

CONCENTRATIONS AND SOURCES OF TRACE METALS IN WATER AND SEDIMENTS
OF THE SOUTH FORK NEW RIVER, ASHE COUNTY, NORTH CAROLINA, AND THEIR
POTENTIAL EFFECTS ON AQUATIC BIOTA

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partial fulfillment of the requirements of the degree of Master of Science in Chemistry

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LIST OF ABBREVIATIONS

EPA	Environmental Protection Agency
NPL	National Priority List
NCDEQ	North Carolina Department of Environmental Quality
NR	South Fork New River
NRT	South Fork New River Tributary
NR 27	Downstream North and South Fork New River confluence
NR 28	New River
KC	Kings Creek
DC	Dog Creek
NNR	North and South Fork New River confluence
ICP-OES	Inductively coupled plasma optical emission spectrometer
XRF	X-Ray Fluorescence
LOI	Loss-on-Ignition
SSC	Suspended Sediment Concentration
OCC	Organic Carbon Content
IQR	Interquartile Range
TEC	Threshold Effect Concentrations
PEC	Probable Effect Concentrations

ABSTRACT

CONCENTRATIONS AND SOURCES OF TRACE METALS IN WATER AND SEDIMENTS OF THE SOUTH FORK NEW RIVER, ASHE COUNTY, NORTH CAROLINA, AND THEIR POTENTIAL EFFECTS ON AQUATIC BIOTA

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The Ore Knob Copper Mine located near Jefferson, North Carolina, operated from 1850 to 1962; mine tailings from the mine were disposed of behind an earthen dam located in the headwaters of Ore Knob Branch, a tributary to Peak Creek. As a result of groundwater contamination, the EPA placed the mine on the National Priority List in 2009. The erosion of mine tailings also released toxic trace metals to Peak Creek, where they were transported to the South Fork New River, an important recreational and ecological resource. The objectives of this study were to determine the source of metals in the South Fork New River and their potential impact(s) on aquatic biota by (1) documenting metal concentrations, and their spatial variations, in water and sediment along the river to locate potential sources, and (2) comparing these metal concentrations to previously developed aquatic effect criteria. Geochemical analyses included the determination of both total and bioavailable concentrations of aluminum, cobalt, copper, iron, and zinc in channel bed sediment as well as their dissolved and total recoverable concentrations in water along a 33 km reach of the South Fork New River. Water and sediment samples were also collected from

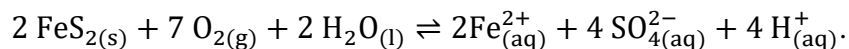
surrounding tributaries, including Peak Creek. Metal concentrations in Peak Creek, below the mine, were relatively high and decreased downstream, indicating that some metals were delivered to the South Fork New River. However, metal concentrations in both water and sediment varied significantly along the river and did not abruptly increase downstream of Peak Creek. These spatial trends suggest that the Ore Knob mine and Peak Creek (which was once devoid of fish) no longer contribute significant quantities of metals to the South Fork New River. Downstream variations in sediment-associated metal concentrations appear to reflect, in part, localized fine-grain sediment deposition, differences in organic carbon content, and the exposure of bedrock outcrops from which sulfidic minerals can be eroded. Comparison of dissolved metal concentrations in water to EPA guidelines found that no sampling sites exceeded acute nor chronic limits. In contrast, numerous (56 %, 94%) of the sampling sites exceeded the NCDEQ total recoverable concentration limits (120 ppb, 7 ppb) for Zn and Cu, respectively. A total of 2 sites exceeded the threshold effect concentration (TEC) for sediments compiled for Zn by MacDonald et al., whereas 3 sites exceed the TEC for Cu. The various criteria used herein suggest differing potential impacts of the metals on aquatic biota along the South Fork New River. However, the bioavailability of the metals is an important consideration. Dissolved concentrations in water, which indicated limited potential impacts, represent highly bioavailable metals. In contrast, metals attached to sediments both on the channel bed and within the water column may in part, be non-bioavailable, particularly given that the metals are largely derived from sulfidic mineral layers in the bedrock. Thus, the impacts to aquatic biota is likely to be limited.

CHAPTER ONE: INTRODUCTION

The Ore Knob Mine located near Jefferson, North Carolina operated from the 1850's to 1962. Tailings materials from the mine were disposed of in a headwater drainage (Ore Knob Branch) that was blocked by an 18 meter-high, 213-meter wide earthen dam. The estimated 550,479 m³ of tailings waste covered an area of approximately 80,937 m² and were characterized by high levels of toxic trace metals including arsenic (As), cobalt (Co), copper (Cu), and zinc (Zn), among others. The tailings were also high in major metals such as iron (Fe).¹ The Environmental Protection Agency (EPA) and NC Department of Environmental Quality (NCDEQ) found that many residences in and near Jefferson had contaminated tap water. The surface water around Ore Knob Branch and Peak Creek contained high concentrations of metals, including manganese (Mn), Fe, Co, and Cu. Ground- and tap-waters were also highly acidic (pH= ~4).² In response to the contaminated ground water, surface water, sediment, and soil in the vicinity of the tailings piles, the copper mine was placed on the Superfund National Priorities List (NPL) in 2009. Between April 2010 to June 2012, the EPA installed 79 potable water sources to replace the domestic supply of water from 64 private wells and 15 springs.³

Metals within the Ore Knob's tailings were presumably associated with sulfide minerals, such as pyrite, chalcopyrite, sphalerite, and pyrrhotite. In addition, erosion had severely damaged the tailings dam, allowing tailings to be transported downstream.^{1,3}

When sulfide minerals are subjected to moisture in the presence of oxygen, they are oxidized thereby releasing trace metals to the water, along with SO_4^{2-} , generating acid drainage in the process:^{2,4}



Microbial activity may also have played a role in the mineral oxidation of sulfur and iron containing minerals, as oxidizing bacteria can catalyze oxidation reactions.⁴

Given that the tailings were “improperly” disposed and stored in the headwaters of Ore Knob Branch, a tributary to the Little Peak Creek and the South Fork New River, contaminated tailings material were likely to have been eroded and transported downstream to the South Fork New River (Figure 1).^{2,5} In fact, in 2009 exploration level investigations found that Ore Knob Branch and Little Peak Creek contained elevated levels of trace metals (in comparison to background values), particularly Cu, Co, Zn, Mn, As, and mercury (Hg).² However, the degree of contamination was such that persons were not expected to “come into contact with these areas (of contamination) with a frequency that would lead to ingestion of a sufficient amount of sediment or soil to cause harm”.¹ Thus, regulatory agencies proposed no further actions on the drainage system other than monitoring. However, the potential impact of metal contaminants on aquatic biota within the riverine ecosystems of the Peak Creek drainage network and the South Fork New River to which it drains is less clear. In fact, the release of acidic, metal-enriched waters from the mine site has resulted in yellow and red-stained sediments indicative of acid mine drainage along Ore Knob Branch, Little Peak Creek and Peak Creek. Based on a 2009 Health Risk Assessment, the long term release of these metals from the mine site left all three

drainages devoid of fish.² Significant loads of trace metals were also known to enter the South Fork New River from Peak Creek.²



Figure 1. Mine site (white arrow) showing the earthen dam across Ore Knob Branch (1) and the disposed of tailings materials (2) within the Peak Creek Drainage system.

In addition to the potential introduction of metals to the South Fork New River from Peak Creek, metals may enter the river system via groundwater inflow and from natural sources, including mineralized rocks that underlie the basin. Little is currently known about the contributions of metals to the South Fork New River from these potential sources.

The introduction of metal contaminants to the South Fork New River is a significant concern as the river has been designated by the State of North Carolina as an “outstanding resource water”. The New River, into which the South Fork New River flows, was the first “National Wild and Scenic River” in the United States. It is also designated as an “American Heritage River” and is one of only four rivers designated as a “State Scenic River”. A portion of

the New River State Park is located along the South Fork New River, and is used for canoeing, fishing, camping, and picnicking.²

High levels of the metal contaminants may affect aquatic biota within and adjacent to the South Fork New River and its tributaries. The oxidation of sulfide minerals may also affect the pH of the water and soil. Acidic waters are known to lead to decreases in fish populations and other aquatic biota. In addition, acidic waters can lead to an increase in dissolved aluminum (Al) concentrations that can result in excess mucus in fish, causing fish to asphyxiate.⁸ High levels of Co can damage the citric acid cycle enzymes and inhibit cellular respiration.⁶ High levels of manganese (Mn) can cause hypoxia in aquatic biota.⁷ High levels of Cu can create dead zones in water bodies such as lakes and alluvial reaches of riverine systems; Cu often frays gills on fish and causes them to lose the ability to regulate the transport of salts in and out of the fish.⁸ Iron can be taken up through water or food; when food is enriched with high levels of Fe it can cause the gut membrane in fish to form thick iron hydroxide encrustations on the gut wall.⁹ Determining the concentrations of these metal contaminants can aid in determining the potential risks posed by these metal contaminants from the Ore Knob Mine on biota within the South Fork New River and tributaries.

Objectives

Trace metal contamination of the South Fork New River near the Ore Knob Mine has not been investigated in detail. The primary objectives of this investigation were to: (1) determine the concentration of metals shown to be elevated in the vicinity of the mine, including Fe, Co, Cu, and Zn in river waters and the channel bed sediment along the South Fork New River and surrounding tributaries, (2) document the spatial variations in metal concentrations along the South Fork New River to determine the potential sources of the metal contaminants in the river,

and (3) determine the potential impact of these trace metals on the aquatic biota using previously developed threshold and probable effect quotients developed by MacDonald et al.¹⁰

CHAPTER TWO: STUDY AREA

Geographic and Geomorphic Setting

The South Fork New River flows northeast for approximately 201 km from Boone, North Carolina to the New River confluence (Figure 2) before flowing through West Virginia and Virginia to join with Gauley River in West Virginia.^{11,12} The overall water quality of the South Fork New River in 2008 was classified as Good-Fair and had Fair Bioclassification.¹¹ The New River Basin, including the South Fork, is designated as an Aquatic Significant Natural Heritage Area on the basis of the occurrence of a variety of rare species, including the federally threatened Bog Turtle, the endangered Purple Wartyback mussel, and the rare, Kanawha minnow, sharpnose darter, tonguetied minnow and Kanawha darter, which are found nowhere else in North Carolina. In fact, the Kanawha minnow is found nowhere else in the world. Two amphibians, the hellbender and common mudpuppy, are found along the South Fork New River.¹³

The South Fork New River belongs to the New River drainage basin with an area of 1950 km². The South Fork New River has a daily mean discharge of 15.4 m³/s.¹⁴ The major underlying geology of Ashe county, which underlies the South Fork New River basin, consists of biotite granitic gneiss, muscovite-biotite gneiss (locally sulfidic), amphibolite, gneiss, metagraywacke, metafelsite, and meta-ultramafic rocks.¹⁵

CHAPTER THREE: METHODS

Sampling Program

Metal contaminants can be found in water or sediment and can be either dissolved within the water column or sorbed to particle surfaces. Dissolved metals are typically considered bioavailable, whereas the bioavailability of sediment-associated metals depends on how they are bound to the particle. Regardless of how metals exist in the riverine system, aquatic biota can uptake these metals after which they can cause various health effects.¹⁶ Previous studies have shown that more than 90% to 99% of the trace metal load is dispersed through river systems attached to sediments (i.e., with the particulate load). These metal-associated sediments are transported through the river system and incorporated into channel bed and floodplain deposits. Thus, the analysis of channel bed and floodplain deposits provides insights into the longer-term (annual to decadal scale) transport of metals through the river as well as the potential impacts of the metals on aquatic biota.¹⁷

During this investigation unfiltered (total recoverable metal content), filtered (dissolved metals) and channel bed sediments were sampled and analyzed. Sampling occurred in August 2018; water and sediment samples were collected from the South Fork New River between the Waggoner Access Point and the Allegany County Access Point (Figure 2). Samples were also obtained from major tributaries including Peak Creek (which drains the mine), Dog Creek, Kings Creek, and the North Fork New River. A total of 36 sediment samples were taken approximately every 1 km along the 33 km study reach of the South Fork New River. In doing so, sampling sites were located, up- and downstream of the tributary mouths listed above. The water samples were collected at approximately at every other site. At each sampling site, the sediments were

collected from the upper 5 cm of the channel bed at multiple locations across the channel and composited (combined) to reduce field variance. The sampling process was aided by a research team that included, Dr. Miller and three geology research assistants. All samples were placed in polyethylene containers, packaged in plastic bags and shipped to WCU for analysis. In addition to the sediment samples, two water samples were collected at each site, one to determine dissolved metal concentrations and the second to determine total-recoverable metal concentrations. Samples obtained for dissolved metal analyses were filtered into pre-cleaned polyethylene bottles in the field using 0.45 μm disposable filtering syringes. The samples were then be acidified (preserved) with approximately 2 ml of 10 % nitric acid, and “double-bagged” in plastic sampling bags.

