Powell River Project 2020-2021

Sediment Particle-Size Fractionation to Quantify Trace-Element Exposure Risk to Freshwater Mussels in the Powell River

Final Research Report

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INTRODUCTION

Freshwater mussel populations in the Powell River have not recovered from decades of decline beginning in the early 1980's (Ahlstedt et al. 2016). Consequently, mussel restoration remains a high priority for conservation managers, whose recovery plans will benefit from knowledge of the factor(s) causing suppression of mussel populations. Toxicity from trace elements, such as those released to waterways during coal mining, is hypothesized as a candidate cause, but further ecotoxicological study is necessary to test that hypothesis. However, such testing requires a thorough characterization of toxicant exposure conditions and pathways for mussels in the Powell River.

One pathway by which mussels may be exposed to trace elements is through feeding on particles containing those elements (i.e., trophic pathway), either by filtering particles from surface water or substrate interstitial (pore) water, or by ingestion of deposited sediments within river substrate. In a prior study sponsored by Powell River Project, we documented substantial trace-element concentrations associated with pore-water particles in the Powell River (Timpano and Jones 2020). Given this abundance of particle-borne trace elements in sediments of the river and the potential trophic pathways of exposure to those sediments, we here tested our hypothesis that trace elements associated with sediment particles in interstices of river substrate are a possible route of exposure to mussels, especially juveniles, in the Powell River. Our hypothesis predicts substantial trace element concentrations are associated with the smallest (< 20 μm) sediment particles, which are most likely to be ingested by juvenile mussels.

A thorough understanding of water quality patterns and mining-origin contaminant concentrations and exposures – in both dissolved and particulate form – in the Powell River will enable experimentation necessary to identify causes of mussel decline and guide restoration efforts. This study generated data required to inform that critical research, which will ultimately inform experiments that will advance the environmental science mission of the Powell River Project to enhance management and restoration of environmental resources affected by mining in the Appalachian coalfield.

OBJECTIVES

The immediate goal of this project is to quantify trace-element concentrations associated with Powell River substrate interstitial sediments, which will ultimately inform design of experiments to evaluate potential for toxicity to freshwater mussels from trace elements associated with substrate interstitial particles. To accomplish this goal, our objectives were:

1) Determine concentrations of trace elements associated with multiple particle-size classes and organic/inorganic forms of pore-water sediment.
2) Assess potential for temporal and longitudinal variation in sediment trace-element content by sampling and conducting analyses at multiple sites along the Powell River during multiple seasons.

METHODS

Interstitial Sediment Sampling

We collected interstitial sediment samples from nine sites (Table 1, Figure 1) with suitable mussel habitat (i.e., shoals) in Powell River using a ¼”- dia. PushPoint sampler (M.H.E. Products, East Tawas, Michigan) inserted into the substrate to a depth of 10 cm. Sediment particles were then extracted from the interstices in a pore-water slurry using a plastic syringe-pump assembly and silicone tubing. We collected a 450 mL composite sample composed of 50 mL aliquots of sediment slurry from nine sampling locations, with each location spaced randomly 3-5 m apart within the mussel shoal. Samples were contained in polyethylene bottles and stored on ice for transport to the laboratory, where they were frozen at -20 °C until processing.

We collected sediment samples in this manner during December 2020 and May 2021. We also collected interstitial sediment using identical procedures in October 2020 with a conceptually-similar though larger diameter (~1” dia.) Drive-Point piezometer (6-inch Model 615, Solinst Canada Ltd., Georgetown, Ontario, Canada).

Table 1. Powell River sampling site information.

Site Name	Site ID	River km	State	County	Latitude	Longitude
Appalachia	APP	293.7	VA	Wise	36.904303	-82.781520
Big Stone Gap	BSG	287.2	VA	Wise	36.863290	-82.785774
Dryden Boat Ramp	DRY	269.1	VA	Lee	36.782730	-82.924930
RT 70 Bridge	RT70	233.5	VA	Lee	36.662840	-83.094310
RT 833 Bridge	RT833	186.8	VA	Lee	36.620998	-83.284952
McDowell Shoals	MCD	171.9	TN	Hancock	36.574995	-83.362610
Upper Brooks Bridge	UBB	153.4	TN	Claiborne	36.535090	-83.441700
Oakley Property	OAK	144.2	TN	Claiborne	36.535754	-83.467856
Yellow Shoals	YEL	136.2	TN	Claiborne	36.527517	-83.507572

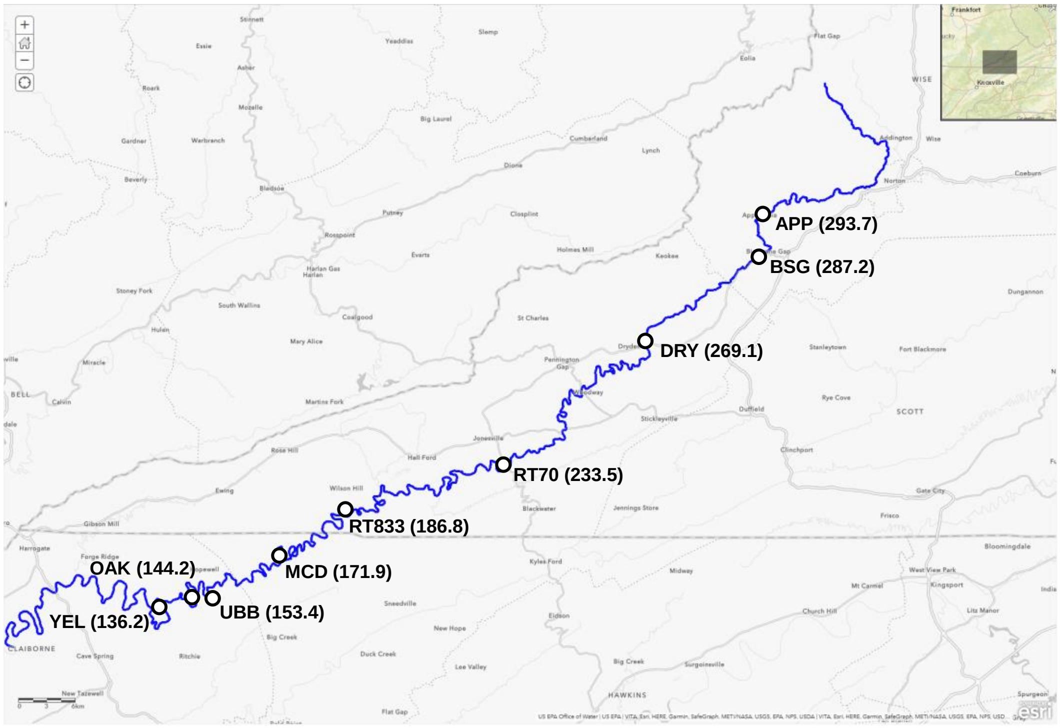

Figure 1. Map of Powell River sampling sites. Site identifiers are shown with river kilometer in parentheses.

