of environmental impact from a known metal source. An area, which was actively mined from 1938 to 1957 and is currently under remediation following the classification as a USEPA Superfund site, has historically contributed concentrations of metals to Railroad Creek leading

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to designation as an impaired waterbody under the U.S. Clean Water Act.[45] The impairment of Railroad Creek is driven by ambient water concentrations of dissolved metals (copper, mercury, and lead) that have been historically in excess of the State water quality criteria for the protection of the aquatic life.

Although dissolved metal concentrations in discrete water samples can often be used to delineate the downstream areas of influence from a source, the accumulation of metals onto biofilms and sediments allows us to analyze a time-integrated sample. Samples for copper and zinc at upgradient sites showed lower concentrations in all of the media (Figure 4). In

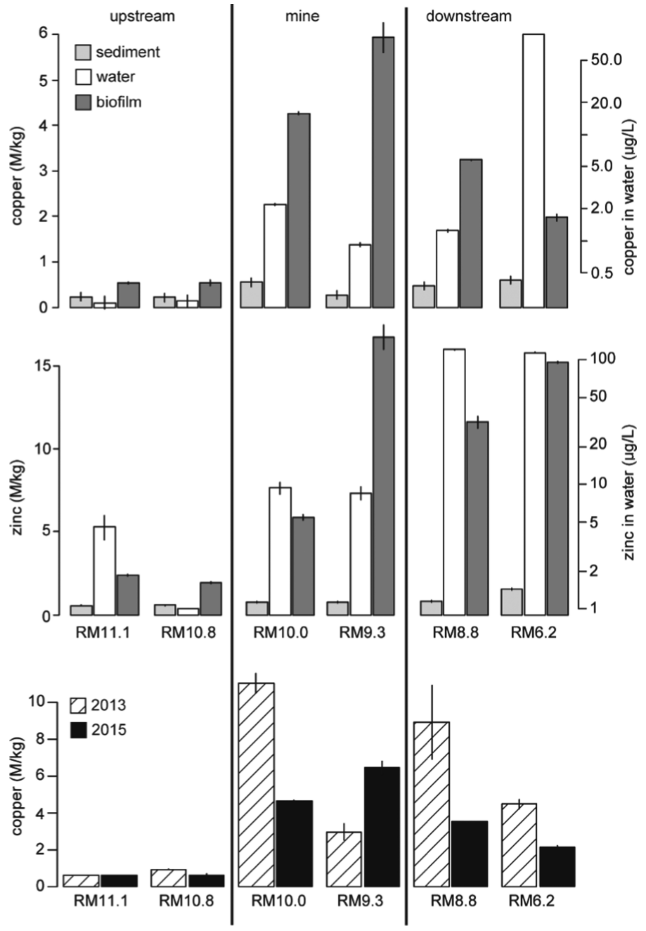

Figure 4. Concentrations of copper (upper) and zinc (middle) in various media from upstream to downstream of the mine site in 2015. (Lower) Concentrations of copper in biofilms in Railroad Creek from 2013 and 2015. Error bars are standard errors of field replicates.

the vicinity of the mine site, concentrations of both copper and zinc increased above the upgradient concentrations; however, the differences were more clearly resolved in the biofilm samples. For instance, the copper concentrations in the sediment at one of the sites in the vicinity of the mine (RM9.3) were not above the variability (±2 SE) of the upgradient/background sample results. Whereas, the water and biofilm results clearly showed the impacts of the former mining activity. The attenuation of Cu and Zn inputs downstream of the mining area was also more clearly resolved using the biofilm over the sediment as a sample media.

Investigations at Railroad Creek between 2013 and 2015 have coincided with ongoing remedial activities at the site (Figure S6). In 2013 and 2015, concentrations of copper in biofilms at sites upgradient of the former mine site were similarly low while concentrations within the mine site varied between years (Figure 4). A number of factors influenced the input of metals near the sample location RM10.0 between 2013 and 2015, including the closing of the mine porthole, rerouting spring melt water around historic tailings, relocation of sections of the stream channel, and the construction of a subsurface barrier between tailings and the stream channel.[46] The biofilm copper results at RM10.0 between 2013 and 2015 reflect this change by decreasing, whereas the results at RM9.3 reflect an increase in the inputs of copper as a result of stream channel alteration and the barrier wall altering groundwater flow patterns (Figure 4). Overall, copper concentrations in biofilms across the impacted sample sites from 2015 in Railroad Creek show evidence that copper inputs have been reduced as a result of the projects. Our work in Railroad Creek has highlighted the sensitivity and efficacy of using the metal concentrations in biofilms as a tool in assessing the spatial and temporal inputs of metals to freshwater.

