Project Final Report

Grant # C9994861

EVALUATION OF SEDIMENT TOXICITY AND MACROINVERTEBRATE COMMUNITIES AT SELECTED SITES IN THE UPPER CUMBERLAND RIVER (KENTUCKY, USA)

Workplan # 9908

Project Period September 2000-October 2001

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Funding for this project (EVALUATION OF SEDIMENT TOXICITY AND MACROINVERTEBRATE COMMUNITY AT SELECTED SITES IN THE UPPER CUMBERLAND RIVER, KENTUCKY, USA) was provided for in part by the U.S. Environmental Protection agency (USEPA) through the Kentucky Division of Water, Nonpoint Source Section, to the Bioassay Section within the Division of Water as authorized by the Clean Water Act Amendments of 1987, 319(h), Nonpoint Source Implementation Grant # C9994861-99. The contents of this document do not necessarily reflect the views and policy of the USEPA, KDOW nor does the mention of trade names or commercial products constitute endorsement. This document was printed on recycled paper.

ACKNOWLEDGMENTS

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EXECUTIVE SUMMARY

Sediment is an integral part of aquatic ecosystems. Resource extraction can adversely affect sediment quality through point and nonpoint source runoff. The objective of this study was to investigate the feasibility of utilizing the benthic macroinvertebrate community, sediment toxicity tests, and sediment chemistry in assessing selected aquatic systems in the Upper Cumberland River Basin that have drained areas of coal mining activity.

Sediment sampling was conducted in the fall of 2000 on seven test sites and two control sites. Quantitative benthic macroinvertebrate samples were collected concurrently with sediment used for chemical analysis and toxicity testing. The sediment was analyzed for metals, selected organic compounds, acid volatile sulfides, and total organic carbon. Both 10-day acute and 28-day chronic toxicity tests were performed using *Hyalella azteca*. Macroinvertebrate data were subjected to parametric and nonparametric analyses.

Six of the seven sites were found to have high levels of contaminants. Only one site exhibited acute toxicity and three showed evidence of chronic effects. Two sites had impaired benthic communities. However, when the results from the chemical analysis, toxicity tests and benthic investigation were considered in a Sediment Quality Triad (SQT) assessment methodology, only one site was determined to show evidence of contaminant induced degradation. Contaminants were considered unavailable at two sites and the benthic response at two sites was not attributable to contaminants. One site was determined to exhibit no contaminant degradation. The remaining site had contaminants available but the macroinvertebrate community response was unaffected by sediment chemistry.

The SQT assessment methodology was a good tool to investigate sediment contamination. More statistically robust and impact sensitive methods of performing chronic toxicity tests should be developed to increase assessment accuracy. Macroinvertebrate sampling strategies should consider ecoregion/subecoregion variability, as well as techniques for reducing within and between site sampling error.

The Bioassay Section gained valuable experience in toxicity testing from this project. The use of the triad approach as an assessment tool was very valuable. It enabled us to look at AVS/SEM (Model), benthic invertebrate data, chemistry and compare these data with our usual toxicity testing. This project led to the discovery of a better protocol for the 28-day toxicity test and it will be used in our next round of testing. We now realize we should have completed a particle size analysis. We gained valuable field experience and expanded our toxicity/chemical database.

INTRODUCTION

The Upper Cumberland River Basin in southeastern Kentucky contains some of the Commonwealths most valuable water resources. The most widespread water quality problem for the Upper Cumberland watershed is associated with the coal mining industry. Point and non-point sources of acid mine drainage from coal deposits, which contain large quantities of sulfides and sulfates, contribute to the problem. Oxidation of iron disulfide exposed by mining initiates the formation of water soluble acidic pollutants which cause the most objectionable characteristics of coal mine drainage. The most significant water quality problem associated with mining occurs when dissolved, suspended, or other solid mineral wastes and debris from mining or mine related operations enter streams, watercourses or ground water. Mine drainage includes not only water flowing by gravity or pumped from underground mines, but also runoff or seepage from surface and strip mines, excavated waste deposits and haul roads. (KDOW, 1975). Resource extraction is a major source of chemically altered sediments (Burton, 1992).

Sediment associated with aquatic systems play a vital ecological role. They serve as a potential reservoir for organic material as well as a sink for industrial contaminants (Call, et al. 2001). Contaminated sediment can be directly toxic to aquatic organisms. Additionally, non-lethal concentrations of chemicals sorbed to sediment can accumulate in benthic organisms and bioaccumulate up the food web. The goal of this study was to document sediment contaminant concentrations, measure sediment toxicity and evaluate negative impacts to the associated macroinvertebrate communities.

Seven potentially impacted sites were selected using data from the Kentucky Nonpoint Source Assessment Report (1999) and two KDOW reference reach sites were selected to provide control sediment. Sediment and macroinvertebrate samples were taken concurrently at each site in the Fall of 2000. Each site was analyzed for toxicity, total organic carbon content, and concentrations of metals, pesticides, and polychlorinated biphenyls (PCBs). Analysis of the benthic communities included identification of taxa to the lowest positive level, calculation of selected metrics, parametric, and nonparametric tests. The primary objective of the analysis was to identify possible toxic materials, evaluate the level of toxicity and relate these findings to the benthic biota.

METHODS AND MATERIALS

All sites were located within the Eastern Kentucky Coalfield Region (Table 1). Material taken from Cranks Creek was collected from two sites located in Cranks Creek Reservoir. The remaining sites were located in the free flowing portions of each stream. At each site, one composited sediment sample was collected and split for chemical analysis and toxicity testing. Three replicate samples were collected for macroinvertebrate monitoring.

Table 1. Site locations used in 10-day acute and 28-day chronic sediment toxicity test.