Particle-Size Fractionation

To test our hypothesis that freshwater mussels – especially juveniles ranging in length from 0.3 mm to 20 mm – could be exposed to trace elements associated with sediment particles, we conducted particle-size fractionation of Powell River interstitial sediments. Size classes were chosen based on particle ingestibility by mussels at different life stages, which is constrained by their mouth size (Table 2). Food particles ingested by juvenile mussels are generally < 20 μm (Vaughn et al. 2008), with smaller particles being available to the youngest juveniles – Lasee (1991) found mouth size of *Lampsilis ventricosa* to be approximately 16 μm at 2-days old. Thus trace elements associated with the two smallest size classes measured here (< 10 μm and 10 – 20 μm) likely pose the greatest risk to juvenile mussels.

Table 2. Sediment particle size classes and their hypothesized fate in streambed interstitial space.

Sediment Particles		**Hypothesized Fate**			
Particle Size Range (μm)	**Description for Mineral Particles**	**Ingested by Smallest Juvenile Mussels (<1 mm)**	**Ingested by Larger Juvenile Mussels (1-20 mm)**	**Ingested by Sub-Adult & Adult Mussels (>20 mm)**	**Future Source of Fine, Ingestible Particles**
< 10	Clay/Silt	X	X	X	
10 to 20	Silt		X	X	
20 to 60	Silt			X	
60 to 120	Silt/Sand			X	
120 to 300	Sand				X

Particle-size fractionation began by thawing the samples, allowing particles to settle at 4 °C to clarity (usually overnight), then pipetting off the overlying water. To the bottle with remaining sediment we added 50 mL of deionized water, capped, and shook for 2 hours at 3 oscillations per second to disaggregate particles. Next, we rinsed the entire sample through a 300-μm nylon sieve with deionized water, discarding the larger fraction. The remaining slurry with sediment < 300 μm was sequentially sieved/filtered with aid of deionized water, a nylon bristle brush, and vacuum through nylon mesh filter membranes of pore size 120, 60, 20 and 10 μm. The solids retained on each filter were collected into a pre-weighed polystyrene weigh boat, dried at 60 °C overnight, then cooled in a desiccator and weighed to determine the dry weight of sediment in each particle-size class. For the < 10 μm fraction, slurry passing through the 10 μm filter was collected into a large shallow polyethylene container and water evaporated at 60 °C until reduced in volume, then transferred to a pre-weighed polystyrene weigh boat and dried and weighed as with other fractions.

Trace-Element Measurement

After drying and weighing each fraction of sediment to obtain particle size distributions, they were ground fine using a borosilicate glass pestle. Approximately 500 mg of ground sample (or the entire fraction if < 500 mg was present) was transferred to a pre-weighed polystyrene weigh boat and dried at 60 °C overnight, then cooled in a desiccator and weighed to obtain the mass of each sediment particle size class from which trace element concentrations were measured.

Ground samples were hot-acid-digested using a microwave digester (Mars6 Express, CEM Corp., Matthews, NC) following USEPA Method 3051. Into each 50-mL digestion vessel we rinsed ground sample (≤ approx. 500 mg) with approximately 5-10 mL deionized water and added 10 mL concentrated trace-metal grade nitric acid. After digestion, samples were allowed to cool overnight then transferred quantitatively by rinsing with deionized water into 50-mL metal-free polypropylene centrifuge tubes. Samples were spun at 2000 rpm for 10 minutes to settle the remaining sediment particles. The supernatant was poured into a 50-mL volumetric flask and brought to volume with deionized water. An additional 0.1 dilution was made from that aliquot to produce a 10-mL sample containing approximately 2% nitric acid for subsequent analysis by ICP-MS.

Note that trace-element concentrations were not partitioned among organic and inorganic fractions as was our original objective. We found no method for such partitioning that would accurately maintain the size and chemical integrity of particles as they would be encountered by mussels *in situ*. Thus, all results are presented based on particle size alone, regardless of type. We also note that during the filtration process, we observed no obvious amount of low-density organic particles in sediment samples (e.g., material with lower settling rates than other particles in the size class), suggesting these interstitial sediment samples are overwhelmingly composed of inorganic particles by mass.

Data Analysis

Data Used

We measured 21 trace elements associated with sediment particles: aluminum (Al), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), tin (Sn), titanium (Ti), uranium (U), vanadium (V), and zinc (Zn).

All analyses were conducted in R statistical software (v. 4.1.1, R Core Team 2021) with test level of alpha = 0.05 unless specified otherwise.

Particle-Size Fractionation and Trace Element Sediment Concentration

We calculated four sediment metrics: two metrics for particle size and two metrics for trace element sediment concentration. First, we calculated *Percent of Sediment Mass* contributed by each particle size class by dividing the mass of each size class by the sum of masses for each of the five size classes and multiplying by 100. This provided insight into the relative abundance of each particle size in sediment. Second, we summed the relative percentage mass contributions for particles < 10 μm and 10 – 20 μm to derive *Percent of Sediment Mass < 20 μm*. This presents a measure of the finest material in the sediment, as well as the relative amount of sediment small enough to be ingested by juvenile mussels. Third, we calculated *Total Element Sediment Concentration* (mg element per kg dry sediment) to provide an estimate of the total concentration of particle-associated trace element present in interstitial sediment. We used measured element concentrations in dilute digestate (via ICP-MS), along with dilution factors and sample mass digested to calculate the mass of each element (mg) present per unit mass (kg) of each size class. The size-class element concentration was then multiplied by the proportion of sample by mass for the respective size class to arrive at the mass (mg) of each element contributed by each size class per kg of whole (< 300 μm) sediment sample. The sum of the element contributions from each of the five size classes yielded the total mass of element (mg) per kg dry sediment. Finally, we calculated a fourth metric, *Element Sediment Concentration < 20 μm*, which represents trace element mass associated with particles ingestible by juvenile mussels. This metric was the sum of size-class element contributions (mg element per kg dry sediment) for the < 10 μm and 10 – 20 μm classes.

In some samples, especially those collected after Oct 2020, the mass of sediment obtained from interstices was insufficient to provide the desired 500 mg of dried sediment at each size class. Many samples contained < 100 mg of dried sediment per size class, with some containing < 10 mg. Thus, some element concentrations in diluted digestate from those samples were below detection limits of the analysis method (BDL). Because calculation of total element mass and element mass contributions by size class rely on quantitative data at each size class, those estimates for samples with a substantial number of values BDL are less reliable. Therefore, we omitted from summary statistics, and spatial and temporal statistical analyses elements with > 25% of samples BDL (Se – 67%, Sn – 46%, Mo – 32%) but include them in summary plots and data tables. We substituted ½ method detection limit (MDL) values for element concentrations that were BDL.

