Washington County Lead Mining District Natural Resource Damage Assessment
Study Plan
Updated March 2023

SURVEY OF FRESHWATER MUSSELS AND SEDIMENT CONTAMINATION OF THE MINERAL FORK AND MILL CREEK

WASHINGTON COUNTY MISSOURI

INTRODUCTION

The Mineral Fork River is the primary drainage system for three of the Washington County Lead Mining District Mines National Priorities List Sites and Mill Creek is within two of the NPL sites. The Washington County Lead Mining District is composed of five NPL sites. The three NPL sites that are in the Mineral Fork watershed are the Richwoods, Old Mines and Potosi sites, which form the eastern boundary of Washington County. Two NPL sites are in the Mill Creek watershed, the Potosi and Old Mines site. Pre-environmental regulation and inefficient mining operations resulted in soil, sediment, and surface water resources contaminated with elevated levels of heavy metals including cadmium, lead, zinc, and barium. The Natural Resource Trustees (US Fish and Wildlife Service on behalf of the U.S. Department of the Interior and the Missouri Department of Natural Resources on behalf of the State of Missouri) are initiating a natural resource damage assessment and restoration (NRDAR) process in 2023. The Trustees are finalizing of a Pre-Assessment Screen and Notice of Intent to Conduct an Assessment, followed by release of a Damage Assessment Plan in 2023. This study plan describes methodologies to sample sediment metals concentrations and freshwater mussel presence and species composition in the Mineral Fork and Mill Creek.

Mineral Fork and Mill Creek are tributaries to the Big River, which is the largest tributary to the Meramec River, an important stream for mussel diversity which supports 45 species including the federally listed Pink Mucket (*Lampsilis abrupta),* Scaleshell *(Leptodea leptodon),* Sheepnose (*Plethobasus cyphyus),* Snuffbox (*Epioblasma triquetra)* and Spectaclecase (*Margaritifera monondonta).* The Trustees have conducted extensive surveys of mussel fauna in the Big River as part of the Big River NRDAR case. Previous mussel surveys in Mineral Fork occurred in 1967, 1977, and 2011 at 3 locations and previous mussel surveys in Mill Creek occurred in 1978 and 2011 at 3 sites.

Due to the lack of freshwater mussel data within Mineral Fork and Mill Creek and the proximity of federally listed mussel species to the extensive heavy metal contamination present throughout Mineral Fork and Mill Creek, the Trustees will evaluate freshwater mussel species richness, relative abundance, and determine heavy metal concentrations in gravel bar and mussel bed sediment at 6-12 sites on Mineral Fork, 2-5 sites on Mill Creek, one reference site on the upper Big River, and an additional reference site to be identified after further evaluation of stream order and mussel fauna, to establish baseline conditions. Additionally, quantitative sampling to determine mussel density and recruitment will be conducted at 3-4 sites; 2 sites on Mineral Fork as applicable and each reference site. The mainstem Mineral Fork from the merger of Fourche A

Renault and Mine A Breton Creek to the confluence with the Big River (approximately 17 river miles), will be the area investigated. The mainstem Mill Creek from the confluence of Fountain Farm Branch to the confluence with Big River (approximately 10 river miles), will be area investigated (Figure 1). Biologists will conduct a desktop review of the area using aerial imagery to identify priority reaches for surveys and will coordinate with landowners for site access. Previously surveyed mussel sites will also be re-surveyed as part of this investigation to analyze whether changes in the mussel communities at those sites have occurred over time. The reference site will be selected from a known reference mussel community on the Big River and that has similar stream order and underlying geology to Mineral Fork. The study objectives are to:

1) Identify freshwater mussel presence/absence within Mineral Fork and Mill Creek;
2) Evaluate species richness and relative abundance of freshwater mussels within the Mineral Fork, Mill Creek, one Big River reference, and one additional reference sites;
3) Determine richness, relative abundance, density, and recruitment data from 2 Mineral Fork, one Big River reference, and one additional reference site;
4) Collect instream sediment from mussel beds and adjacent gravel bars to provide data on the concentrations of heavy metals present within occupied mussel habitat.