Water Body	GPS coordinates	Mile Point	Sample date
Turkey Creek Bell County	N 36d 46 m 06.3 s W 084d 02m 28.3s	**0.3**	**10/31/00**
Little Clear Creek Bell County	N36d 42m 31.8s W083d 44m 29.3s	**0.8**	**10/31/00**
Cranks Creek Harlan County	N36d 04 m 50.2s W 83d 12m 40.7s	**3.0**	**9/20/00**
Cranks Creek Reservoir Harlan County	N36d 44m 50.2s W083d 12m 55.5s	**3.2**	**9/20/00**
Indian Creek Jackson County	N 37d 22m 59.7s W084d 02m 28.3s	**1.5**	**9/19/00**
Crummies Creek Harlan County	N36d 47m 26.8s W083d 13m 02.2s	**1.6**	**9/20/00**
Roaring Paunch McCreary County	N36 d 40m 48.3s W084d 32m 28.0s	**0.1**	**10/30/00**
Laurel Fork* Jackson County	N84d 02m 28.9 s W37d 22m 11.5s	**1.0**	**9/19/00**
Bark Camp Creek* Laurel County	N36d 54m 14.8s W84d 16m 52.2s	**2.5**	**10/30/00**

* = Control site

DESCRIPTION OF MACROINVERTEBRATE ASSESSMENT

The macroinvertebrate samples were collected from the same depositional areas as the rest of the sediment used in the study. The benthic samples taken at Cranks Creek Reservoir were collected with a Petite Ponar dredge. Stream samples were taken by inverting a glass specimen jar into the sediment. All benthic samples were sieved through 300μ Nitex netting in the field. The samples were then preserved in 70 % ethanol and transported to the lab for identification. To reduce sample processing time, samples were subjected to sucrose flotation (Flannagan, 1973). Each sample was examined separately using a stereo-dissecting microscope with magnification to 75X.

Invertebrates were hand picked from samples, identified to the lowest positive taxonomic level and enumerated. Organisms requiring slide preparation for identification, such as Oligochaeta and Chironomidae, were mounted in CMC-10 and dried. Identifications were then made using a phase-contrast compound microscope with magnification to 1000X.

Counts of organisms were converted to number per square meter. Mathematical manipulations included calculation of diversity (Shannon 1948) and evenness (Pielou 1966) values for each sample. In lieu of standard protocols for determining negatively impacted sediment, best professional judgement was used on a site by site basis to designate impaired sites. Raw macroinvertebrate data are in Appendix E.

Macroinvertebrate communities at each site were analyzed by cluster analysis using the Bray-Curtis dissimilarity index and the unweighted pair-group arithmetic averaging (UPGMA) clustering algorithm. These two techniques have been used frequently together and are considered to be robust at classifying distinct objects. Prior to clustering, we transformed taxa densities using $\log_{10}$ (x+1). The technique arranges all clusters into a hierarchy so that the relationships between the different groups are apparent. Cluster analysis produces a tree-like diagram called a dendrogram.

SEDIMENT CHEMICAL ANALYSIS

Sediment was collected in the field for chemical analysis according to protocols established by USGS (1994). The Kentucky Department of Environmental Services (DES) analyzed each sample for metals and selected organics (see Appendix C) to identify suspected toxic material. Chemical contamination is a concept that is not always clearly defined relative to sediment. Traditionally, higher concentrations of potential toxicants are thought to produce higher risks to the biota. However, this assumption is not always true. Some evaluation must be made to estimate the potential risk to aquatic life that the compound may have. The EPA defines sediment criteria as a specific level of protection from the adverse effects of sediment associated pollutants, for beneficial uses of the environment, biota, and human health. Sediment quality criteria are the numerical concentrations of individual chemicals, which are intended to be predictive of biological effects, protective of the presence of benthic organisms and applicable to the range of natural sediments from lakes and streams. A sediment criterion must relate to the level of harm that the contaminant possesses by specifying an appropriate level of protection. Only if the contaminant concentration is less than all of the available criteria can exposure to the sediment, or to organisms that inhabit the sediment, be considered to be without significant risk.

Sediment contaminants primarily consist of heavy metals and persistent organic compounds (New York DEC 1998). Sediment criteria for non-polar organic compounds (e.g. PCBs and PAHs) are derived using equilibrium partitioning method (USEPA, 2000). Sediment criteria for metals are derived from empirically derived values establish by the National Oceanic and Atmospheric Agency (Long and Morgan, 1991). Total organic carbon (TOC) values are important to normalize the bioavailability of organic toxicants between sites (USEPA, 2000).

Simultaneously extracted metals/ acid volatile sulfides analysis was performed by EN CHEM, Incorporated Laboratory in Madison, Wisconsin. Simultaneously extracted metals (SEM) and acid volatile sulfide (AVS) are operationally defined methods for the analysis of sulfide and associated metals in aquatic sediments. The SEM to AVS ratio has been used to clarify the results of bioassay tests of metal toxicants. Sulfide production occurs in organically enriched sediment under anaerobic conditions. Typically, sulfides are restricted to a few centimeters beneath the sediment surface, depending on the influx of organic matter. The AVS-SEM model, developed by DiToro (1985), recognized that AVS is a reactive pool of solid phase sulfide that is available to bond with certain metals and reduce free metal ion concentrations. The AVS-SEM model has been verified for six divalent metals in anoxic sediment (i.e. cadmium, copper, lead, nickel, mercury and zinc). If the molar ratio of toxic metals measured by SEM to AVS exceeds one, the metals are potentially bioavailable. A ratio less than one suggests that the metals in the sediment are non-toxic (ENCHEM SOP WCM-63, April 2000).

DESCRIPTION OF THE 10 DAY TOXICITY TEST

The 10-day sediment toxicity test was performed using *Hyalella azteca* to determine acute effects. Conditions for conducting the test are fully outlined in USEPA(1994). Results were analyzed using ToxCalc, Tidepool Scientific Software. Toxicity was determined when the test site results were statistically different from control sites. Results of all toxicity tests are in Appendix D.