Spatiotemporal Patterns

Statistical analyses included assessment of spatial and temporal differences in Percent of Sediment Mass < 20 µm, Total Element Sediment Concentration, and Element Sediment Concentration < 20 µm. We used linear mixed models (LMMs) with normal error distribution to account for temporal and spatial autocorrelation, as is produced from repeated sampling of the same sites. To assess spatial differences among the nine study sites, we modeled sediment metric as a function of study site as a fixed effect and sample month as a random effect. We assessed differences among the three study months by modeling sediment metric as a function of sample month as a fixed effect and study site as a random effect. Mixed models were constructed using the *nlme* R package (v. 3.1-152; Pinheiro et al. 2021) and tested for significant predictor effects using Wald tests.

Spatial trends were assessed through Mann-Kendall trend analysis of sediment metrics using R package *Kendall* (v. 2.2; McLeod 2011). If statistically significant, spatial trend results are presented as positive or negative relative to the downstream direction, to represent whether sediment metrics increased or decreased moving downstream from the influence of mining land use in the headwaters. We conducted spatial trend analysis separately for each sample month. We did not examine temporal trends for sediment metrics because of the short study span.

Toxicity Potential of Sediment Trace Elements

We estimated toxicity potential for seven of the 21 elements (As, Cd, Cr, Cu, Ni, Pb, and Zn) using consensus-based sediment quality guidelines (MacDonald et al. 2000). We compared the total sediment concentration of each element with its respective probable effect concentration (PEC). We calculated a PEC quotient (PECQ) for each element in each sample by dividing the element concentration by its PEC, then we calculated a mean PECQ for each sample. A PECQ is a standard method of assessing sediment toxicity potential, by comparing the exposure concentration of an element to its PEC. A mean PECQ > 1.0 indicates toxic effects are probable to sediment-dwelling organisms (MacDonald et al. 2000).

In order to gain perspective on the magnitude of the sediment-element exposure risk to mussels in Powell River, we wanted to quantify sediment elements at sites where mussel populations are healthy. Thus, we also compared sediment element concentrations and PECQs for the Powell River during May 2021 with concentrations and PECQs from samples taken in the same month at two sites in the Clinch River where mussel populations have been documented in good condition as recently as 2014 (Jones et al. 2018).

RESULTS AND DISCUSSION

Sediment Particle-Size Fractionation

Particle-size fractionation of interstitial sediment samples (< 300 μm) revealed fine particles constitute the majority by mass (Figure 2). Across all sites and months (n = 27), 70 ± 11% (mean ± sd) of sediment mass was contributed by particles < 20 μm, a size range ingestible by juvenile mussels (Table 3). Relative contribution to sediment mass from particles < 20 μm was significantly different among sites (spatial LMM, $p = 0.0025$), but exhibited no significant longitudinal trends in any month (Mann-Kendall trends, all $p > 0.05$). The spatial differences were attributable to the significantly lower percentage of particles < 20 μm (Tukey multiple comparisons, $p < 0.05$) at the upstream Big Stone Gap site (BSG), in contrast to the higher percentage of particles < 20 μm at three sites downstream (RT70, MCD, and UBB; Figure 2, Table 3). Relative contribution from particles < 20 μm was significantly different among months (temporal LMM, $p = 0.0383$), with Oct 2020 (74%) > Dec 2020 (72%) > May 2021 (65%) (Table 3; Tukey multiple comparisons, $p < 0.05$). This pattern is consistent with a seasonal cycle of colmation and decolmation (Wharton et al. 2017), whereby fine sediment accumulates in the substrate interstices as river flow declines in summer months and into autumn (e.g., as measured in October), then is flushed away as discharge increases over the winter into spring (e.g., as measured in May).

Table 3. Percent of sediment mass contributed by particles < 20 μm (ingestible by juvenile mussels) in the Powell River. As measured for substrate interstitial sediment < 300 μm.

Site ID & River km	**APP 293.7**	**BSG 287.2**	**DRY 269.1**	**RT70 233.5**	**RT833 186.8**	**MCD 171.9**	**UBB 153.4**	**OAK 144.2**	**YEL 136.2**	
Month										***Mean (SD[a])***
Oct 2020	73	66	67	80	77	79	85	64	74	*74 (7)*
Dec 2020	75	45	69	81	75	78	88	77	59	*72 (13)*
May 2021	61	49	60	87	57	76	68	64	65	*65 (11)*
Mean (SD[a])	*69 (8)*	*53 (11)*	*65 (5)*	*83 (4)*	*70 (11)*	*78 (2)*	*81 (11)*	*68 (7)*	*66 (8)*	***70 (11)***