Additionally, through the Comprehensive Environmental Response, Compensation, and Liability Act Superfund program, the Environmental Protection Agency (EPA) is performing ongoing response actions within the Washington County Lead District. EPA has contracted with HydroGeoLogic, Inc. to conduct a remedial investigation/feasibility study (RI/FS) for mine waste (OU3) and surface water and sediment (OU4) in Washington County that will completed in late 2023. Recent data collected by Missouri University of Science and Technology students show surface water in the Mineral Fork and its tributaries had heavy metal concentrations (including Ba, Pb, and Zn) that could adversely impact aquatic life communities Miller 2020, Mortensen 2022). Some concentrations exceeded Probable Effects Concentrations (PEC; MacDonald *et al.* 2000) for heavy metals. However, these locations may not adequately represent heavy metal concentrations in all instream habitat types, and may not fully account for heavy metal exposure experienced by freshwater mussels.

The results of this investigation will be summarized in a final NRDAR injury report and the mussel species data will be included within the state of Missouri's freshwater mussel database (MDC 2017).

METHODS

Qualitative Mussel Sampling

Qualitative sampling in the form of Timed Visual Searches (TVS) will be conducted at approximately 10 sites including a reference sites on the Big River, to evaluate presence/absence, species richness and relative abundance of freshwater mussels. TVS are used to produce a more complete list of species at a given location, including the detection of rare species (Obermeyer 1998, Strayer and Smith 2003). In addition to species richness, a measure of mussel abundance can be expressed as CPUE (Catch Per Unit Effort, expressed as number of mussels per person

hour) and the relative abundance of each species can be expressed as a percentage of the total catch.
TVS will involve visual and tactile searches for live mussels while snorkeling or wading if water is too shallow to snorkel. Tactile searches will include disturbing and fanning gravel substrates by hand and moving cobble and large flat rocks. These techniques are necessary to ensure representative collections of juveniles, smaller species, and individuals buried in the substrate which may not be detectable by visual surveys alone. Mussels will be identified to species and recorded as they are found. On-shore searches of dead shell material will also be conducted on adjacent banks, gravel bars and in raccoon/muskrat middens. Dead shells that are not represented by living species will be collected during timed searches for voucher purposes.

Dead shells collected will be classified as fresh-dead, dead, or subfossil. Fresh-dead shells represent individuals in which the soft anatomy has not fully decomposed, and indicate the individual has recently perished. Dead shells have some luster to the nacre (innermost layer of the shell) and have a relatively intact periostracum (outermost layer of the shell). Subfossil shells have chalky and lusterless nacre and are missing considerable amounts of the periostracum (Buchanan 1980). The rate at which shell material decomposes following the death of a mussel depends on a variety of factors, including whether the shell was above or below the substrate, whether the shell was in the water or immersed, species, and shell thickness. In general, dead shells represent mussels that have been dead for less than a year and subfossil shells represent mussels that have been dead for more than a year.

All habitats will be searched at each site until at least 1.5 person-hours of search time failed to increase the number of mussel species present. Sites will be surveyed by at least two biologists experienced with mussel sampling and familiar with the regional fauna. Searches will be conducted during periods of low flow when aquatic habitats are accessible for visual searches (generally mid to late summer). At each survey reach the sampling method(s), total sampling effort, the number of living specimens of each species found, and species represented by dead shell material only will be recorded. Subjective descriptions will be made of the habitat in which each mussel species is found and of the surrounding stream habitat conditions. The approximate dimensions, location, and general water depth of the site will be described.