DESCRIPTION OF THE 28 DAY TOXICITY TEST

In order to determine the existence of chronic effects in sediment collected for this study, a 28-day test was conducted. In the absence of standardized protocols for such experiments, KDOW Bioassay Section personnel developed their own procedures. Initially, 400 ml of field sediment were placed in each 2.5-gallon aquarium. It was overlain by 2 liters of moderately hard-reconstituted water. The sediment was allowed to settle overnight. Twenty-five *Hyalella azteca* were introduced into each aquarium on the following day. Organisms were less than 14 days old. The organisms were fed 5 ml YCT daily after a one-liter water exchange. Prior to water exchange, the overlying water was measured daily for temperature, pH, and dissolved oxygen. At test end, the sediment was poured out gently into a Pyrex glass dish. Organisms were removed by pipette. If they could not be found, the sediment was sieved through a Standard US #30 screen. All test and control sites had organisms other than *Hyalella azteca*, presumably from latent egg deposition which occurred prior to collection. The sediment was considered to be toxic if mortality was greater than 20 % of the control results.

As with any bioassay technique, the results take careful consideration. *Hyalella azteca* is an organism that is used in sediment toxicity tests because they have contact with the sediment and are easily cultured in the laboratory. Their sensitivity can not be considered representative of all benthic organisms. Additionally, the test has daily water exchanges utilizing moderately hard reconstituted water. The expression of toxicity in the laboratory may not accurately reflect problems in the field. The chemical composition of the synthetic water can moderate the temperature, dissolved oxygen, and pH of the sediment in consideration.

INTEGRATIVE ASSESSMENT

The overall assessment of sediment quality is contingent upon many factors. Possible variables include toxic material concentrations, the synergistic/antagonistic properties associated with the pollutants, sediment depth and oxygen levels, particle size and physical chemistry of the sediment, and temperature. Compounding problems include genetic selection of benthic organisms in low level, chronically polluted streams. Organisms that are typically sensitive to pollution can appear to be quite tolerant (Burton, 1992). It has also been found that the testing process itself can release bound contaminants, yielding different results in the laboratory than are actually found in the field.

To increase the accuracy of identifying chemically altered sediments Chapman (1990), developed the Sediment Quality Triad (SQT). An SQT incorporates benthic community parameters, sediment chemistry and sediment toxicity into the overall assessment process. The

utilization of a multiple component system to evaluate sediment quality reduces the risk of misrepresenting actual conditions.

RESULTS AND DISCUSSION

Table 2 summarizes the results of the SQT and the possible conclusions that can be drawn. Results from sites located in Cranks Creek should be considered with caution. Since both sites were lentic in nature, the macroinvertebrate results are not directly comparable with the lotic control sites. The results from Cranks Creek were not included in the parametric analysis. However, the data were included in the nonparametric cluster analysis. The one way analysis of variance (ANOVA) followed by Tukey's Test was unable to differentiate sites by mean total organisms. However, several sites were significantly different at $p = 0.05$ level utilizing mean taxa richness (Table 3).

Table 2. Triad evaluation of Upper Cumberland sites; Y = evidence of degradation; N = no evidence of degradation

Site	Chemistry	10 day toxicity	28 day toxicity	Benthos	Possible Conclusions
Turkey Creek	Y	N	Y	Y	Evidence of contaminant induced degradation
Little Clear Creek	Y	N	N	N	Contaminants not bioavailable
Cranks Creek	Y	N	N	N	Contaminants not bioavailable
Cranks Creek Reservoir	Y	N	Y	N	Toxic pollutants may be stressing the system
Indian Creek	N	Y	N	N	No evidence of contaminant degradation or other conditions causing response
Crummies Creek	Y	N	N	Y	Contaminants not available or response not due to chemistry
Roaring Paunch	Y	N	Y	N	Toxic pollutants may be stressing the system

Table 3. Descriptors of the invertebrate community of sediments at The Upper Cumberland River Basin stream sites: dominant taxa densities, percent compositions, and selected measures of community quality.

Little Clear Creek	no/m^2	%
UIW/OCS*	701532	36.8
Dubiraphia sp.	261765	13.7
Procladius sp.	146589	7.7
Bezzia/Palpomyia *sp.*	146589	7.7
UIWICS*	136118	7.1
Paralauterborniella sp.	83765	4.4
Tanypus sp.	73294	3.8
Hydra sp.	41882	2.2
Tanytarsus spp.	41882	2.2
Ablabesmyia mallochi	31412	1.6
Nais variabilis	31412	1.6
Bezzia sp.	31412	1.6
Psectrocladius sp.	20941	1.1
Polypedilum halterale gp.	20941	1.1
Cladotanytarsus sp.	20941	1.1
Mean Taxa Richness	14[c]	
Mean Total Organisms/m^2	73294[b]	
Mean Sample Diversity	2.81	
Mean Sample Eveness	0.77	

Crummies Creek	no/m^2	%
UIW/OCS*	1047167	82.6
Stictochironomus sp.	83773	6.6
Tanytarsus spp.	31415	2.5
Paratendipes albimanus	20943	1.7
Polypedilum scalaenum gp	20943	1.7
Mean Taxa Richness	5.67[d]	
Mean Total Organisms/m^2	1267072[b]	
Mean Sample Diversity	0.98	
Mean Sample Eveness	0.39	

Cranks Creek	no/m^2	%
Corbicul fluminea	21292	36.7
UIW/OCS*	13824	23.8
Tanytarsus spp.	6593	11.4
Ablabesmyia mallochi	3067	5.3
Branchiura sowerbyi	1971	3.4
Sphaeriidae	1541	2.7
Dicrotendipes sp.	1319	2.3
Centroptilum sp.	1096	1.9
Cryptotendipes sp.	874	1.5
Paracladopelma sp.	874	1.5
Mean Taxa Richness	19.67	
Mean Total Organisms/m^2	58007	
Mean Sample Diversity	3.43	
Mean Sample Eveness	0.8	