Geochemical Analysis

The water samples were analyzed for a suite of trace metals (Co, Cu, Fe, and Zn) using a Perkin Elmer Optima 4100DV inductively coupled plasma – optical emission spectrometer (ICP-OES). The focus on these four elements (Co, Cu, Fe, and Zn) was related to their relatively high concentrations within the tailings materials² at the Ore Knob Mine, and previously collected data along Little Peak Creek and the South Fork New River.¹ Sample analysis for dissolved and total-recoverable metals in water followed the EPA 200.7 method.¹⁸ The dissolved samples were filtered through 0.45 μm filters and stored in 10 % HNO_3 , when collected in the field and later analyzed. In contrast, the samples for total metals were treated with a combination of 2:1 nitric and hydrochloric acid, heated, refluxed and then brought to a known volume. They were then allowed to sit for at least 24 hours (after they had cooled) to allow digested sediment to settle and then filtered and analyzed. Trace metal nitrate salts were used to prepare all the calibration standards. Replicate analyses were performed on a subset of samples to determine analytical

precision, and analyses of standards and reagent blanks were used to estimate lower limits of detection. Accuracy and precision were generally within 5%.

The sediment samples were analyzed by two different methods. First, X-ray fluorescence spectroscopy (XRF) was used to determine the total metal concentrations within the sediments. These total concentrations provide insight into the mineral structure and composition. Second, the samples were digested in aqua regia and analyzed by ICP-OES. This procedure, often referred to as partial digestion, provides insight into the concentrations of the metals bound to the sediment surface, and which are likely to be more bioavailable.

Prior to analysis, the sediment samples were dried and sieved using nylon screen mesh to obtain the <2 mm sediment fraction. A subsample of the bulk (< 2mm) fraction of sediment samples were then digested in an aqua regia (1 mL nitric acid and 3 mL hydrochloric acid) hot bath (90-95 °C) (Appendix A for complete digestion procedure). Following digestion, the samples were analyzed for major elements (Cu, Fe, Co, and Zn) using ICP-OES. Calibration standards were created using trace grade nitrate salts. Method blanks and reagent blanks were used to determine if there was the presence of contamination between samples during the acid digestion procedure and to determine any signal produced by the detector which was not due to the presence of the analytes. A regression line was fitted to the calibration points and the equation of the line was used to quantify unknown sample concentrations using the Beer-Lambert law.

With regards to the analysis of total metal concentrations in the sediments, a subsample of the dried sediment was ground with a mortar and pestle into fine (<63 µm) particles. This allows the X-rays to penetrate the sediment sample completely and the detector to analyze the X-

rays and compute an accurate spectrum. The ground sediments were then placed in plastic sample cups to which a plastic film (Mylar) had been placed over the bottom. The cap was then placed over the cup to allow the film to be tightly secured to the cup. Sediment was added and packed in, after which a small piece of cotton was placed on top to completely pack the sediment in the sampling cup.

After the analysis, the raw data as oxides from the sediment samples, were converted to concentrations using a set of two standards, USGS-SGR-1b and ORESA-930. These standards were obtained from the USGS and their composition was the closest to the grain composition of the sediment samples analyzed.

For the validation of the standards utilized in this project, calibration curves of known vs. measured were created for elements of 24 OREAS soil standards and 5 USGS soil standards. Then the raw data from the USGS-SGR-1b and ORESA-930 standards were input into each regression line of the metal (calibrated data). The calibrated data was then compared to the known in the respective standards using percent difference. If the percent difference was <10% then the data collected was able to be validated, if 10-20% then the data collected was potentially able to be validated and if >20% then the data collected could not be validated.

Analysis of Sediment Grain Size Distribution of Channel Bed Sediment

Approximately 5 g of the dried sediment was added to a plastic beaker. Subsequently, 40 mL of deionized water was added to a plastic beaker containing the sediments along with the dispersal agent (10% sodium pyrophosphate). The sediment-dispersal mixture was then stirred and allowed to sit for at least 24 hours. After redispersion of the sample mixture, the grain size distribution of the < 2 mm fraction was analysed using a Malvern laser particle size analyser.

Carbon Analysis by Loss-On-Ignition

The sediment samples were dried (~ 70 °C) for 6-8 hours. Approximately ~5-10g of sediment were added to the crucible and massed and recorded. The sediment sample and crucible were added to a muffle furnace and heated at 550 °C for 6 h. Then the samples were put into a desiccator and allowed to cool, and the samples were massed and recorded again. The percent loss-on-ignition (LOI %) was calculated by:

$$\text{LOI \%} = \frac{(\text{sediment sample} - \text{heated sediment sample}) \text{ g}}{(\text{sediment sample}) \text{ g}} \times 100$$

Once the LOI % was calculated for each site, the values were multiplied by a conversion factor of 0.55 due the relationship found between LOI , wet chemical oxidation, soil clay content, and clay correction factor for structural water loss explored at given furnace temperatures by Hoogsteen et al.¹⁹

Analysis of Suspended Sediment Concentration of Channel Bed Sediment

The suspended sediment samples were poured into a vacuum filter apparatus, containing a glass cup, metal tray, and filter paper. Once the water was completely through the vacuum filter apparatus, the filter paper, glass cup, and metal tray were put into an oven and heated at 50°C for an hour. Then the glass cup, tray and filter paper were put in a desiccator to cool. The dried sediment was subsequently massed and divided by the amount of water that was present in the sample giving the suspended sediment concentration in milligrams sediment per liter of water.²⁰

Data Analysis

In order to determine the potential impacts of the analyzed metals on aquatic biota, the resulting geochemical concentration data were compared to existing aquatic guidelines. With respect to water, dissolved metal concentrations were compared to acute and chronic EPA freshwater aquatic biota criteria. Total-recoverable metal concentrations in water were compared to North Carolina standards. The concentrations of metals within sediments were compared to the guidelines provided by MacDonald et al.¹⁰

The sources of metal contaminants within the water and sediments were determined based on spatial trends in metal concentrations along the study reach, and their relationships to geomorphic features. Metal concentrations in both sediments and water were plotted for the length of the South Fork New River study reach (Figure 2) to determine the spatial variations that exist. Observed variations could then be related to known or potential sources of metals (e.g., the Ore Knob Mine, mineralization of bedrock units, and/or groundwater) to assess the significance of each metal source.

CHAPTER FOUR: RESULTS

Morphologic and Sedimentologic Characteristics of the South Fork New River

The channel of the South Fork New River within the study area varies in terms of its morphometric, sedimentologic, and hydrologic characteristics. In some areas, referred to as alluvial reaches, the channel bed and banks were dominated by alluvial sediments, the channel was relatively narrow and deep, and flow velocities were relatively low. The channel bed sediments within these reaches was highly variable, consisting in some instances of fine-grain sediment (fine sediment sized particles, $<63 \mu\text{m}$); in other locations, the bed consisted of coarse-gravel and cobbles that were surrounded by accumulations of sand. These fine-grain sediments contain clay minerals and organic matter which are chemically reactive and can influence metal concentrations.¹⁷ These alluvial reaches were separated by bedrock reaches. Bedrock outcropped along these reaches, although the channel bed also contained coarser sediment, reaching up to boulder sized materials. Accumulations of coarse sand commonly occurred downstream of cobbles and boulders in the channel bed, while sand bars occasionally occurred along the channel margins, particularly downstream of tributaries. Many bedrock reaches exhibited bedrock knickpoints (abrupt breaks in channel slope) that created rapids that possessed highly turbulent flows. The turbulent flows and high gradient prevent finer-grain particles from depositing and less chemically reactive particles to be present in this reach. An example of each reach type has been provided in Figure 3.

Each sampling site was categorized as an alluvial reach or bedrock reach prior to statistically analyzing metal concentrations. Categorization was based on field descriptions of channel form (e.g., width, depth), channel bed composition (e.g., bedrock outcrops, sediment

size), and flow velocities, as well as the occurrence of bedrock knickpoints and rapids. Google Earth images were used to determine the reach type of each sampling site based on the criteria above.

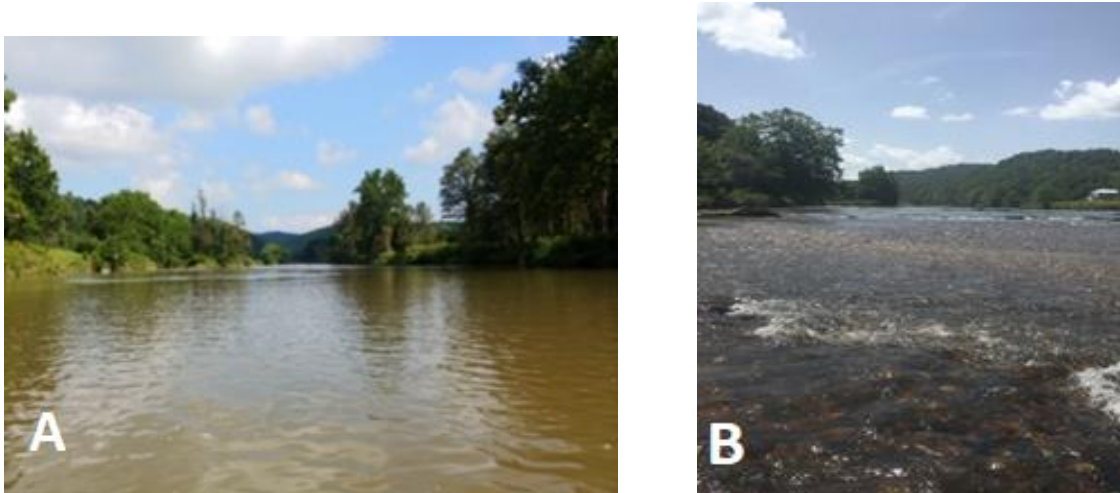


Figure 3. An (A) alluvial reach and (B) bedrock reach along the South Fork New River.

The South Fork New River and adjacent tributaries were primarily dominated by sand sized or larger sediments. In fact, Figure 4 shows that 14 (82%) of the 17 channel bed sediments mostly contained less than 5% clay and silt. Of the total sites with measurable clay and silt, 11 out of 17 sites, were alluvial reaches (Figure 4). The sites with the highest percent of silt and clay were NR 21 (alluvial reach) and NR 26 (bedrock reach, with bar), which contained 21.1% and 31%, respectively. Other sites with above average ($> 4.9\%$) clay and silt were NR 2, NR 3, NR 16, NR 21, and NR 26. Except for NRT 2, sampled tributaries possessed less than the average amount of silt and clay in the main channel. The sample taken at sites NNR 1, NR 27 and NR 28 all possessed less fine sediment than the average found along the South Fork New River.

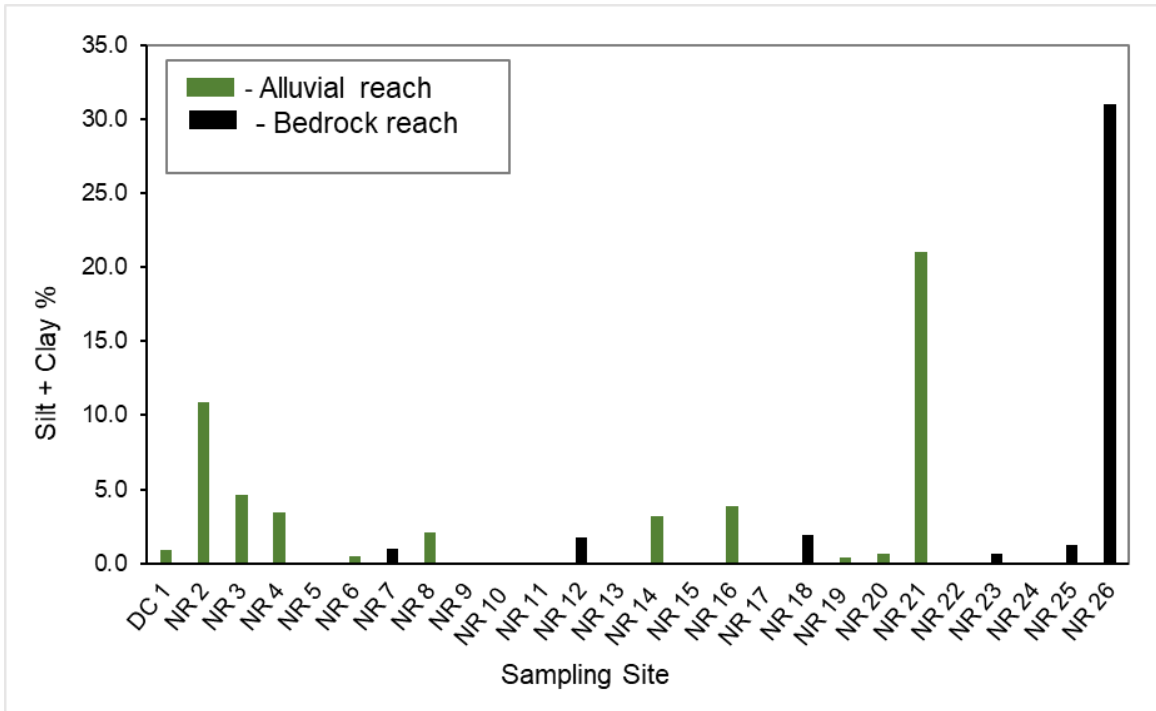


Figure 4. Percentage of fine sediment measured at each sampling site. Some sites exhibited no measurable silt and clay.