[a] SD: standard deviation

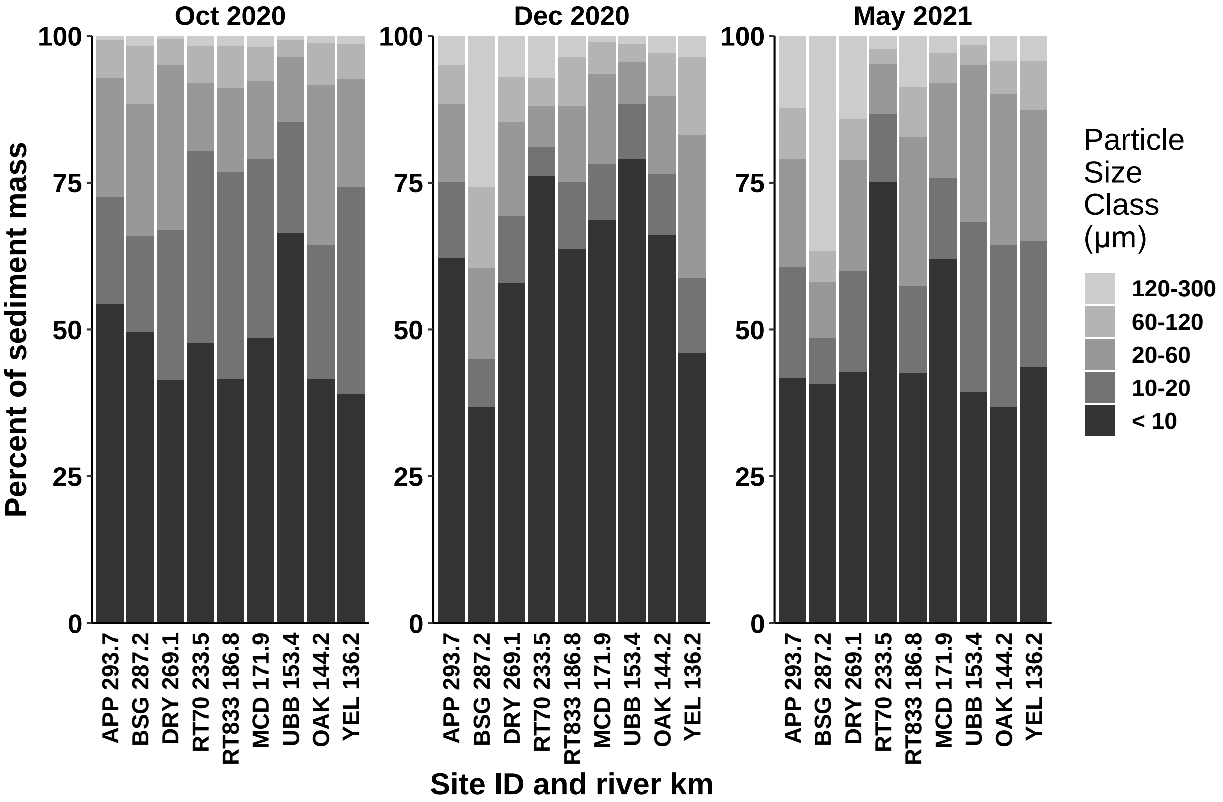

Figure 2. Percent of sediment sample mass contributed by each particle size class for nine sites in Powell River during three months. As measured for substrate interstitial sediment < 300 µm. Sites are arranged moving downstream left to right within each month.

Trace Element Sediment Concentrations

As expected, given their abundance in the earth's crust, Fe and Al were the dominant elements by mass in interstitial sediment samples (Table 4). Third most-dominant was Mn, which is known to be elevated in alkaline mine discharge (Griffith et al. 2012). Among trace elements of potential sediment-toxicity concern (As, Cd, Cr, Cu, Ni, Pb, Zn), Zn, Pb, Ni, Cu, and Cr were consistently in the upper half of dominant elements by mass (Table 4).

Total sediment concentration and concentration contributed by particles < 20 µm differed among sites for most elements (Table 5), but few exhibited longitudinal trends (Table 6). This suggests that sediment element concentrations may not be predicted solely by distance downstream from mining land use. This is supported by the fact we observed concentrations of Zn, Pb, Ni, and Cu to be higher at some mid-river sites (RKM 233.5 and 186.8) than in the upper-most sites (RKM 293.7 – 269.1) (Table 4). Such spatial variability suggests the need to evaluate multiple sites within Powell River to adequately assess sediment exposure risk to mussels.

Table 4. Total concentration (mg/kg) of each element in Powell River substrate interstitial sediment (< 300 µm) by site and month. Elements are arranged in descending order of monthly mean concentration.

Oct 2020	**Site ID & River km**									
	APP	**BSG**	**DRY**	**RT70**	**RT833**	**MCD**	**UBB**	**OAK**	**YEL**	
Element	**293.7**	**287.2**	**269.1**	**233.5**	**186.8**	**171.9**	**153.4**	**144.2**	**136.2**	***Mean (SD[a])***
Fe	29700	28200	30800	27500	24800	24500	24200	24300	20600	*26100 (3220)*
Al	8550	7610	11700	11900	10100	10700	11200	9930	8120	*9990 (1570)*
Mn	1640	1590	2760	893	1890	1280	1550	1940	1120	*1630 (545)*
Zn	174	130	155	174	141	111	145	113	108	*139 (25.6)*
Ba	124	142	176	121	151	118	146	136	106	*136 (21.1)*
Sr	76.3	131.0	89.6	54.1	62.4	44.9	57.6	61.1	49.3	*69.6 (26.8)*
Pb	85.3	36.1	51.0	66.0	211.0	33.1	42.0	38.3	34.4	*66.3 (56.9)*
Ti	45.0	38.6	33.6	47.7	34.9	49.2	48.5	48.1	42.7	*43.1 (6.06)*
Ni	33.4	35.0	39.4	40.4	31.3	30.8	30.1	27.2	22.3	*32.2 (5.68)*
Cu	36.2	38.8	37.0	35.0	41.8	26.7	26.9	22.7	17.5	*31.4 (8.23)*
Cr	16.4	17.3	19.7	22.2	19.1	23.8	22.5	20.8	19.2	*20.1 (2.45)*
Li	18.1	14.6	24.8	26.8	17.7	21.6	21.6	16.6	13.4	*19.5 (4.54)*
V	14.7	15.6	19.3	19.8	18.1	22.2	21.0	20.8	18.3	*18.8 (2.48)*
Co	20.0	16.8	20.3	17.7	17.9	17.7	17.6	21.2	16.0	*18.3 (1.74)*
As	6.26	7.79	7.55	6.09	5.54	5.73	5.97	5.80	5.66	*6.27 (0.828)*
Se[b]	0.106	0.108	1.36	0.933	1.62	1.53	1.68	1.41	0.0855	*0.982 (0.694)*
Sn[c]	0.929	0.759	0.294	3.29	2.36	0.102	0.129	0.0631	0.156	*0.898 (1.16)*
Mo[d]	0.485	0.937	1.31	0.801	0.696	0.609	0.604	0.606	0.530	*0.731 (0.258)*
U	0.607	0.875	0.871	0.972	0.601	0.683	0.681	0.542	0.545	*0.708 (0.159)*
Cd	0.301	0.392	0.337	0.267	0.275	0.246	0.297	0.278	0.229	*0.291 (0.0491)*
Ag	0.0496	0.0146	0.153	0.209	0.0939	0.149	0.0986	0.0703	0.0139	*0.0946 (0.0661)*

Table continues

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[a] SD: standard deviation; [b] Se - 67% of samples below detection limit; [c]Sn – 46% of samples below detection limit; [d]Mo – 32% of samples below detection limit

Table 4 (continued). Total concentration (mg/kg) of each element in Powell River substrate interstitial sediment (< 300 μm) by site and month. Elements are arranged in descending order of monthly mean concentration.