Cranks Creek Reservoir	no/m^2	%
Branchiura sowerbyi	11201	25.1
Sphaeriidae	5052	11.3
Dero trifida.	4830	10.8
Caenis sp.	3289	7.4
Orthotrichia sp.	3067	6.9
Aulodrilus pigueti	2415	5.4
Tanytarsus spp.	1319	3.0
Ablabesmyia idei	1096	2.5
Dicrotendipes sp.	1096	2.5
Polypedilum halterale gp.	1096	2.5
Parachironomus hirtalatus	874	2.0
Paratanytarsus sp.	874	2.0
UIW/OCS*	874	2.0
Bezzia/Palpomyia sp.	667	1.5
Dubiraphia sp.	667	1.5
Enallagma sp.	667	1.5
Labrundinea neopillosella	667	1.5
Procladius sp.	667	1.5
Mean Taxa Richness	16.33	
Mean Total Organisms/m^2	44643	
Mean Sample Diversity	2.87	
Mean Sample Eveness	0.71	

Roaring Paunch	no/m^2	%
Polypedilum scalaenum gp.	157059	27.8
Tribelos jucundum	146589	25.9
Stictochironomus devinctus	83765	14.8
Polypedilum halterale gp.	73294	13.0
Hydroptila sp.	20941	3.7
Procladlus sp.	10471	1.9
Dubiraphia sp.	10471	1.9
Atherix sp.	10471	1.9
Djamabatista sp.	10471	1.9
Tanytarsus spp.	10471	1.9
Nilothauma sp.	10471	1.9
UIW/OCS*	10471	1.9
Gomphidae	10471	1.9
Mean Taxa Richness	6[d]	
Mean Total Organisms/m^2	565413[b]	
Mean Sample Diversity	2.17	
Mean Sample Eveness	0.9	

Bark Camp Creek	no/m^2	%
Stictochironomus devinctus	460707	78.6
UIW/OCS*	41882	7.1
Ephemera sp	20941	3.6
Microtendipes pedellus gp..	20941	3.6
Corynoneura sp.	10471	1.8
Tanytarsus spp.	10471	1.8
Polypedilum scalaenum gp.	10471	1.8
Cladotanytarsus sp.	10471	1.8
Mean Taxa Richness	3.67[a]	
Mean Total Organisms/m^2	586355[b]	
Mean Sample Diversity	1.16	
Mean Sample Eveness	0.64	

Turkey Creek	no/m^2	%
UIW/OCS*	198942	61.3
UIWCS*	52353	16.1
Mean Taxa Richness	3[a]	
Mean Total Organisms/m^2	324589[b]	
Mean Sample Diversity	0.99	
Mean Sample Eveness	0.45	

Indian Creek	no/m^2	%
Tanytarsus spp.	492168	30.9
UIWCS*	293207	18.4
UIW/OCS*	240848	15.1
Parakiefferiella sp.	198962	12.5
Dubiraphia sp.	73302	4.6
Corbicula fluminea	41887	2.6
Stempellinella sp.	41887	2.6
Bezzia/Palpomyia sp.	31415	2.0
Mean Taxa Richness	11.33[c]	
Mean Total Organisms/m^2	75795[b]	
Mean Sample Diversity	2.67	
Mean Sample Eveness	0.76	

Laurel Fork	no/m^2	%
UIW/OCS*	733017	73.7
Dubiraphia sp.	31415	3.2
Bezzia/Palpomyia sp.	31415	3.2
Cladotanytarsus sp.	31415	3.2
Corbicula fluminea	20943	2.1
Nematoda	20943	2.1
Tipulidae	20943	2.1
Centroptilum sp.	20943	2.1
Pisidium sp.	10472	1.1
Heptageniidae	10472	1.1
Tanytarsus spp.	10472	1.1
Dero nivea	10472	1.1
UIWCS*	10472	1.1
Paralauterborniella sp.	10472	1.1
Polypedilum scalenum gp.	10472	1.1
Ceratopsyche sp.	10472	1.1
Mean Taxa Richness	7[d]	
Mean Total Organisms/m^2	994808[b]	
Mean Sample Diversity	1.9	
Mean Sample Eveness	0.7	

Means with same letter are not significantly different (oneway ANOVA followed by Tukey's test; p = 0.05)

*UIW/OCS = unidentified immature oligochaete without capilliform setae; UIWCS = unidentified immature oligochaete with capilliform setae

TURKEY CREEK

The chemical analysis of sediment at Turkey Creek indicated high levels of manganese (see Appendix C). Based on sediment quality criteria, impact from manganese may be considered to be moderate. Sediment toxicity test results indicated no acute effects, but chronic effects were found. The SEM/AVS molar ratio at Turkey Creek was 1.3. Since none of the metals found in high concentration have been verified in the AVS-SEM model, their bioavailability is in question. Macroinvertebrate data (Table 3) indicate Turkey Creek was dominated by immature worms (UIW/OCS, probably *Limnodrilus* sp.). Mean taxa richness was 3.0. These results are typical of streams with high nutrient loads, although the absence of tolerant chironomids (e.g. *Chironomus* and *Dicrotendipes*) would suggest impairment other than organic enrichment. It is important to note that Turkey Creek had no other habitat available in the stream channel other than soft mud. The lack of a sand or sand/gravel constituent in the sediment could limit the number of organisms that can inhabit it. Nonetheless, we agree with the conclusion of the SQT that the impairment is due to (a) sediment contaminant(s).