Table 1. Mean, minimum and maximum quantities of silt and clay, fine sand, organic content (OCC), and suspended sediment concentrations (SSC) for the sampling sites (n=25) along the South Fork New River.

Parameter	Mean (%)	Minimum (%)	Maximum (%)
Silt+ Clay %	4.9	0.3	31
Fine Sand %	17.3	0.6	55.2
OCC %	0.8	0.2	5.2
	Mean (mg/L)	Minimum (mg/L)	Maximum (mg/L)
SSC	23.7	0.8	161.6

As shown in Figure 5, alluvial reaches possessed larger variation in percentage of silt and clay and fine sand; the interquartile range (IQR) was 3.74 % and 32.61 %, respectively, compared to the bedrock reaches which was 1.78 % and 8.25 %, respectively. The alluvial

reaches also had a larger mean of silt and clay percent (3.90 % compared to 1.53 %) and fine sand percent (22.44% and 13.69%) than the bedrock reaches. Median values of silt and clay and fine sand were also higher for alluvial reaches (3.19 % compared to 0.60 %, and 13.40 % compared to 8.82 % for silt and clay and fine sand, respectively). The alluvial reaches had more outliers of silt and clay, whereas the bedrock reaches had more outliers of fine sand (Figure 5). The outliers were determined by multiplying the IQR by 1.5, after which the derived value was subtracted from the first quartile value and added to the third quartile.

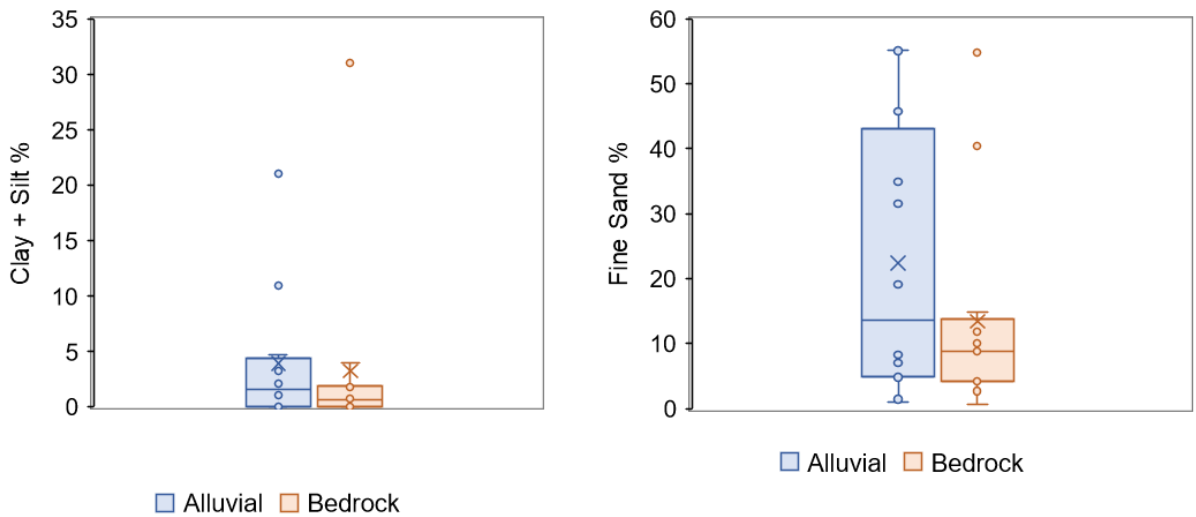


Figure 5. Box and Whisker plot of fine sediment % and fine sand % for alluvial and bedrock reaches. The outer lines represent minimum and maximum values, the first, second and third line of the box represents the first quartile, median and third quartile, respectively; the “x” represents the mean of the data set and the circles on the outside of the whiskers represent the outliers of the data set.

The silt and clay and fine sand percentages were compared for each reach type by using a Mann-Whitney U-test to determine if each site had a difference in grain size.²¹ The test suggest that there was no difference in the two reach types at a significance level of $p < 0.05$.

The organic carbon content (OCC) for each site is shown in Figure 6 and the mean, minimum, and maximum values are provided in Table 1. Of the tributary sites and sites sampled outside of the South Fork New River, NRT 2, PC 1, PC 2 and NRT 3 possessed OCCs that were above the average OCC for the South Fork New River; sites NNR 1, NR 27 and NR 28 were all below the average for the South Fork New River. The percent of OCC in the channel bed sediments remained under 1% for sites identified as bedrock reaches except for sites NR 16 (1.06 %) and NR 26 (5.21 %). OCC values decreased downstream from NR 2 after which it remained under 1 % in alluvial reaches (except for sites NR 2, NR 3, and NR 4). The sites NR 21 (an alluvial reach) and NR 26 (bedrock reach, where sediment was obtained from a side-channel bar) possessed the highest OCC values; 3.58 and 5.21 %, respectively.

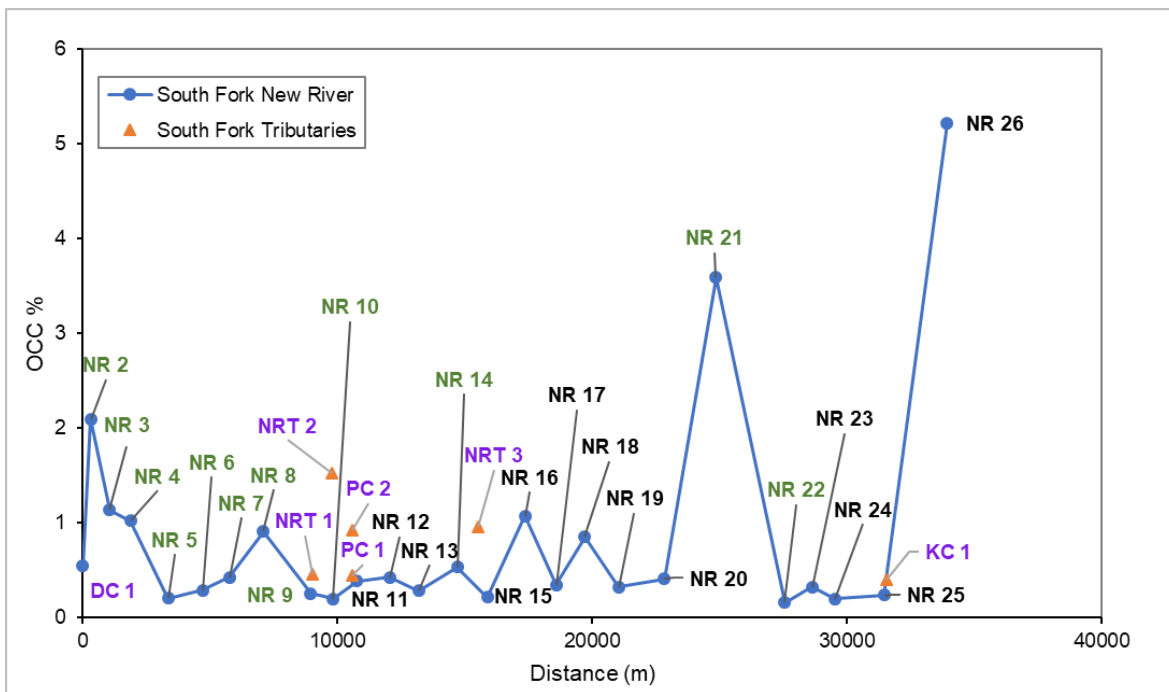


Figure 6. The spatial variation of OCC between sampling sites. The sites classified as alluvial reach are labelled in green, sites classified as a bedrock reach are labelled in black, and tributaries are labelled in purple.

Box and whisker plots, shown in Figure 7, were created to compare the amount of OCC in alluvial and bedrock reaches. The alluvial reaches had a higher IQR, whereas mean and median values were calculated to be 0.90 % and 0.48 %, respectively. In comparison, bedrock reaches exhibited a mean and median of 0.79 % and 0.34 %, respectively. Each site type had one outlier for OCC. The OCC was compared for each reach type using a U-test to determine if there was a difference in carbon content between the two.²¹ The test suggest there was no difference in OCC of the two reach types at a significance level of $p < 0.05$.

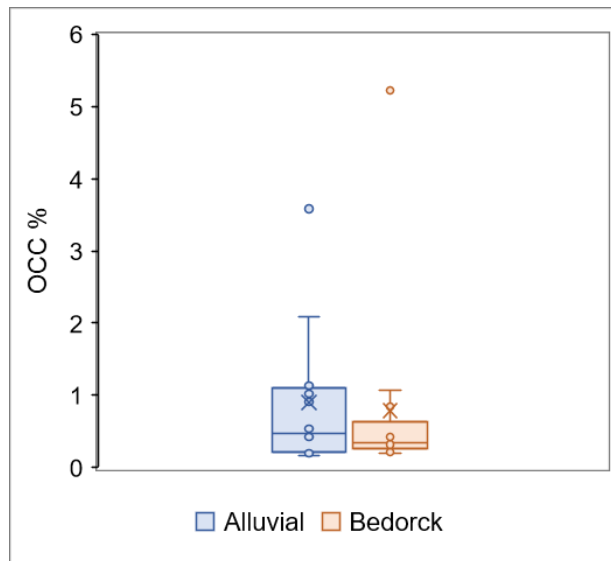


Figure 7. Box and Whisker plot comparing the amount of OCC in alluvial reach sites and bedrock reaches.

The mean, minimum and maximum were calculated for suspended sediment concentrations (SSC) values are given in Table 1. Downstream SSC decreased from NR 2 to NR 4, then increased from NR 6 to NR 13 after which it remained around 20 mg/L for the remainder of the sites (with the exception NR 25 and NR 26) in Figure 8. Site NR 2 (alluvial reach) had the

highest concentration of 162 mg/L (Table 1). Site along the NNR 1 and NR 28 exhibited below average SSC. There were no significant relationships (correlation) between fine sediment, fine sand and OCC in the channel bed and SSC.

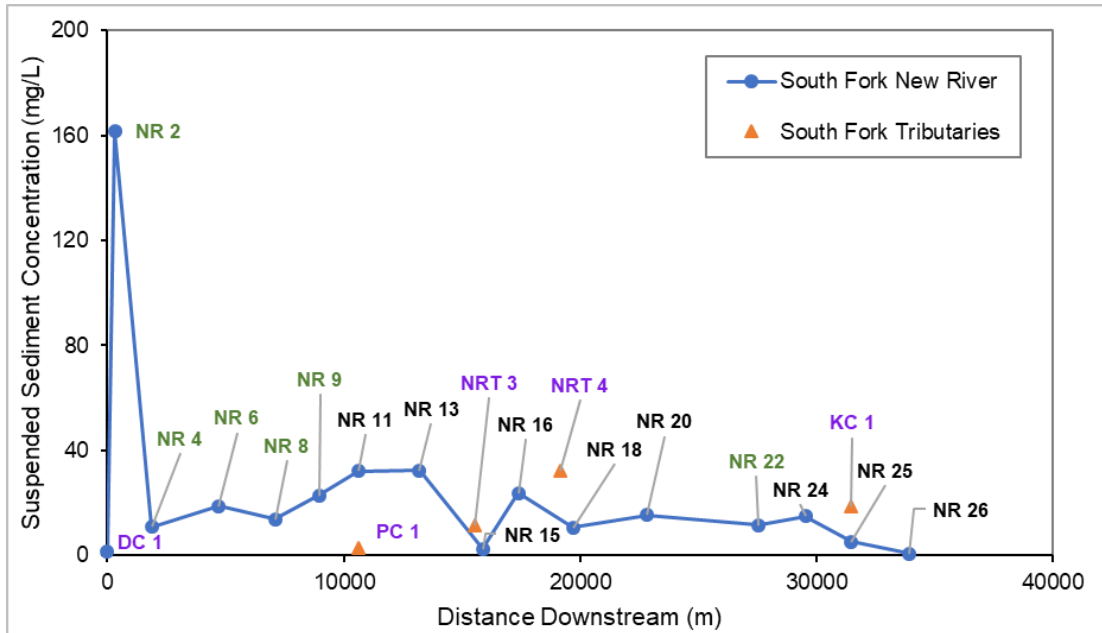


Figure 8. Spatial distribution of suspended sediment concentration in South Fork New River; sites classified as alluvial reach are labelled in green, sites classified as bedrock reach are labelled in black, and tributaries are labelled in purple.