Mussel Bed Delineation and Sampling

Quantitative sampling in the form of quadrats will be conducted at 4 sites including 1 reference site on the Big River and 1 additional reference site, to estimate mussel density, relative abundance, species diversity, and document recruitment (Strayer and Smith 2003). Prior to conducting surveys, the boundary of each mussel bed will be delineated to establish the sampling area. This allows quantitative surveys to be focused on the portion of the channel occupied by mussels and minimizes site variance to provide more accurate population estimates (Strayer and Smith 2003). Visual and tactile searches will be used to determine the linear and lateral extent of the area occupied by mussels. Searches will be conducted systematically in a zig-zag pattern across the channel and in an upstream or downstream direction. Tactile search methods involve disturbing the top layer of substrate by hand to increase detection of mussels at or just below the substrate surface. The boundary will be marked with a Trimble® GeoXT™ where mussel densities drop to less than 1 individual/m^2 as estimated by the diver. Sites will be surveyed by at

least 2 experienced biologists familiar with the regional fauna. Searches will be conducted during periods of low-flow to increase access and reduce sampling of unsuitable habitat.
Following mussel bed delineation, at least 30 0.25m^2 quadrats will be evenly spaced within the mussel bed boundary using a systematic sampling approach with 3 random starts (Smith *et al.* 2001, Strayer and Smith 2003, Roberts *et al.* 2016). Exact number of quads will be determined once the area of the mussel bed is delineated.

Quadrats will be positioned on the stream bottom at each identified sampling point and all visible mussels will be collected. Following initial visual surveys, large cobble and flat rocks will be removed and remaining substrates will be excavated to a depth of approximately 10cm. Samples will be sieved through a floating 7mm screen and sorted for mussels. Length and age (counting external growth lines) will be estimated, and species recorded for each individual mussel prior to returning them to their original quadrat location. Any dead species not represented by live individuals will also be noted.

Sediment Sampling

The goal of sampling sediment is to determine concentrations of heavy metals in stream sediments in occupied mussel habitats. Sediment sampling will be conducted at each site where live mussel data are collected. Approximately 5 to 10 kilograms of sediment will be collected at each location and GPS readings will be recorded.

Sediment sampling methods are based on those used by Roberts et al. 2016 and are similar to sediment sampling and analysis used by EcoAnalysts and CERC on the Spring River (EcoAnalysts 2018). Two composite substrate samples will be taken at each site where live mussels are identified, one within the mussel bed and one from an adjacent gravel bar. Samples will consist of five subsamples or aliquots taken from random points. Sediments will be collected from relatively slow-moving water near physically adequate mussel habitat consisting of riffle/run complexes with relatively stable gravel sized particles and from adjacent depositional gravel bar areas. Each composite sample from mussel habitat will be collected from water less than 15 cm (6 inches) deep. The five aliquots will be placed in a high-density polyethylene (HDPE) mixing vessel using a plastic scoop, homogenized, and then spooned into a Ziploc® brand 1 gallon size freezer bag. Samples will be labeled and placed in a cooler for transfer to the US Fish and Wildlife Service Columbia, Missouri office (USFWS office) for drying and XRF analysis and then transferred to the U.S. Geological Survey Columbia Environmental Research Center (CERC) for further chemical analysis. Used HDPE vessels and collecting scoops will then be placed in a storage bag for decontamination including a nitric acid rinse for later reuse. Sample labels will include a unique sample identifier, site name, date, and initials of collector. At sites where visible mine waste is present, additional composite samples may be collected for heavy metal and grain size analyses.

Duplicate sediment material will be collected at certain sampling locations for the purpose of quality control/verification of metals analysis. Duplicate samples will be selected to reflect a relative range of metal concentrations: high, medium, and low, based on heavy metal concentration in EPA's recent (2020-21) sampling data. One quality control (QC) sample will be analyzed for every tenth sample, or one QC sample will be collected by each team per day,

whichever number is greater. Two separate bags should be collected with alternating spoonfuls of sample placed in each bag.

Sediment samples will be recorded in a logbook and a chain of custody form. The chain of custody form will be maintained with the samples and will accompany the samples to the USFWS office and CERC laboratory. The samples and chain of custody will be signed over to the sample custodian at the USFWS office (if different from the collector) and at CERC.