LITTLE CLEAR CREEK

The chemical analysis of sediment at Little Clear Creek indicated high levels of magnesium, aluminum and iron (see Appendix C). Based on sediment quality criteria, impact from manganese and iron may be considered to be moderate. Sediment toxicity test results indicated that no acute or chronic effects were found. The SEM/AVS molar ratio at Little Clear Creek was 0.6. Therefore toxicity due to metals in the AVS/SEM model is unlikely. Like Turkey Creek, none of the metals found in high concentration have been verified in the AVS-SEM model. Macroinvertebrate data (Table 3) indicated Little Clear Creek was dominated by immature oligochaetes (UIW/OCS, probably *Limnodrilus* sp.). Mean taxa richness was 14.0, this value is statistically similar to Indian Creek. Cluster analysis (Figure 1), however, indicates that the community is unlike any of the rest of the study sites. We agree with the SQT conclusion that the sediment contaminants are not available.

CRANKS CREEK

The chemical analysis of sediment at Cranks Creek indicated high levels of copper, nickel and iron (see Appendix C). Based on sediment quality criteria, impact from copper, nickel and iron may be considered to be moderate. Sediment toxicity test results indicated that no acute or chronic effects were found. The SEM/AVS molar ratio at Cranks Creek was 7.2. The AVS-SEM model predicts that metal toxicity is likely, at least from copper and nickel. Macroinvertebrate data (Table 3) indicated Cranks Creek was dominated by the Asian clam, *Corbicula fluminea*, immature oligochaetes (UIW/OCS, probably *Limnodrilus* sp.) and midges of the genus *Tanytarsus*. Mean taxa richness was 19.67. We agree in part with the SQT assessment. Although sediment contaminants are available, they are not causing an impact. Unsurprisingly, the Cranks Creek sites were grouped together in the cluster analysis.

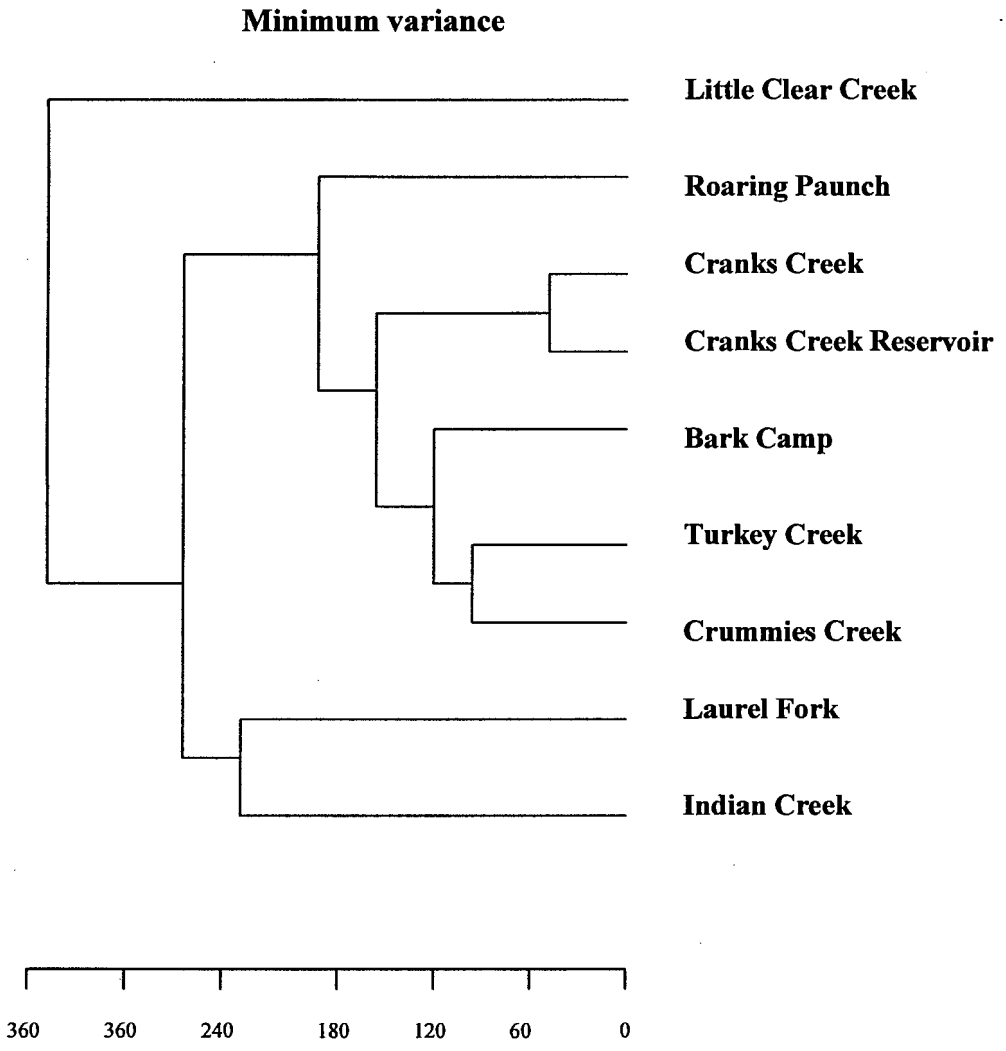

Figure 1: Results From URGMA Cluster Analysis

CRANKS CREEK RESERVOIR

The chemical analysis of sediment at Cranks Creek Reservoir indicated high levels of copper, nickel, mercury and iron (see Appendix C). Based on sediment quality criteria, impact from copper, nickel, mercury and iron may be considered to be moderate. The SEM/ AVS molar ratio at Cranks Creek Reservoir was 0.6. The AVS-SEM model predicts that metal toxicity is unlikely, at least from mercury, copper and nickel. The ten day sediment toxicity test results indicated no acute effects, but chronic effects were found in the 28 day test. A high level of bis (2-Ethylhexyl) phthalate (45 mg/kg) was detected in the sediment chemistry analysis. Macroinvertebrate data (Table 3) indicated Cranks Creek Reservoir was dominated by the tubificid, *Branchiura sowerbyi*, sphaeriid clams and the naidid worm *Dero nivea*. Mean taxa richness was 16.33. It should be noted that this site was covered with a mat of the musk-grass *Chara* sp. and the depth of the site was 3 to 4 meters. The musk-grass supported many taxa not associated with sediment and inflated the overall taxa richness. The water depth probably lowered sediment temperature and created anoxic conditions. Both of these factors reduce sediment toxicity *in situ*. Conditions in the laboratory likely promoted sediment toxicity through increased temperature and aerobic conditions. We agree with the SQT assessment that the benthos response is not due to sediment contaminants.