Sediment-Associated Metal Contamination

Total Sediment-Associated Metal Concentrations

The mean, minimum and maximum for the total metal concentrations measured in channel bed sediments in comparison to crustal averages are presented in Table 2. The present crustal averages provided a measure of what may be expected under natural (uncontaminated) conditions within the riverbed sediments.²² Of the sites exhibiting Zn and Cu concentrations above the crustal average (12 and 8 sites, respectively), half were in alluvial reaches and half were bedrock reaches. Tributary sites DC 1, NRT 2 and NRT 3 exceeded crustal average values

for Zn, whereas NRT 2, PC 1 and PC 2 exceeded crustal values for Cu. All sites along the South Fork New River, except NR 22, and all tributary sites were above the crustal average for Co. Other sites NNR 1, NR 27 and NR 28 were above the crustal average value of Co and NR 27 was above the Zn crustal average.

There was no systematic downstream changes in the total metal concentrations within the channel bed sediments; rather concentrations varied site to site significantly in Figure 9. Though, bioavailable Co and Fe metal concentrations displayed similar downstream behavior (Figure 9 C, D). The tributary site along Peak Creek (PC 2) near the mine, exhibited concentrations greater than the mean for Cu and Fe: concentrations for Co, Zn, Cu and Fe immediately downstream of the confluence with the South Fork New River (NR 11) decreased below the concentrations of Peak Creek (PC 1). The concentrations immediately downstream of the South Fork and Peak Creek confluence did not abruptly increase above the concentrations observed upstream of the confluence.

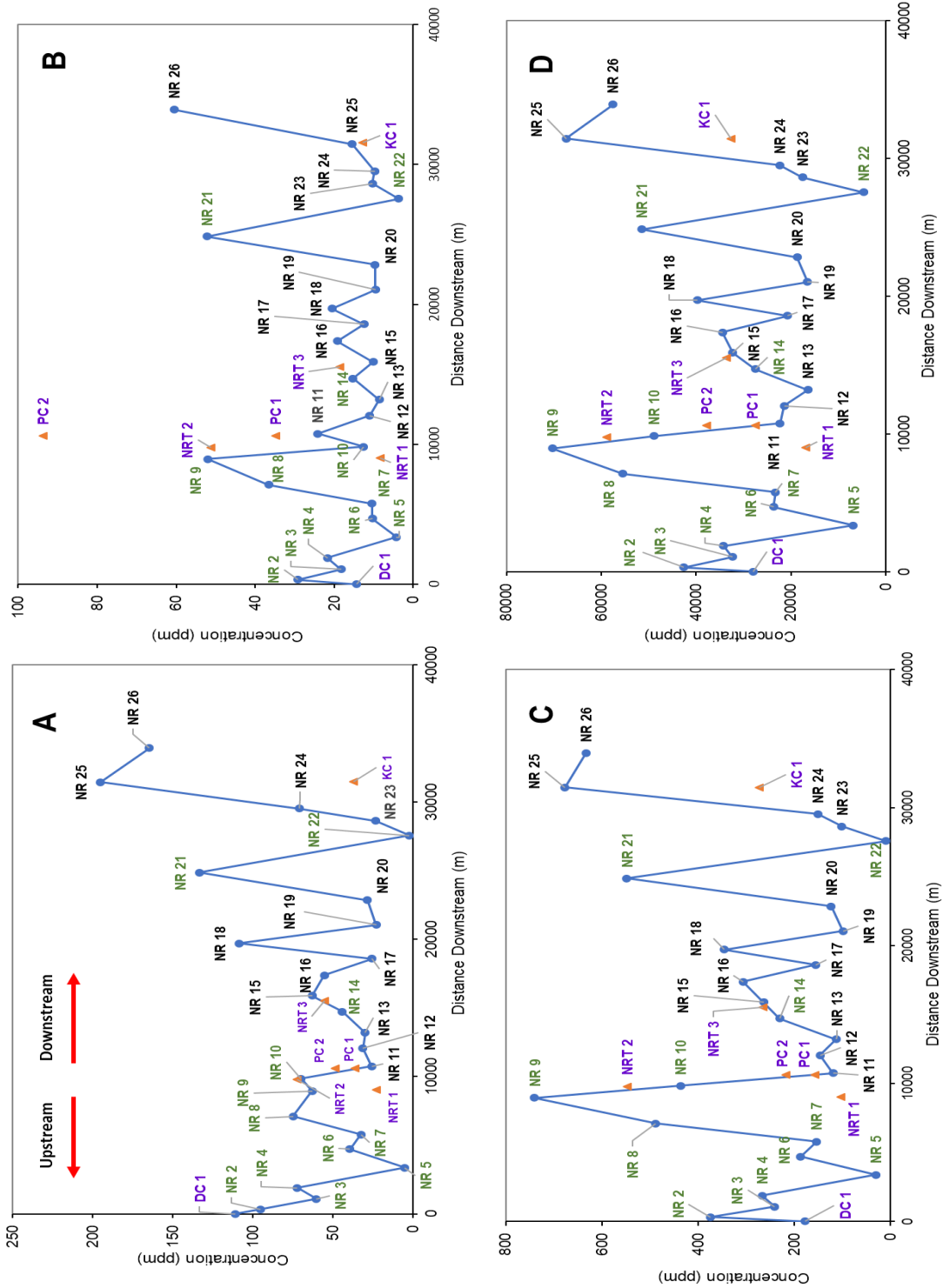


Figure 9. Spatial distribution of total (A) Zn, (B) Cu, (C) Co, and (D) Fe concentrations in channel bed sediment; the direction of the sites relative to Peak Creek (PC 1 and PC 2) is shown by the red arrows, the sites classified as alluvial reaches are labelled in green, sites classified as bedrock reaches are labelled in black, and tributaries are labelled in purple.

Table 2. Mean, minimum and maximum values for total metal concentrations in sediment for sampling sites (n= 25) along the South Fork New River.

	Mean (ppm)	Minimum (ppm)	Maximum (ppm)	Crustal Average (ppm)
Total Zinc	63.35	2.17	195.14	50
Total Copper	19.35	3.67	60.47	20
Total Cobalt	273.84	9.12	739.92	10
Total Iron	32183	4676	70362	-

Box and whisker plots in Figure 10 were created to compare the total Fe, Cu, Co, and Zn concentrations in sediment between alluvial and bedrock reaches. Except for Zn, alluvial sites had larger IQR, median, and mean, total concentrations in sediment than the bedrock sites. Bedrock sites had one outlier (site NR 26 for all the metals) while alluvial sites lacked outliers. The total metal concentrations in sediment were compared for each reach using the Mann-Whitney U-test to determine if there was a difference in total metal concentration.²¹ The test results suggest that there was no difference in the total concentrations in sediment for the two reach types at a significance level of $p < 0.05$.

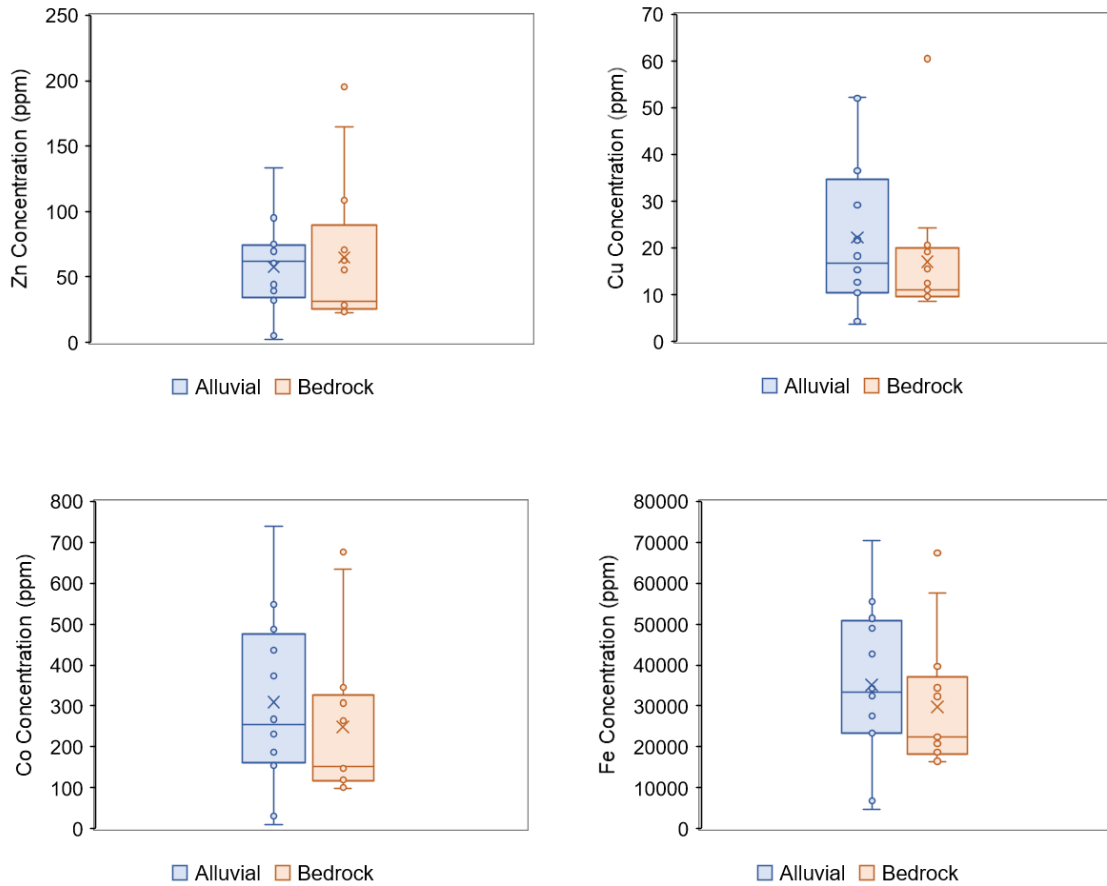


Figure 10. Box and Whisker plots comparing total metal concentrations in sediment (ppm) between alluvial and bedrock reaches along the South Fork New River.

Pearson correlation coefficients in Table 3 showed that strong relationships (significance level at $p < 0.001$) occurred between total Fe concentrations and total Co and Zn concentrations. Total Cu concentrations were strongly related to total Co concentrations and moderately related to total Zn concentrations. Total Co concentrations were also strongly correlated to total Zn concentrations.

Table 3. Correlation coefficients of total metal concentrations in sediment ($^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$).

	Total Fe	Total Cu	Total Co	Total Zn
Total Fe	1			
Total Cu	0.75 ^{**}	1		
Total Co	0.99 ^{***}	0.77 ^{***}	1	
Total Zn	0.76 ^{***}	0.46	0.77 ^{***}	1

Bioavailable Sediment-Associated Metal Concentrations

Bioavailable metal concentrations were determined using a partial digestion. These metals are thought to be weakly or moderately bound to particle surfaces and can be more readily accumulated by biota than metals strongly bound to sediments or contained within minerals structure¹⁷. The mean, minimum and maximum bioavailable metal concentrations in sediment are presented in Table 4.

As shown in Figure 11, no systematic downstream changes occurred in bioavailable sediment-associated concentrations. Tributary site NRT 2 exceeded the average for all the bioavailable metal concentrations determined for the mainstream of the river; PC 2 (immediately downstream of the mine) exceeded the averages for Zn, Cu, Co and Fe, whereas PC 1 exceeded average concentrations for Zn, Cu, and Fe (Figure 11 B, C, D). Site NRT 3 exceeded the average concentrations for Zn and Co. The concentrations of all bioavailable metals were significantly correlated with one another ($p < 0.001$) (Table 5).