Dec 2020	**Site ID & River km**									
	APP	**BSG**	**DRY**	**RT70**	**RT833**	**MCD**	**UBB**	**OAK**	**YEL**	
Element	**293.7**	**287.2**	**269.1**	**233.5**	**186.8**	**171.9**	**153.4**	**144.2**	**136.2**	***Mean (SD[a])***
Fe	29100	20300	20300	18200	14600	15400	29300	23200	22200	*21400 (5250)*
Al	9060	7290	8750	10100	7710	8250	16100	11100	8820	*9690 (2670)*
Mn	850	1320	1300	471	770	523	1320	938	784	*919 (330)*
Ba	146	113	127	112	99.2	89.8	141	113	96.4	*115 (19.5)*
Zn	156	106	108	128	81.3	79.0	110	78.2	91.9	*104 (25.7)*
Ti	63.4	70.6	52.5	65.9	36.8	49.4	69.4	62.4	84.9	*61.7 (13.9)*
Sr	44.1	119	60.5	45.2	31.7	32.5	45.2	45.0	56.8	*53.4 (26.4)*
Pb	105	25.1	42.7	31.4	110	36.2	44.6	34.7	26.8	*50.7 (32.8)*
Cu	92.5	37.3	40.5	48.3	27.3	25.6	34.7	20.9	29.4	*39.6 (21.5)*
Ni	29.8	30.8	28.6	35.2	22.2	23.1	33.9	27.8	26.1	*28.6 (4.42)*
Li	20.8	17.7	20.6	27.6	17.4	19.4	37.2	21.8	15.9	*22.0 (6.61)*
Cr	22.7	20.8	17.4	20.5	11.7	18.7	25.1	28.3	27.8	*21.4 (5.27)*
V	17.0	12.3	14.3	16.6	13.0	14.6	27.4	21.4	18.7	*17.2 (4.76)*
Co	14.6	11.8	13.1	12.8	11.3	16.4	16.5	16.5	13.0	*14.0 (2.06)*
As	6.88	5.92	5.09	3.58	3.84	3.90	10.0	5.66	4.02	*5.44 (2.05)*
Sn[c]	18.8	0.281	0.520	1.89	0.105	0.0918	0.0488	0.0885	0.150	*2.44 (6.16)*
Se[b]	0.520	2.11	0.906	3.76	0.175	1.08	0.366	0.663	1.13	*1.19 (1.12)*
U	0.780	0.586	0.723	0.766	0.642	0.569	0.760	0.567	0.503	*0.655 (0.104)*
Cd	0.273	0.278	0.223	0.350	0.176	0.135	0.208	0.183	0.215	*0.227 (0.0646)*
Ag	0.208	0.208	0.124	0.181	0.120	0.123	0.118	0.134	0.0852	*0.145 (0.0436)*
Mo[d]	0.00692	0.0281	0.169	0.0329	0.119	0.00919	0.369	0.174	0.0151	*0.103 (0.121)*

Table continues

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[a] SD: standard deviation; [b] Se - 67% of samples below detection limit; [c]Sn – 46% of samples below detection limit; [d]Mo – 32% of samples below detection limit

Table 4 (continued). Total concentration (mg/kg) of each element in Powell River substrate interstitial sediment (< 300 μm) by site and month. Elements are arranged in descending order of monthly mean concentration.

May 2021	**Site ID & River km**									
	APP	**BSG**	**DRY**	**RT70**	**RT833**	**MCD**	**UBB**	**OAK**	**YEL**	
Element	**293.7**	**287.2**	**269.1**	**233.5**	**186.8**	**171.9**	**153.4**	**144.2**	**136.2**	***Mean (SD[a])***
Fe	26600	20100	24200	21000	17700	19100	23400	24200	19900	21800 (2920)
Al	8770	7090	9420	10200	8560	9320	11400	10200	8290	9260 (1260)
Mn	1280	427	1410	472	893	539	991	891	1100	888 (352)
Ba	115	79.2	118	100	96.2	95.2	114	109	107	104 (12.4)
Zn	111	86.2	107	112	90.5	85.6	96.1	88.7	82.4	95.5 (11.6)
Sr	89.2	63.3	69.8	46.5	43.1	37.2	38.8	32.6	47.6	52.0 (18.5)
Ti	58.8	38.5	22.2	40.4	33.3	35.0	38.5	40.5	41.6	38.8 (9.58)
Pb	26.2	20.1	35.8	28.7	63.9	26.8	34.8	48.2	33.1	35.3 (13.3)
Cu	37.5	44.0	25.4	28.3	23.6	21.9	26.4	20.5	19.8	27.5 (8.17)
Ni	32.2	24.5	29.9	31.4	23.3	24.8	29.5	26.0	24.3	27.3 (3.41)
Li	19.4	17.9	22.1	25.0	17.5	20.5	25.4	21.1	13.6	20.3 (3.73)
Cr	17.2	15.6	16.8	17.9	12.9	16.6	17.8	18.5	22.4	17.3 (2.53)
V	13.5	11.5	14.6	14.1	12.6	15.5	19.2	25.1	17.5	15.9 (4.17)
Co	16.9	9.5	13.2	11.7	11.4	9.98	14.1	26.1	13.6	14.0 (5.05)
As	5.14	5.34	6.03	3.82	3.52	4.36	6.56	12.90	5.40	5.89 (2.81)
Sn[c]	0.0362	0.153	0.233	9.40	0.290	0.220	0.220	0.246	0.113	1.21 (3.07)
Mo[d]	0.459	0.787	1.23	0.539	0.481	0.463	0.689	0.679	0.897	0.692 (0.254)
U	0.468	0.556	0.759	0.715	0.541	0.635	0.657	0.639	0.523	0.61 (0.0951)
Se[b]	0.392	0.667	0.885	0.105	0.455	0.154	0.085	0.0813	0.394	0.358 (0.283)
Cd	0.248	0.167	0.230	0.147	0.192	0.142	0.189	0.268	0.218	0.200 (0.0442)
Ag	0.0419	0.0711	0.133	0.144	0.104	0.109	0.0286	0.0361	0.0585	0.0807 (0.0431)

[a] SD: standard deviation; [b] Se - 67% of samples below detection limit; [c]Sn – 46% of samples below detection limit; [d]Mo – 32% of samples below detection limit

Table 5. Spatial and temporal mixed model statistical significance for each element and sediment metric.

	Spatial (among 9 sites)		**Temporal (among 3 months)**	
Element	**Total Concentration**	**Concentration from Particles < 20 μm**	**Total Concentration**	**Concentration from Particles < 20 μm**
Ag	ns	ns	*	*
Al	**	**	ns	ns
As	ns	ns	ns	ns
Ba	*	ns	**	***
Cd	ns	ns	**	ns
Co	*	*	**	***
Cr	ns	ns	*	*
Cu	*	ns	ns	ns
Fe	*	ns	**	**
Li	***	**	ns	ns
Mn	**	*	ns	ns
Ni	**	***	*	**
Pb	**	**	ns	*
Sr	**	**	*	ns
Ti	*	*	ns	ns
U	*	**	ns	*
V	**	**	ns	ns
Zn	ns	***	ns	ns

p-value codes: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = $p \geq 0.05$. Model p-values are results of Wald test for overall significance of differences in sediment concentration (mg/kg) of each element among sites (spatial models) or months (temporal models) for each sediment metric. Determined for each element/sediment-metric combination using a linear mixed model of sediment element concentration as a function of site as a fixed effect and sample month as a random effect (spatial models), or sediment element concentration as a function of sample month as a fixed effect and site as a random effect (temporal models).

Table 6. Spatial (downstream) Mann-Kendall trends for sediment concentration of each element and sediment metric by month.