INDIAN CREEK

The chemical analysis of sediment at Indian Creek indicated no high levels of metals or organics (see Appendix C). Sediment toxicity test results indicated acute effects were present but chronic toxicity was not found. The SEM/AVS molar ratio at Indian Creek was 4.7. The AVS-SEM model predicts that metal toxicity is likely, however no elevated levels of the model metals were found. Macroinvertebrate data (Table 3) indicated Indian Creek was dominated by midges of the genus *Tanytarsus*, aquatic worms (UIWCS and UIW/OCS) and the midge, *Parakiefferiella* sp. Mean taxa richness was 11.33. The cluster analysis indicated that this site was most similar in macroinvertebrate composition to the control site at Laurel Fork. We believe the result of the toxicity test is most likely a false positive. The SQT method of sediment assessment uses multiparameters in anticipation of these results. We agree with the SQT assessment that there is no evidence of contaminant degradation.

CRUMMIES CREEK

The chemical analysis of sediment at Crummies Creek indicated high levels of cadmium, copper, manganese, nickel and iron (see Appendix C). Based on sediment quality criteria, impacts from cadmium, copper, manganese, nickel and iron may be considered to be moderate. Sediment toxicity test results indicated that no acute or chronic toxicity was evident. The SEM/AVS molar ratio at Crummies Creek was 1.2. The AVS-SEM model predicts that metal toxicity is likely but the value is still low, at least from cadmium, copper and nickel. Macroinvertebrate data (Table 3) indicated Crummies Creek was dominated by the immature oligochaetes (UIW/OCS, probably *Limnodrilus* sp.). Mean taxa richness was 5.67. Cluster analysis indicated that this site was most similar to Turkey Creek, these results are typical of systems with high nutrient loading. Straight pipes are a suspected problem on Crummies Creek

(Dave Harmon, pers. com.). This site also supported other organisms that are tolerant of organically enriched streams (e.g. *Chironomus* sp. and *Poypedilum scalaenum* gp.). We agree with the SQT assessment that the benthos response is due to factors other than sediment toxicity.

ROARING PAUNCH

The chemical analysis of sediment at Roaring Paunch indicated high levels of iron (see Appendix C). Sediment toxicity test results indicated no acute toxicity, but chronic toxicity was evident. The SEM/AVS molar ratio at Roaring Paunch was 3.9. The AVS-SEM model predicts that metal toxicity is likely. Macroinvertebrate data (Table 3) indicated Roaring Paunch was dominated by midges, *Polypedilum scalaenum* group, *Tribelos jucundum*, *Stictochironomus devinctus* and *Polypedilum halterale* group. Mean taxa richness was 6.00. Statistically, this site is no different from Crummies Creek or Laurel Fork, although the cluster analysis indicates the community structure is unlike the other sites in the study. Since this site was within 50 meters of the confluence of the study stream and the Big South Fork Cumberland River, the community may be influenced by the latter more than the former. We agree with the conclusion of the SQT that pollutants may be stressing the system.

CONCLUSIONS

In retrospect, the Cranks Creek Reservoir sites should not have been included in this study. The lack of comparable controls leaves the macroinvertebrate data open to speculation. Otherwise, the SQT methodology is a viable tool for assessing sediment impairment. There is need for improvement. It is apparent that more robust methods need to be developed to integrate sediment chemistry, sediment toxicity and the sediment biotic components. The chronic 28-day test should include multiple replicates to examine statistical significance with controls, as well as utilizing an end point other than mortality (e.g. growth) to detect more subtle chronic effects.

Other descriptors of the macroinvertebrate community need to be investigated or developed to differentiate between ecoregion and subecoregion variability. Similarity Indices or an index involving tolerance values may prove useful in this regard. Larger sample sizes may reduce sample variance. Moreover, a larger database restricted to sediment dwelling organisms, could greatly improve diagnostic efficiency. Clean, least impacted sediment should be vigorously examined in order to produce baseline data to provide a benchmark. Also, a particle analysis of the sediment collected at each site could shed light on between site variance. Another issue of concern was the occurrence of other organisms in the 28 day tests. It is presumed that these indigenous taxa hatched from eggs deposited in the sediment before it was collected. This pool of potential competitors with test organisms could alter results.

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Appendix A. Financial and Administrative Close-out

1. List all outputs

Milestone	Result
Sample 8 sites	Samples 9 sites
Run Toxicity Tests	Run Toxicity Tests
Run Chemical Analysis	Run Chemical Analysis
Annual and Final Close Out Report	Report submitted

Additional outputs included macroinvertebrate identification.

2. Summarization of Budget expenditures

Project Budget

Budget Summary

	BMP Imp	Project Man.	Public Ed	Monitoring	Tech-ass	Other	Total
Personnel				$23,614.00			
Supplies			,				
Equipment							
Travel							
Contractual							
Operating Costs				$9,719.00			
Other							
TOTAL	$ 0	$0	$0	$33,333.00	$0	$0	$0

3. There was no equipment purchased with project funds.

4. There was one special condition.

The following project must have an approved quality assurance plan before water quality monitoring begins. The quality assurance plan is attached in Appendix B.