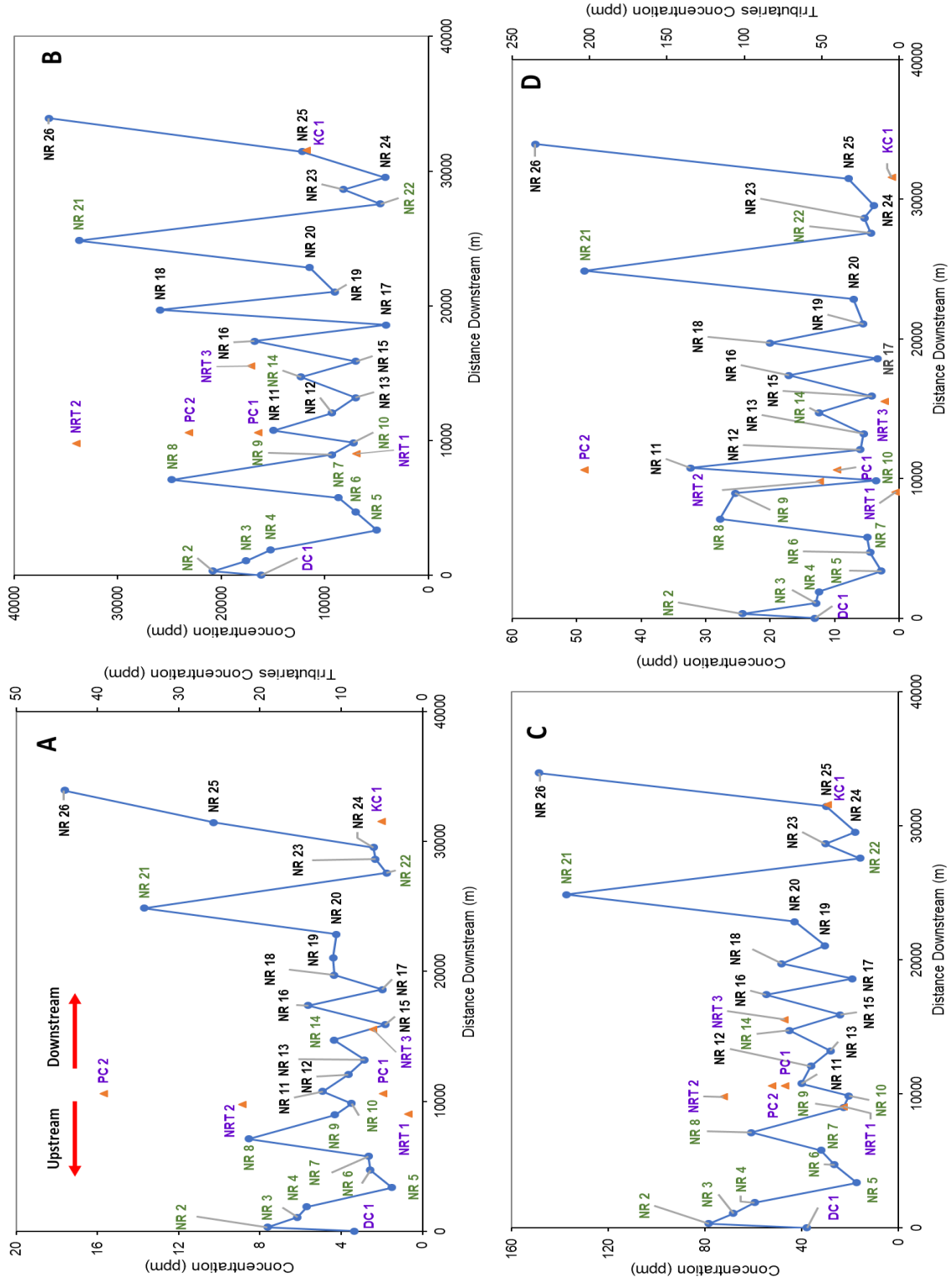


Figure 11. Spatial distribution of bioavailable (A) Co, (B) Fe, (C) Zn and (D) Cu concentrations in sediment. The direction of the sites relative to Peak Creek (PC 1 and PC 2) is shown by the red arrows, sites classified as alluvial reaches are labelled in green, sites classified as bedrock reaches are labelled in black, and tributaries are labelled in purple.

Table 4. Mean, minimum and maximum values for bioavailable metal concentration in sediment, for sampling sites (n= 25) along the South Fork New River.

Metal	Mean (ppm)	Minimum (ppm)	Maximum (ppm)
Bioavailable Zinc	45.11	15.91	148.68
Bioavailable Copper	14.32	2.77	56.42
Bioavailable Cobalt	5.09	1.50	17.66
Bioavailable Iron	13458	4099	36691

Table 5. Correlation coefficients of bioavailable metal concentrations in sediment (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).

	Bioavailable Fe	Bioavailable Cu	Bioavailable Co	Bioavailable Zn
Bioavailable Fe	1			
Bioavailable Cu	0.85***	1		
Bioavailable Co	0.82***	0.79***	1	
Bioavailable Zn	0.90***	0.83***	0.87***	1

Box and whisker plots seen in Figure 12 were created to compare bioavailable Fe, Cu, Co, and Zn concentrations observed in alluvial and bedrock reaches. The alluvial sites had a larger IQR, median, and mean, of all bioavailable metal concentrations in sediment, than bedrock sites. Bedrock sites had at most two outliers (sites NR 25 and NR 26) and alluvial sites had one outlier (site NR 21) (Figure 12). The bioavailable metal concentrations in sediment were compared between alluvial and bedrock reaches using the Mann-Whitney U-test.²¹ There was no apparent difference in the bioavailable concentrations in sediment for the two reach types ($p < 0.05$).

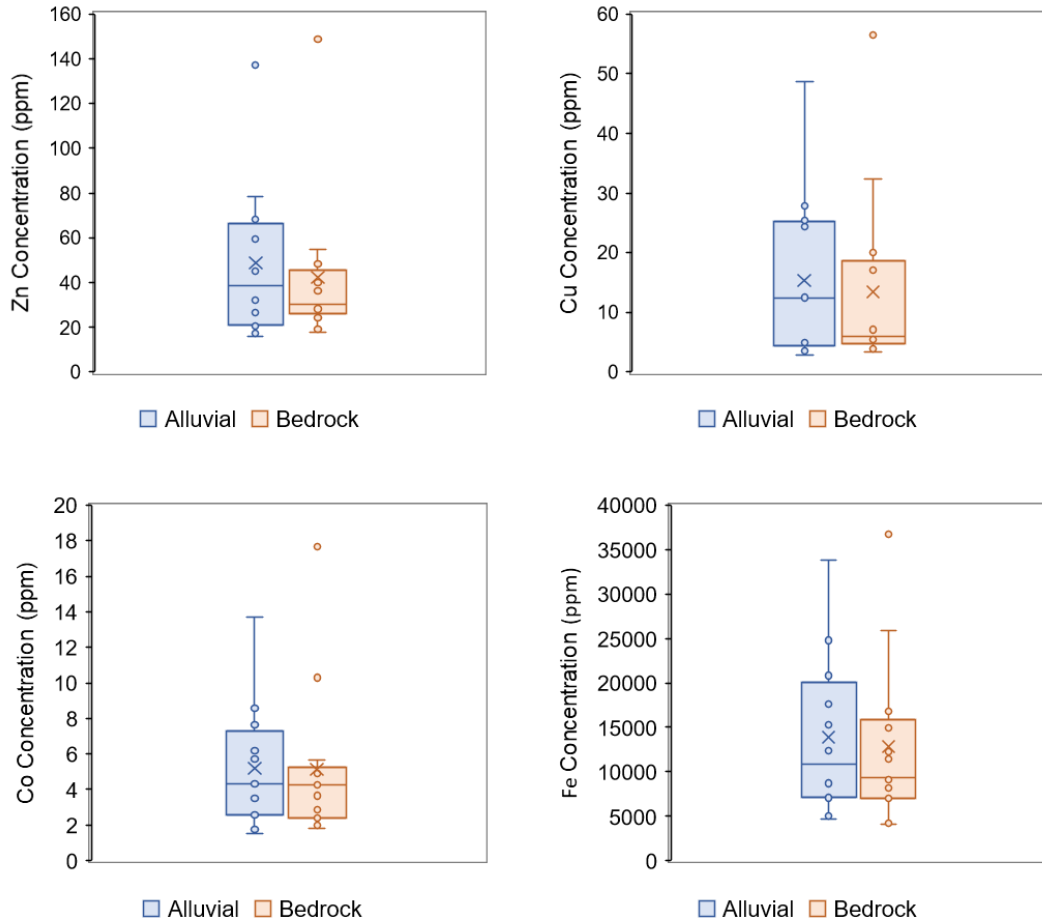


Figure 12. Box and Whisker plots of bioavailable metal concentrations in sediment (ppm) for alluvial and bedrock reaches along the South Fork New River.

The bioavailable metal concentrations in sediment were also compared to threshold effect concentrations (TEC) and probable effect concentrations (PEC) compiled by MacDonald et al.¹⁰ as shown in Table 6. The TEC is a lower guideline and PEC is an upper guideline set for assessing the possibility aquatic ecosystems may experience adverse effects from specific metal concentrations.¹⁷ Sites NR 21 and NR 26 exceeded the TEC for Zn, whereas sites NR 11, NR 21, and NR 26 exceeded the TEC for Cu. One tributary site (PC 2, located near the mine) exceeded the TEC and PEC for Cu. TEC and PEC for Fe and Co were not provided by MacDonald et al.¹⁰

Table 6. The TEC and PEC for trace metals in alluvial sediments in freshwater ecosystems based on the MacDonald et al. Also shown are the range of concentrations reported for the sampling sites and the number (percent) of sites where TEC and PEC were exceeded.

Metal	TEC (ppm)	PEC (ppm)	Main river sites above TEC	Main river sites above PEC	Tributaries above TEC	Tributaries above PEC
Zinc	121	459	2 (8%)	0 (0%)	0 (0%)	0 (0%)
Copper	31.6	149	3 (12%)	0 (0%)	2 (33%)	1 (17%)

Metal Contaminants in Water

Total Recoverable Metal Concentrations in Water

The mean, minimum and maximum total recoverable metal concentrations in water are provided in Table 7. Approximately 80 % of the sites sampled for total recoverable metal concentrations in water were classified as bedrock reaches (13 of 16).

Along the channel bed of the South Fork New River, total recoverable Zn and Cu concentrations were consistently around 200 and 100 ppb, respectively (Figure 13 A, B), with the exception of sites NR 8, NR 22, and NRT 4. The total recoverable Co concentrations ranged from undetectable to 1 ppb (Figure 13 C). The downstream pattern for total recoverable Fe concentrations decreased from sites NR 2 to NR 8, then increased at sites NR 11, before decreasing again until NR 25 and KC 1 (Figure 13 D). Site NNR 1 was the only sampling location outside of the South Fork New River that exceeded the average for all metals. Total recoverable Zn in water had the highest correlation with total recoverable Cu concentrations in water. Total recoverable Co in water was correlated with total recoverable Fe (Table 8).