Uptake of Metals and Biological Impacts. Although there has been a great deal of study on the uptake and cycling of metals by biofilms,[2] the impacts of metal uptake on higher trophic levels are not usually assessed.[16] The bioaccumulation of metals and metalloids is complex and is affected by exposure routes, regulation of metals by the organism, and metal bioavailability.[47] There is a pathway of metal exposure and partitioning in freshwater food webs that starts with the uptake of metals by biofilms. Furthermore, contaminant impacts on the base of the food web have the potential to influence the community composition or ecological health of an important food source. As a means to assess the impact of measured concentrations of metals in the tissues of biofilms from Railroad Creek, we described the community structure of periphyton (mainly diatoms) and macroinvertebrates. In a PCA of the periphyton tissue metals, the first two axes explained 68% of the variance across the six sites and 2 years of data (Figure S7). We observed a strong relationship ($r^2 = 0.73$; $p = 3.7 \times 10^{-4}$) between the total burden of metals in the biofilm matrix (PCA axis 1 scores) and a metric of acidophilous diatom taxa (Figure 5). As the total burden of metals increased in the biofilm tissues, the number of acidophilous taxa increased. Thus, there was a detectable response in the diatoms to a gradient of pH that was not observed in the water samples, since instantaneous in situ measures of pH did not differ considerably among sites. The implication of the relationship between the periphyton community structure and metal burden in biofilms is that the chronic exposure of metals and the concomitant variability in pH favor certain diatom species niches. This response was only observable by examining the biological communities directly, rather than relying on more traditional measures of water quality.

At the same sites where biofilms were sampled in Railroad Creek during 2015, the macroinvertebrate B-IBI scores were calculated. B-IBI scores showed a loss of ecological health from upstream to downstream of the metal-impacted stream sections (Figure 5). B-IBI scores were the highest at the two upstream sites not impacted by mine tailings (excellent ecological health) and high at the uppermost site where much of the remediation efforts took place, with much lower scores (fair to poor ecological health) at the downstream sites. We examined several possible explanatory variables to this gradient of B-IBI and found that there was no relationship

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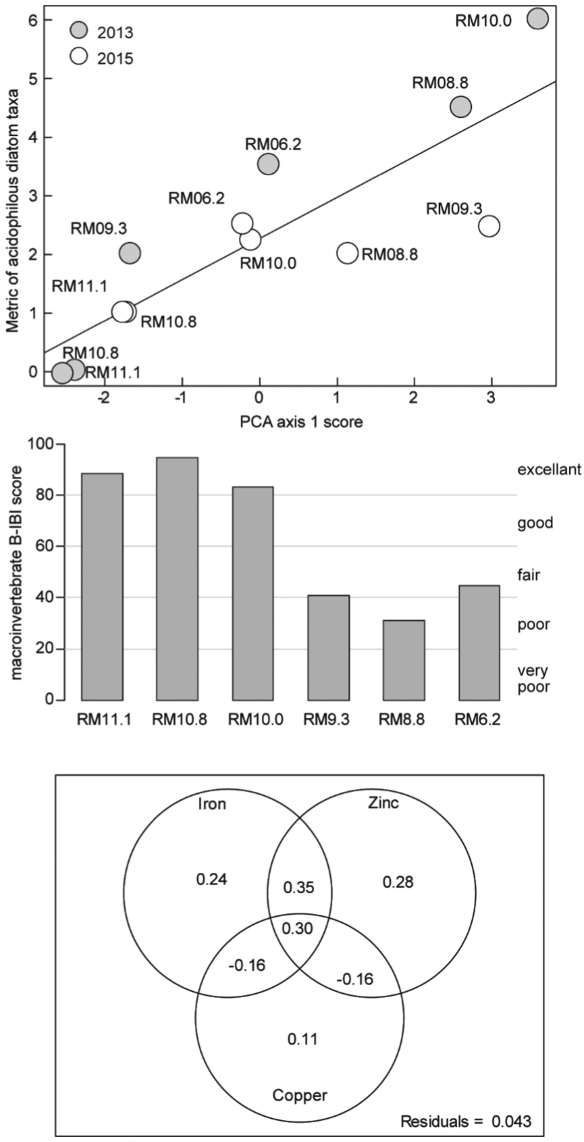

Figure 5. (Upper) linear regression of acidophilous diatom taxa against PCA axis 1 of the metal burden in biofilm tissues (r^2 = 0.73; p = 0.00037). (Mid) bar chart of the macroinvertebrate B-IBI scores from 2015 from upstream (left) to downstream (right). (Lower) Venn diagram of the variance partitioning of B-IBI as the response variable and biofilm copper, zinc, and iron concentrations as the explanatory variables; individual fractions are described in the diagram, while cumulative variance explained is 96%.

between B-IBI scores and metal concentrations in the sediment or surface water. However, there was a significant correlation between the B-IBI scores and the concentrations of the three most abundant metals in biofilms (Cu, Zn, and Fe); the three metals explained greater than 95% of the variation in B-IBI scores (Table S9). Partitioning of the variance between the B-IBI scores and the metal concentrations in biofilms showed that the greatest amount of pure variance was explained by zinc and iron, respectively (Figure 5). Covariance between zinc and iron was also high, as was the covariance between all three metals. This strong explanatory relationship suggests that metal concentrations of biofilms are a much more sensitive and accurate indicator of possible impacts on ecological health than sediments or water concentrations.