Appendix B. Quality Assurance / Quality Control Plan for Sediment Toxicity Testing To Monitor Sediments in the Upper Cumberland Watershed

Methods for Culturing and Conducting Toxicity Tests with *Hyalella azteca*

(Third Edition)

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DISCLAIMER

THE MENTION OF BRAND NAMES OR COMMERCIAL PRODUCTS DOES NOT CONSTITUTE ENDORSEMENT OR RECOMMENDATION FOR USE OF THE PRODUCT.

PREFACE

This document is a product of the educational pilot project sponsored by the Natural Resources and Environmental Protection Agency. The project provides incentive to employees to obtain additional education that will directly enhance their job duties. The graduate level course entitled "Special Problems in Biology" was specifically tailored to assist the Bioassay Section establish the basis for a sediment toxicity program. The main objectives of the course included culturing, sampling, testing, quality assurance, and statistical analysis. This document has been prepared as part of the quality assurance portion of the course work.

MODERATELY HARD RECONSTITUTED WATER

Purpose

The purpose of preparing moderately hard reconstituted water is to supply chemically defined water for maintaining test cultures and for conducting toxicity tests. This water is a standard synthetic dilution water prepared with deionized water and reagent grade chemicals specified in the EPA Methods Manual 600/R-94/024 (1994).

Equipment and Reagents

120 Liter Carboy
Aerator
Plastic tubing
Stir boxes (2)
2 liter jugs (2)
Scale
Meters (pH, DO, Temp., Conductivity)
Titration for Hardness and Alkalinity

NaHCO
$CaSO_4$
$MgSO_4$
$CaCl_2$
KCl
Deionized Water

Procedure

1. To prepare 100 liters of reconstituted water, place 75 liters of deionized water in the carboy.

2. Add 5 grams of $CaSO_4$ and 5 grams of $CaCl_2$ to a 2 liter aliquot of deionized water and mix on a stir plate until salts dissolve. Overnight stirring is recommended.

3. Add 3 grams of $MgSO_4$, 9.6 grams of $NaHCO_3$, and 0.4 grams of KCl to a second two liter aliquot of deionized water. And mix on a stir plate for at least 30 minutes. Assure all salts are dissolved prior to use.

4. Pour the two 2-liter aliquot containing the dissolved salts into the 75 liters of deionized water and fill the carboy to 100 liters with DI water.

5. Aerate the mixture for at least 24 hours prior to chemical analysis and use. Aerate the mixture for the duration of use not to exceed 14 days.

Quality Assurance

1. Temperature, conductivity, dissolved oxygen, hardness, alkalinity, and pH should be taken on each water batch. Each batch should be numbered and logged into the culture water log book along with meter readings.

Chemistry	**Acceptable Range**
Temperature	**23 + or - 1 degree**
Conductivity	**330 to 360**
Dissolved Oxygen	**below 40% unacceptable**
Hardness	**90-100 mg CaCO3/L**
Alkalinity	**50-70 mg CaCO3/L**
pH	**7.8 - 8.2**

2. If chemistries fail to meet the acceptable range, the water must be discarded and the procedure redone.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates (1994).

Section: SOP #2
Revision: 01
Date: 10/98

HYALELLA AZTECA CULTURE MAINTENANCE

Purpose

The quality of the health and reproduction of cultured organisms is essential to the quality of toxicity testing data. *Hyalella azteca* are cultured in-house to assure this quality and provide ease in organism availability.

Equipment and Reagents

Hyalella azteca
Plastic containers 2-8 liter capacity
Nitex screening
Aerators and air stones
Temperature control
Light timer
Screens size 35,50, and 60
Culture log book
Dechlorinated tap water
Culture Water (SOP #1)
Food (SOP #3)

Procedure

1. A mass culture is maintained in a 10 gallon aquarium using dechlorinated tap water. They are also kept in one section of the recirculating water system used for culturing fish. The Hyalella azteca cultured in the fish system are maintained as backup. They are fed the same as the fish (flakes and brine shrimp). The volume of water in the static aquarium is dependent on the number of organisms. The water level is maintained at about four to six inches deep.The system is a static system and is constantly aerated. Nitex screening is used as substrate.

2. Feed all Hyalella cultures daily. Static mass culture receives 15 ml of spirallina algae. YCT is fed every other day (15 ml). Feed on weekends as schedule allows.

3. Prior to feeding on Mon.,Wed., and Fri., do a half water change on the static cultures. Perform water changes by siphoning at least half the water from the aquarium while allowing the organisms to remain under water. Replenish with dechlorinated tap water.

4. Restart cultures with known age young every five to six months or as needed.

5. When young of a specific age range is needed for testing, collect adults and separated 24 hours prior to collecting the young. Collect young by placing a #60 screen under the #35 screen.

Maintain adults if needed, and culture young not longer than 14 days. Try to keep organisms underwater as much as possible during the separation process.

Quality Assurance

1. The organisms should appear healthy, behave normally, feed well, and have low mortality in cultures.

2.All feeding and water change activities are logged daily in the daily maintenance log book.

3. Monthly reference toxicant tests are performed to assure organism health.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates (1994).

HYALELLA AZTECA FOOD PREPARATION

Purpose

The preparation of quality food for the cultured organism is essential for the overall success of any testing procedure. This procedure assures quality food for culturing as well as toxicity testing.

Equipment and Reagents

Blender
Plastic Storage Containers
Scale
Weigh boats
Refrigerator
Yeast
Alfalfa loose or pelleted
Trout chow
DI water
Spirallina

Procedure

1. Combine five grams of trout chow in one liter of DI water. Aerate for seven days. Put in the refrigerator and allow to settle over night.