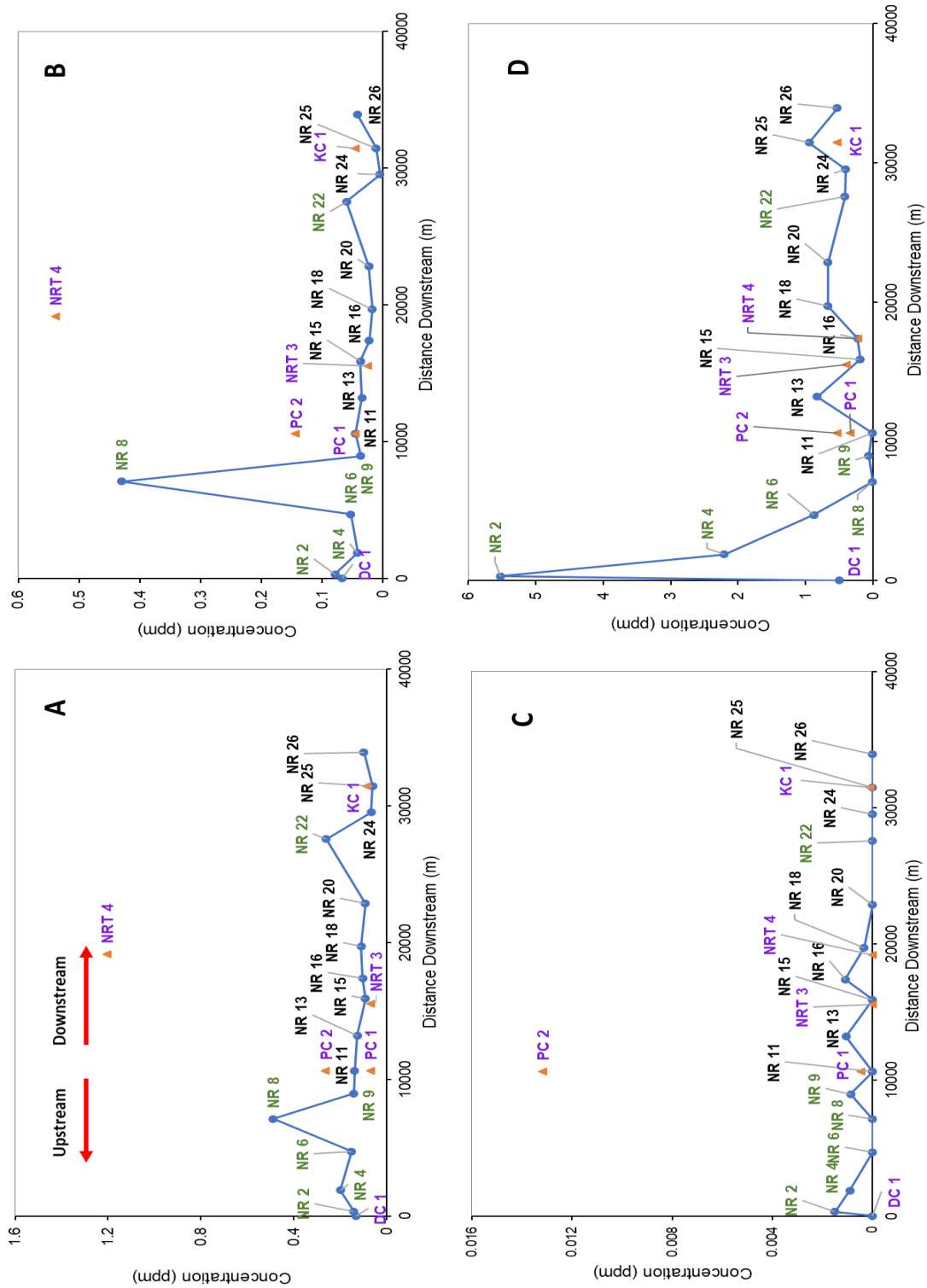


Figure 13. Spatial distribution of total recoverable (A) Zn, (B) Cu, (C) Co and (D) Fe concentration in water. The direction of the sites relative to Peak Creek (PC 1 and PC 2) is shown by the red arrows, sites classified as alluvial reach are labelled in green, sites classified as bedrock reach are labelled in black, and tributaries are labelled in purple.

Table 7. Mean, minimum and maximum values of total recoverable metal concentrations in water for sampling sites (n=16) along the South Fork New River.

Metal	Mean (ppb)	Minimum (ppb)	Maximum (ppb)
Zinc	150	60	490
Copper	60	5	430
Cobalt	0.4	0	1.5
Iron	80	5	5522

Table 8. Correlation coefficients of total recoverable metal concentrations in water ($^{***} p < 0.001$, $^{**} p < 0.01$, and $^{*} p < 0.05$).

	Total Zn	Total Cu	Total Co	Total Fe
Total Zn Water	1			
Total Cu Water	0.92 ^{***}	1		
Total Co Water	-0.09	-0.14	1	
Total Fe Water	-0.08	-0.07	0.62 [*]	1

The total recoverable metal concentrations in water were compared to the NCDEQ acute and chronic values for Zn, Cu, and Fe (guidelines for Co have not been developed) in Table 9. All sites, except NR 24, exhibited Cu concentrations that exceeded chronic guidelines. Of the sites that exceeded Zn and Fe, the majority were alluvial reaches (7 out of 8 sites, 88% for Zn and 2 out of 2 for Fe, 100%). The highest concentrations of Zn were found at sites NR 8 and NRT 4, whereas the highest concentrations of Cu were measured at sites NR 11 and PC 2. Sites DC 1 and PC 2 exhibited the highest total recoverable Co concentrations, and NR 2 and NR 4 possessed the highest total recoverable Fe concentrations. The sites sampled out of the South Fork, NNR 1 and NR 27, exceeded the average for Cu and NNR 1 exceeded the average for Zn.

Table 9. NC Department of Environmental Equality (NCDEQ) chronic and acute guideline values for total recoverable metal concentrations in water compared to measured metal concentrations measured along the South Fork New River and its tributaries. No guideline values are available for Co.

Metal	Chronic (ppb)	Acute (ppb)	Main river sites above chronic	Main river sites above acute	Tributaries above chronic	Tributaries above acute
Zinc	120	120	9 (56%)	9 (56%)	2 (40%)	2 (40%)
Copper	7	-	15 (94%)	-	5 (100%)	-
Iron	1000	-	2 (13%)	-	0 (0%)	-

Dissolved Metal Concentrations in Water

The mean, minimum and maximum for dissolved metal concentrations in water are provided in Table 10. The dissolved Zn concentrations were consistently around 5 ppb for all sites along the river (Figure 14 A). Dissolved Cu concentrations were generally below 0.9 ppb, whereas Co concentrations were under 1 ppb at all sites, except for sites DC 1, NR 15 and NR 26 (Figure 14 B, C). Dissolved Fe concentrations generally increased downstream in both alluvial and bedrock reaches (Figure 14 D). Dissolved concentrations in water at site PC 2 were above the average measured along the South Fork New River for Zn, Cu and Co; tributary site PC 1 was above the average for Cu and Co. NRT 4 was above the average for Zn and Co, and NRT 3 was above the average for Co. Site NNR1, on the North Fork New River, was above the average for all metals except for Fe. There were no statistically significant correlations between the dissolved metal concentrations in Table 11.

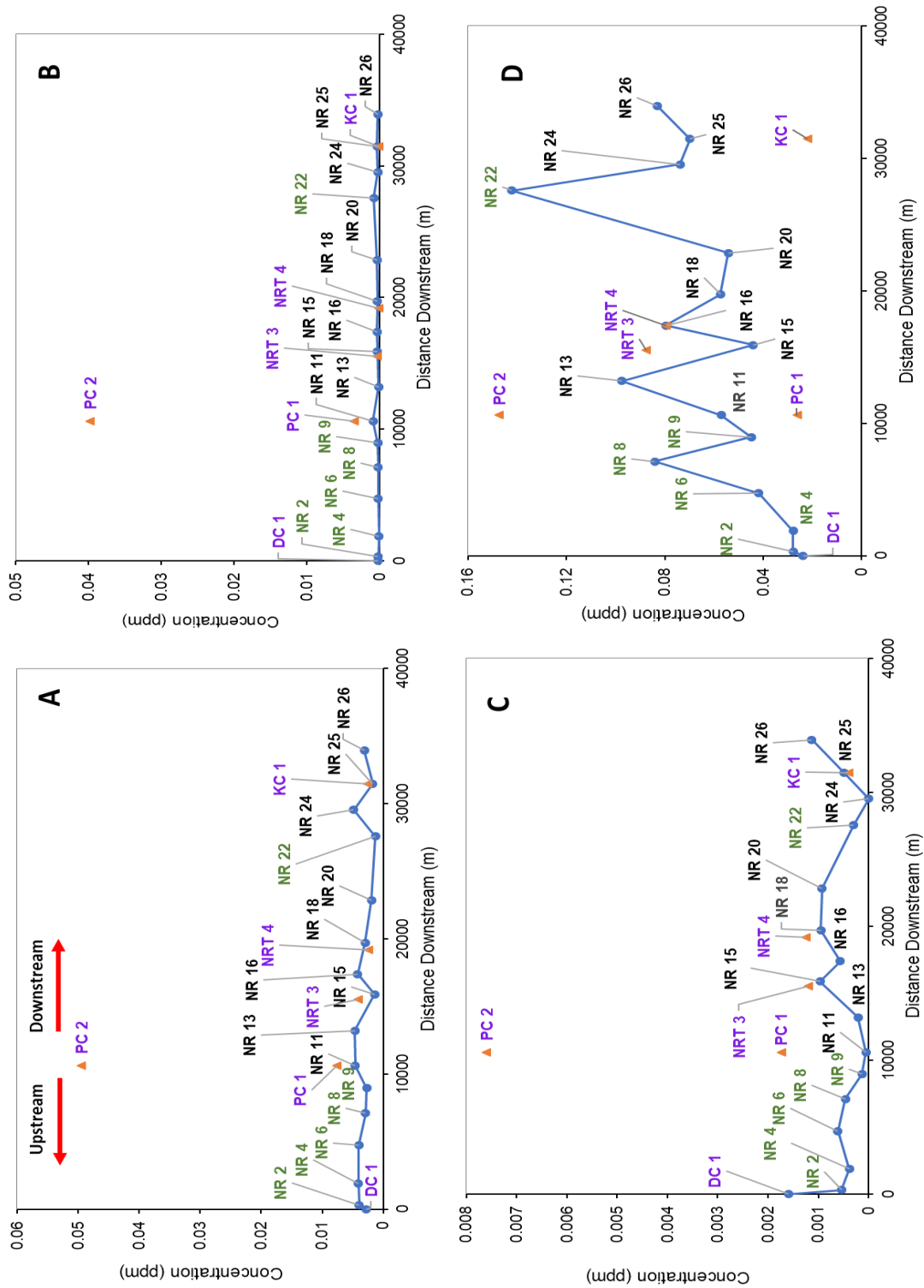


Figure 14. Spatial distribution of dissolved (A) Zn, (B) Cu, (C) Co, and (D) Fe concentrations in water. The direction of the sites relative to Peak Creek (PC 1 and PC 2) is shown by the red arrows, sites classified as alluvial reaches are labelled in green, sites classified as bedrock reaches are labelled in black, and tributaries are labelled in purple.

Table 10. Mean, minimum, and maximum for dissolved metal concentrations in water for sampling sites (n=16) along the South Fork New River.

Metal	Mean (ppb)	Minimum (ppb)	Maximum (ppb)
Zinc	3.2	1.3	4.9
Copper	0.31	0	0.9
Cobalt	0.58	0	1.6
Iron	60	24	142

Table 11. Correlation coefficients of dissolved metal concentrations in water ($^{***} p < 0.001$, $^{**} p < 0.01$, and $^{*} p < 0.05$).

	Dissolve Zn Water	Dissolve Cu Water	Dissolve Co Water	Dissolve Fe Water
Dissolve Zn Water	1			
Dissolve Cu Water	-0.18	1		
Dissolve Co Water	-0.45	-0.25	1	
Dissolve Fe Water	-0.23	0.4	-0.16	1

None of the sampled sites exhibited dissolved metal concentrations that exceed chronic or acute EPA freshwater criteria. Similarly, no tributary sites exceeded chronic EPA Fe standards, except site NNR 1 (with a concentration of 2456 ppb), which exceeded the chronic guidelines. As shown in Table 12, tributary site PC 2 exceeded chronic and acute EPA guidelines for Zn and Cu, whereas site NRT 4 exceeded chronic guideline values for Cu: chronic and acute dissolved concentrations for Co are not provided by the EPA.

Table 12. The chronic and acute values for dissolved metal concentrations of based on the Environmental Protection Agency (EPA) freshwater criteria compared to concentrations measured along the South Fork New River and its tributaries (*metal is dependent on hardness of water).

Metal	Chronic (ppb)	Acute (ppb)	Main river sites above chronic	Main river sites above acute	Tributaries above chronic	Tributaries above acute
Zinc	36	36	0 (0%)	0 (0%)	1 (20%)	1 (20%)
Copper*	2.7	3.6	0 (0%)	0 (0%)	2 (40%)	1 (20%)
Iron	1000	-	0 (0%)	-	0 (0%)	-

Relationships between Parameters Measured in Channel Bed Sediments and Water

There were no significant correlations between the other total recoverable metals in water and total metals in sediment. There were no significant correlations between the total recoverable metals in water and bioavailable metal concentrations in sediment. SCC was strongly ($p < 0.001$, 0.01 , respectively) related to total recoverable Fe ($r = 0.87$) and Co ($r = 0.66$) concentrations in water. There were no significant correlations between SSC and dissolve metal concentrations in water.