	Total Concentration			**Concentration from Particles < 20 μm**		
Element	**Oct 2020**	**Dec 2020**	**May 2021**	**Oct 2020**	**Dec 2020**	**May 2021**
Ag	ns	-	ns	ns	-	ns
Al	ns	ns	ns	ns	ns	ns
As	-	ns	ns	ns	ns	ns
Ba	ns	ns	ns	ns	ns	ns
Cd	ns	ns	ns	ns	ns	ns
Co	ns	ns	ns	ns	ns	ns
Cr	ns	ns	ns	ns	ns	ns
Cu	-	-	-	ns	ns	-
Fe	-	ns	ns	ns	ns	ns
Li	ns	ns	ns	ns	ns	ns
Mn	ns	ns	ns	ns	ns	ns
Ni	-	ns	ns	ns	ns	ns
Pb	ns	ns	ns	ns	ns	ns
Sr	ns	ns	-	-	ns	-
Ti	ns	ns	ns	ns	ns	ns
U	ns	-	ns	ns	ns	ns
V	ns	ns	+	ns	ns	+
Zn	-	ns	ns	ns	ns	ns

Trends determined using site-wise means at test level $\alpha = 0.05$. Negative trends indicate concentrations are decreasing in the downstream direction; positive trends indicate concentrations are increasing in the downstream direction. Trend codes: ns = trend was not statistically significant; **(-)** = trend was statistically significant and negative; **(+)** = trend was statistically significant and positive.

Linking Trace Elements to Particle Sizes

On average across elements and sites, the vast majority (80%) of trace-element concentration is contributed by particles small enough (< 20 μm) to be ingested by juvenile mussels (Table 7). This agrees with the established pattern wherein the majority of trace elements associated with aquatic sediments are contributed by the smallest particles, a consequence of the high surface area-to-mass ratio of those particles (Horowitz 1985). The finding that most trace-element mass is associated with ingestible particles supports the hypothesis that juvenile mussels may experience substantial exposure to trace elements if sediment particles are ingested.

Table 7. Mean percent of total element concentration in interstitial sediment (< 300 μm) contributed by particles < 20 μm in the Powell River, by site and month.[a]

Site ID & River km	**APP 293.7**	**BSG 287.2**	**DRY 269.1**	**RT70 233.5**	**RT833 186.8**	**MCD 171.9**	**UBB 153.4**	**OAK 144.2**	**YEL 136.2**	
Month										***Mean***
Oct 2020	77 (11)	76 (7)	74 (4)	87 (7)	85 (7)	87 (6)	91 (4)	72 (4)	85 (6)	*81 (7)*
Dec 2020	83 (10)	73 (9)	83 (4)	86 (9)	84 (3)	84 (6)	94 (3)	87 (3)	77 (4)	*83 (6)*
May 2021	75 (4)	74 (6)	72 (5)	88 (4)	68 (3)	81 (4)	75 (4)	72 (6)	73 (4)	*75 (6)*
Mean	*78 (4)*	*74 (1)*	*76 (6)*	*87 (1)*	*79 (10)*	*84 (3)*	*87 (11)*	*77 (9)*	*78 (6)*	***80 (4)***

[a] For each site and month, value is the mean of 18 elements: Ag, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sr, Ti, U, V, Zn. Excluded: Mo, Se, and Sn were below detection limits in > 25% of samples.

Toxicity Potential of Sediment Trace Elements

We calculated PECQs for individual elements as well as the seven-element mean PECQ for each sediment sample. The grand mean of individual element PECQs (n = 189; 27 samples x 7 elements) was 0.27 (Table 8). Across all samples, Ni (0.60) and Pb (0.40) had the highest individual mean PECQs, followed by Zn (0.25) and Cu (0.22). Arsenic (0.18), Cr (0.18), and Cd (0.05) mean PECQs were lower still (Table 8). Seven-element mean PECQs varied among study sites and months, ranging from 0.19 to 0.47 across all samples (Table 8).

Downstream trends in mean PECQs were nominally negative but not statistically significant in any month (Mann-Kendall p = 0.0763, 0.2510, 0.7540, in Oct, Dec, and May, respectively). There was no overall spatial difference in mean PECQs among sites over all months (LMM p = 0.0916), but there was a temporal difference in mean PECQ among months (LMM p = 0.0188), with mean PECQ lowest in May 2021 (Figure 3, Table 8). This temporal pattern is reasonable given that element-laden very fine sediment (< 20 μm) is lowest in relative abundance in May (Table 3), thus reducing total sediment concentrations and hence PECQs.