The importance of using metals in the biofilm matrix to assess the biological health of higher trophic levels was highlighted in a study of 23 New Zealand streams.[21] Here, the authors used genetic techniques to show that impacts on the ciliate communities of the streams were more closely related to the metal burden in biofilms than bulk sediment metal concentrations. Biofilms are a direct measure of the transport and integration of metals into the base of the food web of the stream. The possible mechanism by which metals in biofilm tissues more accurately relate to the impairment of macroinvertebrates, compared to sediments, may have to do with physical processes governing the fate and transport of metals. The sediment present in Railroad Creek is generally coarse (medium sands) with a low total organic carbon content. This reduces the available binding sites for metals to adsorb to the sediment,[48] thus reducing the usefulness of the sediment as an integrator of total metals in the stream. Conversely, the organic matrix of biofilms provides a larger area for adsorption in addition to the active uptake of bioavailable metals by the periphyton. Understanding the relative importance of adsorptive processes and biological uptake of metals is complex and as stated previously is a function of many environmental conditions.[48] In acidic streams impacted by mine drainage, the formation and precipitation of hydrous ferric oxides (HFOs) can also affect the cycling and uptake of other metals like Cu.[2] In addition, HFOs can reduce stream productivity by negatively influencing the habitat quality.[49,50] In Railroad Creek, the importance of the biofilm Fe content on the macroinvertebrate communities may be related to the precipitation and binding of Zn and Cu into HFOs associated with the biofilms and/or the degradation of the habitat. The assessment of the binding capacity of HFOs at Railroad Creek site requires further study.

The hyporheic zone and the flux of metals (Cu, in particular) from porewaters into surface waters have been highlighted as an important pathway impacting the toxicity of surface waters to biota.[51] In Railroad Creek, we know that the groundwater−surface water interactions are important for the spatial distribution of metals. Indeed, engineered remedial activities altering the groundwater−surface water connections were tracked by metal concentrations of the biofilms. Overall, we have provided evidence that the biofilm communities and metal burden are responding and recording the fluxes of metals to the creek, which have impacts on macroinvertebrate communities (B-IBI scores). Impairments to the biotic communities of Railroad Creek would not always be inferred when the sediment and water chemistry are assessed using state water and sediment quality criteria.[52,53] We, therefore, advocate for the integration of biofilm tissues to explain and assess biological impacts on streams from metals and other toxics.

Based on the importance of biofilms to stream food webs and the recognition that toxic contaminants accumulate on and within this matrix, biofilms have the potential to be an effective tool in documenting the spatial and temporal variability of contaminant sources and possible ecological impacts on higher trophic levels. Through multiple investigations in different rivers and streams in Washington State, we have shown that: (1) stream and river biofilms effectively sequester and bioconcentrate halogenated organics, such as PCBs and PBDEs, (2) similar to lake and marine phytoplankton, PCBs reach the thermodynamic equilibrium between water and biofilms at a log K_{ow} in the range of 5−7, while PBDEs have

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slower uptake rates and potentially do not reach equilibrium, (3) metal concentrations in stream biofilms are a more accurate description of adverse impacts on the stream biota than bed sediments or water, and (4) organics and metal concentrations in biofilms accurately reflect the location of contaminant sources.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b02865.

Site map and study locations (Figure S1); linear relationship between contaminant concentrations in biofilms vs water for both PCBs and PBDEs (Figure S2); bioaccumulation of PCBs (Figure S3); PCB source location map (Figure S4); PCB congeners of biofilms (Figure S5); source locations and selected remedial activities on Railroad Creek (Figure S6); principal components analysis (Figure S7); laboratory recovery of labeled PCB standards for samples (Table S1); summary of the recovery results (Table S2); summary of the laboratory method (Table S3); laboratory quality control data (Tables S4 and S5); PCB concentrations in biofilm and water samples (Table S6); ANCOVA results of PCBs (Table S7); summary statistics for the segmented regression analysis (Table S8); diagnostics of the variance partitioning analysis (Table S9) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: whob461@ecy.wa.gov. Phone: 360-407-7512.

ORCID

William O. Hobbs: 0000-0001-7321-0779

Author Contributions

The manuscript was written through contributions of all of the authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

B-IBI benthic index of biotic integrity

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