CHAPTER FIVE: DISCUSSION

Source(s) along the South Fork New River

Spatial Patterns in Metal Concentrations

Determining the source of metal contaminants to riverine systems is an essential component of most site assessment programs, and a wide range of approaches have been developed over the past three decades to identify the source and source contributions of metals to a river.¹⁷

Historically, contaminant sources have been identified by interpreting spatial (downstream) patterns in contaminant concentrations.^{17,23} Metal concentrations, for example, often increase abruptly downstream of contaminant inputs (source), and then decrease downstream as a result of dilution, exchange with the floodplain, sediment storage, geochemical processes and biological uptake.¹⁷ Advantages to using spatial patterns for source analysis are that the process can be applied to both riverbed and floodplain deposits, can be completed rapidly by using samples collected during a single sampling operation, and the data are relatively easy to collect and interpret. Disadvantages to this analysis are that multiple sources can be present within a specific sampling reach (sources can overlap), inhibiting their identification. The number and frequency of samples collected influences the ability to identify sources within the river, and abrupt downstream variations may be influenced by erosion and deposition processes within and around the channel, rather than an influx of metals from a source. For example, streams that are characterized by relatively wide valleys, low gradients, and gentle flows (alluvial reaches) are often characterized as zones of sediment-associated metal deposition; thus, these zones may possess relatively high metal concentrations and appear as abrupt increases in metal concentrations along a river (i.e., it appears to be a source, when in reality it is not). In addition,

the analysis cannot be used to find a specific source or sources in a system, rather the relative area of the source can be determined.²³

Based on previous studies and reports on the Ore Knob Mine^{1,2}, it can be hypothesized that previously released tailings from the mine and its associated tailings pile were stored along the Peak Creek drainage system. Peak Creek could be a source of these sediment-associated metals that could be reworked from the drainage and transported to the South Fork New River.^{2,24} The metal concentrations measured along Peak Creek (sites PC 1 and 2) were relatively high upstream, close to the mine, but decreased downstream significantly (over a ~ 2 km distance), potentially by dilution, prior to entering the South Fork New River (site PC 1) (Table 13). Seen in Table 13, these data are consistent with the range of concentrations measured by the EPA and NCDEQ for the Peak Creek drainage system. Sites PC 1 and PC 2 exceeded the background concentrations (uncontaminated sediments and soils) established in the research study performed by the EPA and NCDEQ. Although the elevated concentrations of metals at PC 1 suggest that metals were entering the South Fork New River from Peak Creek, metal concentrations did not abruptly and significantly increase in either water or sediments downstream of its mouth (Figures 9, 11, 13). Also, metal concentrations at sites upstream of NR 11 (the mouth of the South Fork New River and Peak Creek) were relatively higher than at PC 1 along Peak Creek. These spatial patterns in concentrations occurred in bioavailable metals in sediment, total recoverable metals in water and dissolved metals in water. If Peak Creek represented a significant metal source, there would be a significant increase in concentrations in the alluvial reaches immediately downstream of Peak Creek (sites NR 12-NR 26) due to the characteristics of alluvial reaches. Since there was no increased concentrations in the alluvial reaches, the factors controlling the variability in the spatial distribution of metals along the South

Fork New River were investigated such as type of underlying geology, grain size, and presence of organic matter.

Table 13. Comparison of current research, EPA, and NCDEQ bioavailable metal and total recoverable concentrations in channel bed sediments from Peak Creek.

Metal	Recent Research (2018) (mg/kg)		USEPA (2009) (mg/kg)	NCDEQ (2008) (mg/kg)
	PC 2	PC 1		
Bioavailable Zn	52	47	31	14-6900
Bioavailable Cu	203	40	64	5.7-9900
Bioavailable Co	39	5	-	3.7-500
Bioavailable Fe	23108	16454	-	6400-940000
Bioavailable Al	6970	4194	-	2900-13000
	Recent Research (2018) (µg/L)		USEPA (2009) (µg/L)	NCDEQ (2008) (µg/L)
Total Zn Water	262	8	-	3.2-850
Total Cu Water	144	46	-	0.56-330
Total Co Water	13	0.48	-	0.58-8.5
Total Fe Water	536	337	-	72-12900

The underlying geology (bedrock units) of the South Fork New River contains sulfidic minerals such as pyrite, chalcopyrite and sphalerite. Sulfides contain the studied metals within their mineral structure, meaning these metals are not bioavailable unless the minerals are broken down. As a result, the metals are considered to be in the residual phase, that fraction of the sediment that can only be broken down by the weather (decomposition) of the minerals.²² The inspection of Google Earth images combined with field observations showed that bedrock exposures in the channel bed increased downstream. This led to the hypothesis that the erosion of exposed sulfidic layers which are known to exist in the bedrock could cause variability in metal concentrations within the given reaches. Tributaries also possessed high total and bioavailable concentrations of Cu and Zn in sediment reiterating that these metals could be naturally high because tributaries are background or uncontaminated.¹⁷

In addition to coming from the underlying bedrock units, the studied metals could be partially derived from anthropogenic sources in upstream and tributary areas. There are, for example, farm lands located along the South Fork New River and its adjacent tributary basins. A wide range of agricultural additives (e.g., fertilizers, pesticides, herbicides, lime, etc.) contain the examined metals. Moreover, it is important to remember that the Ore Knob mine was placed on the National Superfund Priorities list because of groundwater contamination. The general groundwater flow direction is from upland areas around the mine and tailings pile toward the South Fork New River. Thus, sites upstream from Peak Creek, and closest to the mine, could be contaminated by the movement of groundwater flowing through bedrock fractures lined by sulfidic minerals to the river. Such processes, which could not be directly documented herein, may explain the higher concentrations in both water and sediments upstream of Peak Creek.

Other sources influencing the spatial patterns on the South Fork New River could be the amount of fine-grain sediment, including organic matter, present at a given sampling site. This led to a comparison of a sites' sedimentology and chemical composition of both alluvial and bedrock reaches. Alluvial reaches, in general, contain more fine-grain sediment than bedrock reaches due the flow conditions that characterize the two sites as previously discussed in Figure 5. These fine-grain sediments contain chemically reactive clay minerals and other reactive constituents (e.g., Fe and Mn oxides and hydroxides) which can store (sorb) more metal ions than sand-sized particles (the latter of which are typically dominated by quartz).¹⁷ Their reactive nature is due to their larger surface area and generally higher electrical charge.¹⁷ Organic matter is also found within fine-grain sediments and contains reactive functional groups that can sorb trace metals in water to their surface.¹⁷ Thus, alluvial sites which possessed relatively high levels of OCC and fine-grained sediment had higher metal concentrations than the bedrock sites

(Figures 5, 7, 10, 12). This also implies that alluvial sites possess more trace metals by weight (mass) than bedrock reaches. Though alluvial reaches possessed higher metal concentrations and fine sediment percentages, many reaches possessed limited amounts of fine-grain sediment and OCC. Rather, the high gradient and rapid flow conditions found along most alluvial reaches resulted in channel beds predominantly composed of sand and coarser sediment so the deposition of fine grain sediment was minimal. Site NR 26 was classified a bedrock reach and this site possessed the highest percentage of silt and clay and the highest total and bioavailable concentrations of all sites sampled. This sample was obtained from a large bar along the channel margin at the site and not from the channel bed itself: this was due to lack of fine grain sediment present in the channel bed at this site. The large peak seen at NR 26 in the spatial figures for total and bioavailable metals in sediment represents a unique depositional environment, this allowed for accumulation of relatively fine sediment that was not sampled at the other sites.

Although the alluvial and bedrock reaches did not exhibit statistically different sedimentologic characteristics or metal concentrations, metal concentrations and sediment sizes did occur as shown on the whisker plots in Figures 5, 10, 12. The lack of statistical differences appeared to be related to (1) the relatively few bedrock and alluvial reaches that were sampled, and (2) the occurrence of a few outlier sites that possessed very large amounts of fine sediment. In general, then, the coarse-grained nature of the channel bed limited the role that channel bed sediment characteristics played in controlling the overall spatial pattern in metal concentrations along the river.

Correlation analyses are often used to assess the source and behavior of metals in aquatic systems. Metals that are correlated are generally assumed to be derived from a similar source and tend to behave similarly within the aquatic environment.²⁵ The strong correlations observed

herein between the total metal concentrations in sediment indicate that all metals could be coming from the same source (the underlying bedrock) and, in terms of dispersal, behaved the same. Total Fe and Al concentrations had the lowest correlation of the total metals in sediment. This could be because Fe, when oxidized, precipitates and forms iron hydroxide coatings on channel bed and bank sediments, whereas Al is associated with (found in) the structure of clay minerals.¹⁷ Bioavailable concentrations of the metals were also strongly correlated with one another, supporting the idea that the metals are all derived from the same source.

Potential Effects of Metal Contamination on Aquatic Biota

A commonly asked question with regards to contaminated rivers is whether the measured metal concentrations are high enough to pose a significant threat to the aquatic environment. Multiple approaches have been used to address this question; one of the most common methods to assess the impacts of contamination on aquatic biota is to compare measured metal values to background values and/or experimentally determined guideline criteria suggestive of biotic impacts.¹⁷ Here, metal concentrations measured along the South Fork New River and its tributaries were compared to crustal averages and three previously developed standards, including: the guidelines compiled for sediments by MacDonald et al.;¹⁰ the NCDEQ total recoverable metal concentrations criteria for freshwater systems;²⁶ and the EPA chronic and acute threshold criteria for freshwater ecosystems.²⁷

Comparison of Total Metal Concentrations in Sediment to Upper Crustal Averages

Average upper continental crustal concentrations have been widely used to help understand the composition and chemical differentiation of the continental crust as a whole and the Earth in general.²⁸ Upper crustal averages are mainly determined through large-scale sampling programs that collect and analyze a variety of rock types such as shale, granite, and other sedimentary

rocks.²⁹ These crustal averages provide an overview of metal concentrations worldwide and have been extensively applied as guidelines for anthropogenic enrichment where data from local background materials (sediments, rocks, soils) are not widely known. However, their application to specific drainage systems has been criticized because, in many instances, they do not effectively characterize the rocks that underlie a specific drainage basin, such as the South Fork New River. Nonetheless, these upper continental data are useful when local data are missing.

Herein, average upper crustal concentrations in granite were selected to assess background concentrations because the underlying geology is mostly composed of felsic metamorphic rocks (e.g., gneiss) that are most comparable to granites. In comparison to these average crustal values shown in Table 2, the channel bed sediments at most sites (56% and 96%) along the South Fork New River were enriched in Zn and Co; some sites (32%) were also enriched in Cu. Though many of the sites along the channel and tributaries were above crustal averages, the South Fork New River and its adjacent tributaries are known to have sulfidic layers (i.e., layers containing an abundance of sulfide minerals) within the bedrock units. Thus, metal concentrations within the underlying rocks and the sediments derived from them may be naturally high in these metals. The background concentrations established by the NCDEQ for Cu (38 ppm) was higher than the crustal average, whereas Zn (48 ppm) was similar to the crustal averages. These concentrations were taken around the Ore Knob Mine site.¹ The EPA on the other hand, collected background concentrations upgradient of Ore Knob Branch along Peak Creek, the upstream of Little Peak Creek along Low Gap Branch and upstream of the South Fork New River. They determined background concentrations for Cu to range from 2.8 to 4.7 ppm and Zn concentrations were 11 ppm.² These concentrations did not exceed crustal averages. The data collected by the NCDEQ and EPA show that significant spatial differences occur in the

geological materials in the area. However, given that the mean and median concentrations of Cu and Zn measured along the South Fork river were similar to or below the NCDEQ background values, it appears that the metals were derived from localized natural sources, and were elevated by local sulfide enrichment in the bedrock.