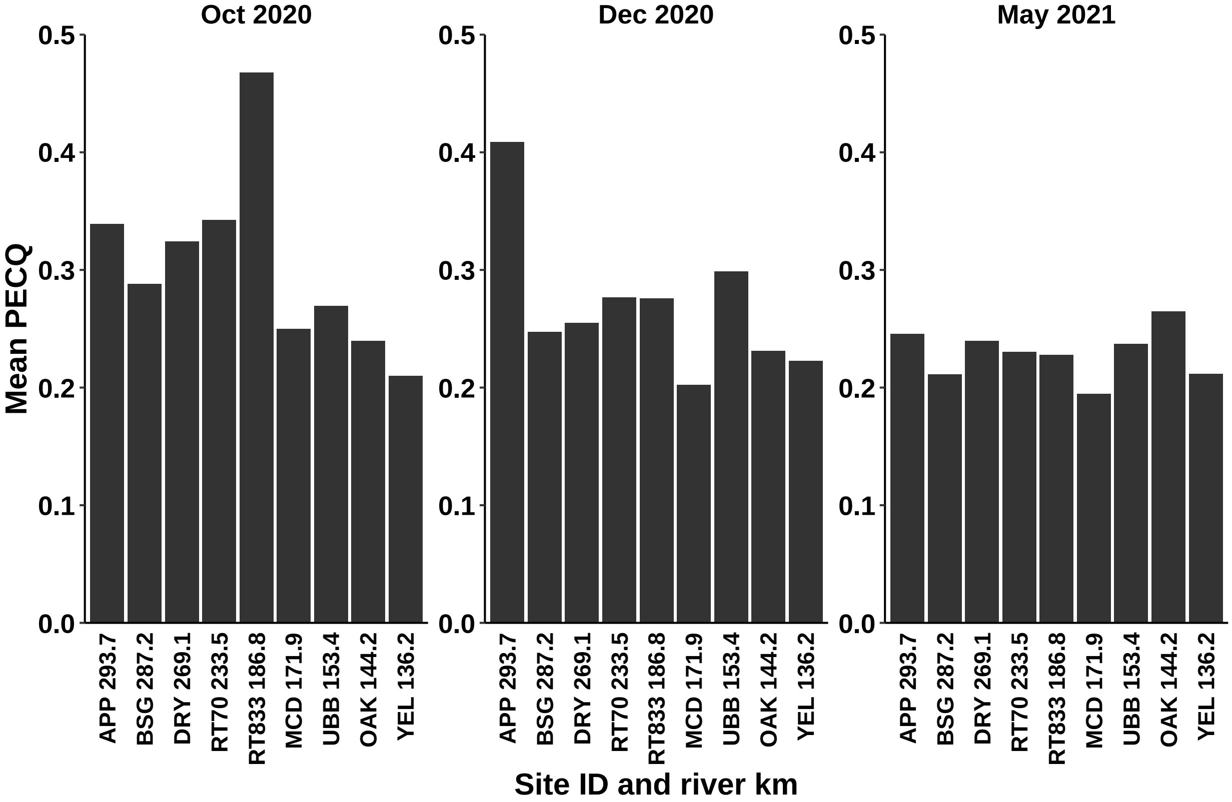

Figure 3. Mean probable effect concentration quotient (PECQ) of seven elements (As, Cd, Cr, Cu, Ni, Pb, Zn) at each of nine sites during three months. Mean PECQ ≥ 0.2 is in the range of toxicity concern (Wang et al. 2013).

Although grand mean PECQ (0.27) was < 1.0, Powell River sediment trace elements may yet pose toxicity risk to mussels. First, evidence suggests PECQ = 1 may not be protective of this sensitive faunal group, as PECs were developed based overwhelmingly on sediment-toxicity data from an insect and amphipod (Wang et al. 2013). Instead, mussels may be sensitive to concentrations of sediment trace elements much lower than their PECs, as toxic effects to mussels have been observed in mining-influenced sediment with multiple-metal mean PECQs ranging from 0.2 to 0.4 (Wang et al. 2013). Second, the grand mean PECQ masks elements with higher individual PECQs, as well as sampling sites or months with higher mean PECQs. For example, the higher PECQs for Ni, which range from 0.46 to 0.83 across all sites and months (Table 8), were above the 0.2 to 0.4 range at which toxicity has been observed (Wang et al. 2013). Similarly, Pb may be of greater concern than most other elements at certain times and places, with PECQ above average (> 0.27) in 15 of 27 samples, ≥ 0.4 in 7 of 27 samples, and as high as 1.65 in one sample (Table 8). In addition, certain locations and times had higher mean PECQs, suggesting spatiotemporal variability in toxicity risk. Third, higher mean PECQs at certain sites or times of year suggest greater toxicity risk to mussels at those places and/or times, emphasizing the need to consider spatiotemporal variability when assessing sediment toxicity risk.

Table 8. Probable effect concentration quotient (PECQ) for individual elements in Powell River substrate interstitial sediment (< 300 μm) by site and month. Elements are arranged in descending order of monthly mean PECQ. Bold and italic values are PECQ ≥ 0.2, the range of toxicity concern (Wang et al. 2013).

		Site ID & River km									
	PEC[a]	**APP**	**BSG**	**DRY**	**RT70**	**RT833**	**MCD**	**UBB**	**OAK**	**YEL**	
Element	(mg/kg)	**293.7**	**287.2**	**269.1**	**233.5**	**186.8**	**171.9**	**153.4**	**144.2**	**136.2**	**Mean**
Oct 2020											
Ni	48.6	***0.69***	***0.72***	***0.81***	***0.83***	***0.64***	***0.63***	***0.62***	***0.56***	***0.46***	***0.66***
Pb	128	***0.67***	***0.28***	***0.40***	***0.52***	***1.65***	***0.26***	***0.33***	***0.30***	***0.27***	***0.52***
Zn	459	***0.38***	***0.28***	***0.34***	***0.38***	***0.31***	***0.24***	***0.32***	***0.25***	***0.23***	***0.30***
Cu	149	***0.24***	***0.26***	***0.25***	***0.24***	***0.28***	*0.18*	0.18	0.15	0.12	***0.21***
As	33.0	0.19	***0.24***	***0.23***	0.18	0.17	0.17	0.18	0.18	0.17	0.19
Cr	111	0.15	0.16	0.18	***0.20***	0.17	***0.21***	***0.20***	0.19	0.17	0.18
Cd	4.98	0.06	0.08	0.07	0.05	0.06	0.05	0.06	0.06	0.05	0.06
Monthly Mean		***0.34***	***0.29***	***0.32***	***0.34***	***0.47***	***0.25***	***0.27***	***0.24***	***0.21***	***0.30***
Dec 2020											
Ni	48.6	***0.61***	***0.63***	***0.59***	***0.73***	***0.46***	***0.47***	***0.70***	***0.57***	***0.54***	***0.59***
Pb	128	***0.82***	***0.20***	***0.33***	***0.25***	***0.86***	***0.28***	***0.35***	***0.27***	***0.21***	***0.40***
Cu	149	***0.62***	***0.25***	***0.27***	***0.32***	0.18	0.17	***0.23***	0.14	***0.20***	***0.27***
Zn	459	***0.34***	***0.23***	***0.24***	***0.28***	0.18	0.17	***0.24***	0.17	***0.20***	***0.23***
Cr	111	***0.20***	0.19	0.16	0.18	0.11	0.17	***0.23***	0.26	***0.25***	0.19
As	33.0	***0.21***	0.18	0.15	0.11	0.12	0.12	***0.30***	0.17	0.12	0.16
Cd	4.98	0.05	0.06	0.04	0.07	0.04	0.03	0.04	0.04	0.04	0.05
Monthly Mean		***0.41***	***0.25***	***0.26***	***0.28***	***0.28***	***0.20***	***0.30***	***0.23***	***0.22***	***0.27***
May 2021											
Ni	48.6	***0.66***	***0.50***	***0.62***	***0.65***	***0.48***	***0.51***	***0.61***	***0.54***	***0.50***	***0.56***
Pb	128	***0.21***	0.16	***0.28***	***0.22***	***0.50***	***0.21***	***0.27***	***0.38***	***0.26***	***0.28***
Zn	459	***0.24***	0.19	***0.23***	***0.24***	***0.20***	0.19	***0.21***	0.19	0.18	***0.21***
Cu	149	***0.25***	***0.30***	0.17	0.19	0.16	0.15	0.18	0.14	0.13	0.18
As	33.0	0.16	0.16	0.18	0.12	0.11	0.13	***0.20***	***0.39***	0.16	0.18
Cr	111	0.16	0.14	0.15	0.16	0.12	0.15	0.16	0.17	***0.20***	0.16
Cd	4.98	0.05	0.03	0.05	0.03	0.04	0.03	0.04	0.05	0.04	0.04
Monthly Mean		***0.25***	***0.21***	***0.24***	***0.23***	***0.23***	0.19	***0.24***	***0.26***	***0.21***	***0.23***
Grand Mean		***0.33***	***0.25***	***0.27***	***0.28***	***0.32***	***0.22***	***0.27***	***0.25***	***0.21***	***0.27***

[a] PEC: probable effect concentration (MacDonald et al. 2000)

Sediment Trace Elements in Clinch River

We compared May 2021 sediment metrics and PECQs from the Powell River to those from two sites in the Clinch River where mussel populations have been documented in good condition as recently as 2014 (Jones et al. 2018). One site in the Clinch River is in Virginia, upstream from the bulk of mining land use, and one site is in Tennessee, downstream from the bulk of mining land use, in a zone of mussel recovery. Comparisons between rivers of percent of sediment mass < 20 μm and percent of total element concentration contributed by particles < 20 μm provide no evidence that the Powell River is obviously different from Clinch River with respect to sediment size distribution and trace element size-partitioning (Table 9). These data do not suggest factors such as relatively more fine sediment in the Powell River or absence of trace elements associated with small, ingestible particles in the Clinch River are likely explanations for observed differences in mussel populations between the two rivers.