Metals Analysis

Sediment samples will initially be screened for metals (Pb, Zn, Cd, and Ba) concentrations using an XRF meter followed by Inductively Coupled Plasma or Atomic Adsorption at the CERC laboratory. The XRF analysis will be completed using a 2007 Thermo Niton Xl3t 600 XRF (Thermo Scientific, Billerica, MA) following EPA method 6200 (EPA 2007). Samples will be allowed to air dry for seven days or until less than 20% moisture has been achieved. A portion of each sediment sample will be sieved to less than 2 mm. Both the less than 2 mm and the bulk sample will be analyzed by XRF. Samples will be thoroughly mixed within the Ziploc® bag by shaking and/or hand manipulation. Each sample will then be analyzed for 90 seconds by placing the instrument directly against the bag with the sediment in full contact with the portion of the bag in contact with the XRF window. An arithmetic mean will be calculated from three separate readings for each sample, with the sample fully mixed and shaken between each reading and used as the best representation of the sample metals concentrations. The use of the XRF to analyze ex-situ samples according to the above methods has been used in other Southeast Missouri NRDAR assessment studies (Roberts et al. 2009 and 2016) and the data have shown correlation with ICP/MS. Data generated through XRF analysis will be compared to ICP/MS data to determine whether results of metals concentrations between the methods are comparable.

A suite of calibration verification check samples will be used to check the accuracy of the XRF instrument and to assess the stability and consistency of the analysis for the analytes of interest. Check samples will be analyzed at the beginning of each working day, during active sample analyses, and at the end of each working day. The measured value for each target analyte should be within ±20 percent (%D) of the true value for the calibration verification check to be acceptable. If a measured value falls outside this range, then the check sample should be reanalyzed. If the value continues to fall outside the acceptance range, the instrument should be recalibrated, and the batch of samples analyzed before the unacceptable calibration verification check will be reanalyzed (USEPA 1998).

Following XRF analyses, samples will be submitted to CERC for analysis of total Pb, Zn, Cd, and Ba using Inductively Coupled Plasma or Atomic Adsorption following EPA method 3050b "Acid Digestions of Sediment, Sludges, and Soils".

A summary of the analytical parameters and methods are provided below:

Table 1. Analytical Parameters

Sample Type	Analytical Method	Analyte	Fraction analyzed	Estimated Number of samples
Contamination characterization	Office/laboratory XRF and ICP or AA EPA 3050b	Pb, Zn, Cd, Ba	Bulk and <2mm	20
QC samples	Office/laboratory XRF and ICP or AA EPA 3050b	Pb, Zn, Cd, Ba	Bulk and <2mm	3

Table 2. Budget

Study Component	Quantity	Costs
Personnel	~240 hours field work (3 biologists); 204 hours field prep, data analysis, report writing, and review	$29,000
Travel	Lodging, M&IE, and vehicle costs for 3 biologists for 8 days in the field	$4,600
Metals analyses	23 samples analyzed by CERC	$6,451.50
Total		$40,051.50

Table 3. Timeline

	Jan-2023	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan 2024	Feb	Mar
Field Work Planning															
Field Surveys															
Data Analysis															
Report Writing															

Data Management

The Service will retain all data and associated metadata related to the mussel surveys which is anticipated to include:

- qualitative mussel data (species lists, presence absence data, numbers of individuals)
- bank survey data (dead shell classification)
- habitat descriptions
- substrate classification
- water quality data
- quantitative mussel data (species lists, numbers of individuals, density, length, age)

- Further analyses of collected data
- XRF analysis of sediment

A final data release will be made publicly available by the Service consistent with applicable law and regulations.

Upon completion of sediment lab analyses, CERC's Supervisory Research Chemist will retain the data and associated metadata. A final data release will be made publicly available by CERC consistent with applicable law and regulations.