Comparison of Bioavailable Metal Concentrations to MacDonald et al. Guidelines

The potential impacts of toxic metals attached to channel sediments are often assessed using sediment quality standards or sediment quality guidelines. Two guidelines, which may be further subdivided into distinct ranges, are often used.¹⁷ The lower guideline concentration, or threshold effect concentration (TEC), is intended to represent a level below which the contaminant will cause no adverse effects. The upper guideline, or probable effect concentration (PEC), represents a value above which adverse effects are likely (probable). MacDonald et al.¹⁰ developed a consensus-based TEC and PEC (based on a geometric mean of multiple research sediment quality guidelines) that can be utilized over a relative wide range of freshwater aquatic environments.¹⁷

Bioavailable metal concentrations were determined by partial digestion methods, the method utilized in the project was previously discussed. This method can be a poor descriptor of the metals that aquatic biota can accumulate. A fraction may be tightly bound to the particles or be absorbed with sulfide minerals such that it is difficult for aquatic biota to accumulate the metals.²² Thus, the bioavailable concentrations measured herein by partial digestion is not necessarily an exact measurement of the true bioavailable metal concentrations within the sediment. The bioavailable concentrations measured in channel bed sediments of the South Fork New River were compared to the consensus TEC and PEC values compiled by MacDonald et al.¹⁰ The comparison found that sites NR 21 and NR 26 exceeded the TEC for Zn and sites NR

11, NR 21 and NR 26 exceeded the TEC for Cu. Site NR 21 was an alluvial reach with one of the highest fine sediment contents measured along the river, whereas NR 26 was a bedrock reach, with a large bar located along the channel banks. The sampled bar had the highest fine sediment content measured along the river. The high concentrations measured at these sites may have been influenced by their relatively fine-grained nature of the sampled sediments rather than the input of anthropogenic metals. Tributary sites NRT 2 and PC 1 exceeded the TEC for Cu, whereas PC 2 exceeded the PEC for Cu. Site PC 2, downstream of the mine, exceeded the PEC for Cu so it has a high probability of posing issues for aquatic biota.

While some sites exceeded TEC and PEC for Cu and Zn, it does not necessarily mean that aquatic life was going to be affected. These values are simply guidelines that do not fully consider the bioavailable concentrations of sediment-associated metals (as noted above). It is possible, particularly given the hypothesis that a large portion of the metals may have been derived from sulfide layers in the bedrock, that a portion of the metals were non-bioavailable. Miller and Mackin²², for example, demonstrated using sequential extraction analysis that metals derived from sulfide layers in the bedrock of the Little Tennessee River in North Carolina were largely non-bioavailable. In order to get a more precise assessment of bioavailability, a sequential extraction process similar to that used by Miller and Mackin²² could be utilized to assess the metals that possess a direct risk to the aquatic biota. Sequential extraction examines metal speciation. There are two assumptions associated with the use of this method: 1) trace metals are associated with discrete phases within the sediment, such as organic matter, Fe and Mn oxides and hydroxides and sulfide minerals and 2) treating the sediment in a stepwise method to increasingly more aggressive chemical reagents will produce extracts containing metals of from a specific phase or are bound to the particle through a specific mechanism.¹⁷

Comparison of Metal Concentrations in River Water to EPA and NCDEQ Guidelines

Two sets of epidemiological criteria were used to analyze the impacts of pollutants on freshwater aquatic life: dissolved metal concentration guidelines provided by the EPA and total recoverable metal concentrations provided the NCDEQ.^{26,27} The EPA assesses the potential impacts of contaminants on freshwater and saltwater aquatic biota using both acute and chronic criteria. The EPA criteria are based on the dissolved metal concentrations in water and, for some dissolved metals, other parameters such as hardness, pH, and/or salinity effect metal toxicity. Subacute effects of some contaminants are also factored in using the results of longer-term tests that examine the impacts of the contaminants on sensitive life stages of an organism. These analyses typically measure growth and reproductive success of selected organisms as well as mortality.¹⁶

These acute and chronic values can be applied to both benthic and surface water organisms. These guideline values are determined using four measurements: 1) an acceptable acute test requires at least one species of freshwater animal in at least eight different families; these families include Salmonidae, Osteichthyes, phylum Chordata, planktonic crustaceans, benthic crustaceans, insects, and phylum other than Arthropoda or Chordata as well as a family of any order of insect or any phylum not already represented; 2) acute-chronic ratios with species of aquatic organisms from at least three different families, including fish, invertebrate, and acutely sensitive freshwater species; 3) acceptable acute tests on freshwater algae or vascular plants; and 4) at least one acceptable bioconcentration factor determined with an appropriate freshwater species, if maximum permissible tissue concentrations are available. Once these four measurements are determined, then a final acute value can be formulated. The guideline values are estimated based on a concentration of the contaminant that results in a cumulative probability of mortality of 0.05 (5%). The final chronic value is determined in the same manner as the final

acute value, or it can be determined by dividing the final acute value by the final acute-chronic ratio.³⁰

Comparison of the EPA guidelines shows that no sites along the South Fork New River exceeded chronic or acute limits for the dissolved trace metals of interest (Table 12); however, tributary sites PC 1 and PC 2 located downstream of the mine exceeded the criteria for Zn and Cu. The NNR 1 site exceeded acute guideline for Fe. It is not entirely clear why the concentrations of Fe measured along the North Fork New River exceed the acute limit for Fe. Thus, further research is needed to fully characterize and understand these high values.

The NCDEQ defines acute standards for aquatic biota based on effects that occur rapidly as a result of short-term exposure: exposure is considered relative to the organism's life span. Chronic effects occur when an effluent or toxicant produces adverse effects as a result of a repeated or long-term exposure; the effects also include lethal and sublethal responses such as abnormal growths and/or reproduction.³¹ The NCDEQ standards, in contrast to the EPA criteria, are based on total recoverable concentrations in water. This method is a more inclusive approach as the term "total recoverable" encompasses both dissolved metals and metals attached to particulates in the water column.

Nine sites along the main channel of the South Fork New River exceeded the NCDEQ chronic and acute limits for total recoverable Zn, and the majority of sites exceeded the chronic criteria for Cu in Table 9. For Zn, these sites were located mainly upstream of Peak Creek that were characterized by alluvial reaches. In the case of Cu, all sites, except NR 24, located both upstream and downstream of the Peak Creek exceeded the total recoverable Cu criteria. The tributary sites PC 1 and PC 2, located downstream of the mine, also exceeded guideline values

Cu and Zn, while sites NRT 2, NRT 4 and KC 1 exceeded guidelines for Cu. The spatial distribution in these sampling sites implies that there were factors other than metal inputs from the Ore Knob Mine via Peak Creek that led to the exceedance of the guideline values. Sites NR 2 and NR 4 exceeded chronic limits for total recoverable Fe. These sites were located upstream of the mine along alluvial reaches with measurable fine sediments so this could be the reason for an isolated increase in this metal concentration.

The guidelines put forth by the EPA²⁷ produced findings that contrast with those produced by comparing metal concentrations to the NCDEQ²⁶ and MacDonald et al.¹⁰ guidelines. Dissolved concentrations represent metals that are completely bioavailable to aquatic biota. None of the sites exceeded chronic nor acute values, suggesting that the biota were not subjected to a health threatening environment. In contrast, the NCDEQ total recoverable concentrations may not all be bioavailable. As noted above, the metals of both channel bed sediments and suspended particles may be non-bioavailable. Thus, while exceeding the threshold guidelines, the actual impact on biota may be limited.

Another important finding related to metal impacts is that the metals appear to be coming primarily from the underlying bedrock. If this is the case, biota along the South Fork New River would have been exposed to relatively high metal concentrations prior to mining operations, and as suggested by Miller and Mackin for the Little Tennessee River, would have likely adjusted to these metal levels.²² In summary, then, it appears that the impacts on aquatic biota are likely to be limited. However, additional analyses, such as sequential analysis, that can assess metal bioavailability should be conducted to verify this conclusion.

CHAPTER SIX: CONCLUSIONS

After comparing results to crustal averages, epidemiological guidelines and sediment quality guidelines, it was found that the South Fork New River and its surrounding tributaries were high in Zn and Cu concentrations. With regards to crustal averages, all sites possessed high total Cu and Co concentrations, and the majority were high in total Zn. These crustal averages only provide a rough estimation for background concentrations in the sediments. The South Fork New River sampling sites compared to EPA guidelines determined that dissolved concentrations of each metal were below acute and chronic values while the NCDEQ guidelines determined sites and tributaries were high in total recoverable Zn and Cu concentrations. The MacDonald et al.¹⁰ sediment guidelines determined that channel bed and tributary sediments were high in bioavailable Zn and Cu. Based on all the comparisons, the South Fork New River and its surrounding tributaries possess high concentrations of Zn and Cu concentrations within the water column and sediments that could potentially impact biota.

Peak Creek was hypothesized to be the main source of metal contamination along the South Fork New River due to the Ore Knob Mine located in its headwaters (Ore Knob Branch). Previous studies conducted by the EPA² and NCDEQ¹ found some of the highest concentrations in Cu and Zn, along with other metals in Peak Creek. The mine tailings were sources of metals in the early years before site remediation. Thus, the metals transported in and through Peak Creek may have been reduced, yet in 2018 samples collected from Peak Creek showed that the concentrations remained high. The concentrations did not cause an abrupt, significant increase in concentrations immediately downstream of Peak Creek within the South Fork New River. The underlying geology of the South Fork New River is known to contain sulfidic layers within the

bedrock units, such as pyrite, sphalerite, and chalcopyrite. These metals are locked within the crystalline structure and can only become bioavailable when exposed and broken down by weathering. As these minerals are eroded from the bedrock, they may accumulate in the channel bed. Thus, the bedrock appears to be the predominant source of the metals, rather than mining at Ore Knob Mine. Given the source of the metals, the concentrations measured in comparison to guideline values, and the potential for a large portion of the metals to be non-bioavailable, the impacts of the metals on aquatic biota appears to be limited.

Future research of the South Fork New River could more fully examine enrichment factors and geoaccumulation index on the trace metals of interest in this project. These parameters can further assess natural mineralization or contamination sourcing based on pollution levels of soils. Sequential extraction methods could also be used to more fully assess the real bioavailable fraction.³² One also could study the tissue samples of some aquatic biota within the river to better understand if these metals are bioaccumulated in the tissues and shells of aquatic organisms, along with pH and Eh values to understand the speciation of the metals within the water column.

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APPENDIX A

ACID DIGESTION PROCEDURE

1. Preheat hot plate/water. Add a few ounces of water to crystallizing dish and place on hot plate.
2. While preheating, weigh and record 0.2-0.5 grams of dried sample, place in PP bottle.
3. Using a graduated cylinder and pipette, measure 3mL HCL and place in sample bottle. With a different cylinder and pipette, measure 1mL Nitric Acid, place in same sample bottle and cap it. Always add nitric acid to HCl, never the other way around (other info on this acid combo, called acid regia, is on wall next to the hood).
4. Once water in crystalizing dish has reached 90-95C add samples (4 per dish) and start timer. Make sure water in the dish complete covers the bottom portion of sample bottles.
5. Digest in water bath for approx. one hour. It is important to maintain water temperature at 90-95C. Hot plate will not accurately reflect water temperature. It will fluctuate periodically so it's important to check temperature frequently.
6. Every 10-15 minutes tighten the bottle lids using gloves (bottles will be hot!!). Internal pressure is beneficial for digestion. Try not to squeeze bottles too tightly or pressure will be released.
7. After approx. one hour, digestion is complete. Turn off hot plates and let samples cool. Failure to let samples cool will result in harmful vapor escaping when opening the lid.
8. Add a few mL of UP water to the PP bottle. Poor into flask. Add more UP water to bottle and shake up, making sure to cover every bit of bottle in order to retrieve every drop of solution. Use a squirt bottle to get the sides and lid of the bottle. Continue rinsing and adding to the flask. Finish filling to volume with pipette. If overfilled sample must be redone.
9. *Concentration and known volume are extremely important therefore it is imperative that it's never overfilled.
10. Once filled to volume place the flask cap on and shake until solution is homogenous.
11. Pour only a few mL of solution back into the same PP bottle. Cap it and shake making sure to cover all sides and lid of bottle. Pour that solution into waste. Then pour solution from flask into the same PP bottle, cap it and store until analysis.
12. Add UP water to volumetric flask, cap, and shake vigorously. Make sure water touches all sides of flask. Pour water into waste container. Repeat 2 times (3 rinses total). RINSE 3 times THOROUGHLY
13. Continue to next sample.

APPENDIX B

U- values calculated for comparison of alluvial reach sites and bedrock reach sites

Hypothesis		
Alluvial Reach median- Bedrock Reach median == 0		
	U-value	P-value
Silt + Clay %	63	0.4295
Fine Sand %	59	0.3125
OCC %	70	0.6818
Bioavailable Fe	68	0.6031
Bioavailable Al	61	0.3681
Bioavailable Cu	74	0.8493
Bioavailable Co	72	0.7642
Bioavailable Zn	72	0.7642
Total Fe	57	0.2627
Total Cu	60.5	0.3576
Total Co	59	0.3125
Total Zn	68	0.6031
Total Al	66	0.5287