Where the rivers differ, however, is in total sediment concentrations, and thus PECQs, for certain trace elements. Examining seven elements of toxic concern (As, Cd, Cr, Cu, Ni, Pb, Zn) between rivers reveals nominally lower total concentrations and PECQs in the Clinch River, where mean PECQs (7 elements) were 0.16 (upstream of mining) and 0.19 (downstream of mining), as compared with mean PECQs ranging from 0.19 to 0.26 (mean = 0.23) in the Powell River (Table 10). Notably, individual PECQs for some elements at some sites in Powell River were 1.4 to 4.8 times higher than the highest observed value in the Clinch River. Among elements with PECQs > 0.1, the greatest relative differences in maximum PECQs between rivers (Clinch vs. Powell) were observed for Pb (0.15 vs. 0.50), Zn (0.17 vs. 0.24), Cu (0.17 vs. 0.30), and As (0.08 vs. 0.39). Nickel was more comparable between rivers, but still higher in the Powell River, especially as compared to the Clinch River site upstream from most mining (0.39 vs. 0.66). Chromium (Cr) was slightly higher in the Clinch River downstream from mining (0.20 vs 0.26) and Cd did not exceed 0.1 in either river (Table 10).

Our observation of healthier mussel populations associated with lower sediment concentrations for several elements of toxic concern is evidence to support sediment trace elements as a candidate cause of mussel suppression in the Powell River. However, more data are needed, especially from the Clinch River, to verify these initial observations. In addition, it is unknown if or how such differences vary throughout the year. We note that the differences between rivers described here are for the time of year when fine sediment (Table 3) and mean PECQs (Table 8) are lowest in the Powell River. Both metrics are highest in Oct 2020; additional data from the Clinch River during autumn would help address this knowledge gap.

Table 9. Comparison of sediment metrics for Powell River and Clinch River substrate interstitial sediment (< 300 μm) in May 2021.

	Powell River		***Clinch River***		
	Mean	**Range**	**Upstream of Mining (VA)**	**Downstream of Mining (TN)**	**Mean**
Percent of Sediment Mass < 20 μm					
	65	49 – 87	50	84	67
Percent of Total Element Concentration Contributed by Particles < 20 μm					
Zn	77	70 – 90	64	91	78
Ni	77	68 – 91	63	92	78
Cr	76	69 – 87	63	91	77
Pb	76	68 – 89	61	86	74
Cu	74	67 – 88	60	91	76
As	73	65 – 82	51	87	69
Cd	72	65 – 88	62	88	75

Table 10. Probable effect concentration quotient (PECQ) for individual elements in Powell River and Clinch River substrate interstitial sediment (< 300 μm) in May 2021. Elements are arranged in descending order of monthly mean PECQ in Powell River. Bold and italic values are PECQ ≥ 0.2, the range of toxicity concern (Wang et al. 2013).

		Powell River		***Clinch River***		
Element	**PEC**[a] (mg/kg)	**Mean**	**Range**	**Upstream of Mining (VA)**	**Downstream of Mining (TN)**	**Mean**
Ni	48.6	***0.56***	***0.48 – 0.66***	***0.39***	***0.57***	***0.48***
Pb	128	***0.28***	0.16 – ***0.50***	0.15	0.08	0.11
Zn	459	***0.21***	0.18 – ***0.24***	0.17	0.15	0.16
Cu	149	0.18	0.13 – ***0.30***	0.14	0.17	0.16
As	33.0	0.18	0.11 – ***0.39***	0.08	0.06	0.07
Cr	111	0.16	0.12 – ***0.20***	0.16	***0.26***	***0.21***
Cd	4.98	0.04	0.03 – 0.05	0.03	0.01	0.02
	Mean	***0.23***	0.19 ***– 0.26***	0.16	0.19	0.17

[a] PEC: probable effect concentration (MacDonald et al. 2000)

CONCLUSIONS

Size-fractionation and trace-element analysis of sediment particles (< 300 μm) from nine sites in the Powell River at three times of year revealed five key findings. First, very fine particles dominate substrate interstitial sediment samples, with an average of 70% of sediment mass contributed by particles < 20 μm, a size ingestible by juvenile mussels. Second, among trace elements of potential sediment-toxicity concern (As, Cd, Cr, Cu, Ni, Pb, Zn), Zn, Pb, Ni, Cu, and Cr were consistently in the upper half of dominant elements by mass. Third, the vast majority (80%) of trace-element mass present in sediment is associated with particles ingestible by juvenile mussels (< 20 μm). Fourth, total sediment concentrations of Cu, Ni, Pb, and Zn were consistently elevated to potentially toxic levels, especially Ni and Pb which occasionally exceeded 60 – 80% of their respective sediment quality guidelines. Finally, spatiotemporal patterns were variable, as sediment concentrations of most elements did not decline predictably downstream from major mining land use. However, a temporal pattern appears to exist, with sediment concentrations of most elements higher in autumn and lower in spring, a pattern consistent with elements being associated with very fine sediment, which itself exhibits a seasonal pattern of colmation and decolmation.

Our findings support our hypothesis that juvenile freshwater mussels could be exposed to trace elements associated with sediment particles. As we predicted, substantial amounts of trace elements are present in the Powell River and are associated with the finest particles readily-ingested by juvenile mussels. Given that such particles are present at all sites spanning over 150 river kilometers, it is possible that freshwater mussels, especially juveniles, are at substantial risk of exposure to potentially toxic trace elements associated with these particles.

These results can be used to inform further research, including experimental ecotoxicological exposures, to identify causes of mussel decline in the Powell River and other Appalachian waters. Findings can also aid refinement of sediment-quality criteria to include more data on freshwater mussels, a fauna historically under-represented in such criteria. These outcomes advance the environmental science mission of Powell River Project by generating knowledge that will aid management and restoration of Powell River water quality and mussel populations, both of which are important environmental resources affected by mining in the Appalachian coalfield.

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