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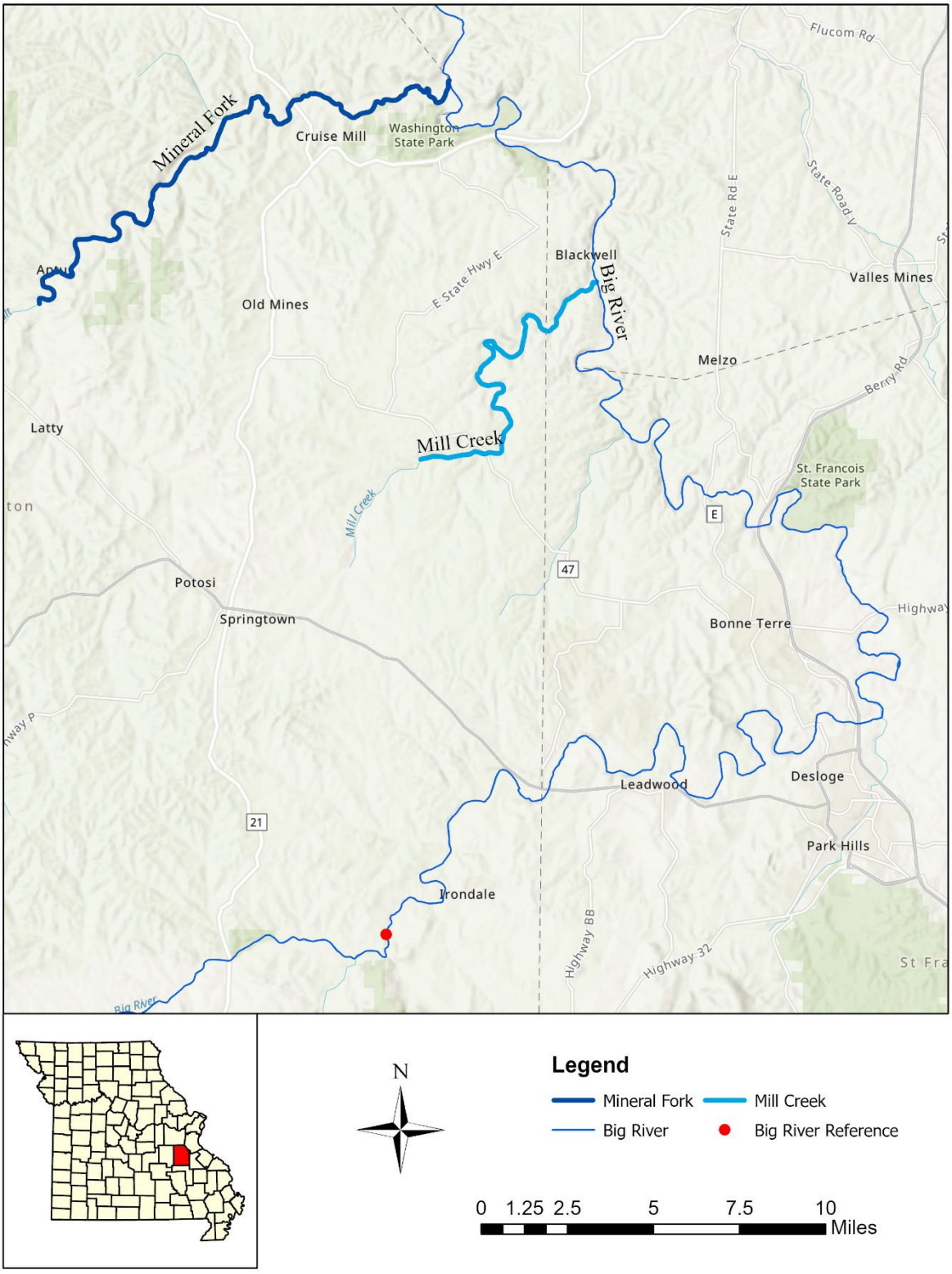

Figure 1. Mineral Fork and Mill Creek Project Location.