2. Combine 5 grams of alfalfa with one liter of DI water. Grind and allow to settle overnight.

3. Decant or siphon the supernatant from the alfalfa and the trout chow. Set aside.

4. Combine 5 grams of yeast and one liter of DI water. Stir.

5. Combine equal volumes of the supernatants and the yeast.

6. Store in small screw top plastic containers and freeze until needed.

Quality Assurance

1. Log date of food and batch number in log book to assure age.

Section: SOP #4
Revision: 02
Date: 10/01

SEDIMENT COLLECTION, HANDLING, AND STORAGE

Purpose

Sediment collection, handling and storage may change the physical, chemical, or biological characteristics of the sediment. This procedure has been established to maintain the sediment integrity of field collected samples.

Equipment and Reagents

Wildco hand core sediment sampler
Plastic ziplock baggies
Stainless steel bucket
Stainless steel spoon
Cooler
Plastic gloves
Hip boots or Chest waders
Ice
#10 screen

Procedure

1. Assess stream for acquiring a representative sample. Also assess where the sediment may be located, depth of sediment, flow patterns, older deposits, organic silts, different depositional areas.

3. Collect a representative sample. If collecting by boat, use dredge or core sampler. If wading the stream, collect sample with spoon. The total volume needed for testing is 800 ml. Collect at least 1 liter of sediment. Sediment should be collected with as little disruption as possible.

4. If the sediment is not collectable by the core sampler due to shallow depth or sandy texture, collect with stainless steel spoon and annotate collection description on the chain of custody.

5. Composite the samples in the stainless steel bucket and stir.

6. Screen sediment through a #10 screen to remove other organisms and large rocks.

7. Pour sediment into plastic Nalge low density polyethelene bottles. Keep overlying water to a minimum. Double bag sample for transport.

8. Store sample in cooler with ice for transport. Sample should be stored at 4 degrees C and should never be frozen.

9. Samples can be stored for up to 8 weeks at this temperature.

Quality Assurance

1. Once samples are collected, general safety precautions should be taken. Dispose of plastic and disposable gear, -wash hands and exposed areas with soap, and all equipment should be properly cleaned with soap, 10% nitric acid, and acetone.

2. Complete chain of custody for files.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates, and USGS. 1994 Guidelines for the Collecting and Processing of Samples of Stream Bed Sediment for the Analysis of of Trace elementsand Organic Contaminants for the National Water Quality Assessment Program. Open File Report # 94-458.

Section: SOP # 5
Revision: 01
Date: 04/97

HYALELLA AZTECA: WHOLE SEDIMENT TOXICITY TEST

Purpose

The purpose of this test is to detect through the use of cultured organisms, toxicity that may be found in whole sediments. This is a 10 day renewal test using *Hyalella azteca*. Overlying water is changed daily and organisms are fed daily. The endpoint monitored at the end of the test is survival.

Equipment and Reagents

Water flow through system
pH meter
DO meter
Conductivity meter
hardness and alkalinity titration
stir boxes
hardness and alkalinity buffers
thermometer
light board
light/magnifier
stainless steel spoon
disposable pipette
temperature controlled room with light timer
Data sheets
Glass dishes for organism recovery
Moderately hard reconstituted water (SOP # 1)
Hyponex potting soil
YCT
sediment samples
Hyalella azteca

Procedure

1. The test chamber allows for one set of controls and two testing sites (24 test chambers). This is a 10 day test, however the preparation starts at **day -1.** It is called this because 1 day prior to inserting the organisms the sediment must have time to reach optimum temperature as well as have time to settle. So with the sediment in front of you, probably straight from the cooler, put the sediment in a clean stainless steel pan. Any pore water that has separated from the sediment should be reincorporated back into the sediment. Allow for as little sediment disruption as possible.

2. 100 ml of sediment should be added to each cup. There will be 8 cups per test. Prepare control dilutions by using potting soil (100 ml also).

3. 175 ml of moderately hard reconstituted water (SOP#1) should be added to the test cups. This includes controls.

4. **Day 0**. Measure water quality by removing with a syringe enough water to measure pH, conductivity, alkalinity, hardness, DO, and temperature. Take an equal volume from each replicate. This will be approximately 10 ml for a total volume of 80 ml. This should be enough volume for meters.

5. Do water exchange by pouring 2100 ml of moderately hard reconstituted water into each pan of the flow through system. This allows for a complete water exchange in each test chamber. 6 test chambers x 175 ml x 2 = 2100 ml. Water exchange should cause minimal sediment disruption. Assure all syringes and cups are lined up.

6. Transfer organisms. Put 10 organisms in each test chamber. Transfer by keeping under water at all times.

7. Feed 1.5 ml of yct daily after each water exchange.

8. **Day 1-9.** Measure DO and temperature daily by technique described in step 4. Renew overlying water daily with a two volume water exchange and feed 1.5 ml of YCT. Log measurements on log sheets and note any observations in regard to organism activity.

9. **Day 10.** Measure temperature and DO. End the test by collecting organisms. Count and mark data sheet. Try to spend the same amount of time collecting organisms on each cup. If the hyalella are dead there will little to no trace. This can lead to excessive amounts of time being spend on retrieval.

10. Test conditions, general activity schedule, and test acceptability requirements from EPA publication 600/R-94/024 (1994) are included as Appendix # 1.

11. After test has ended, dispose of test chambers and test organisms.

12. Results from data sheets can be entered on TOXCALC computer program for statistical analysis and results.

Quality Assurance

1. 96 hour reference toxicant tests are performed on a monthly basis.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates (1994).

96 HOUR REFERENCE TOXICANT TEST USING *HYALELLA AZTECA*

Purpose

Reference toxicity test are performed to assure the quality of the organisms used in toxicity testing. These tests help evaluate the sensitivity and health of the organisms by exposing them to a known toxicant. This is a 96 hour static test.

Equipment and Reagents

Hyalella azteca
30 ml test cups
Brood boards
scales
pH meter
conductivity meter
DO meter
thermometer
data sheets
alkalinity and hardness titrator and buffers
volumetric flasks
gallon jugs

Sodium chloride
moderately hard reconstituted water
YCT

Procedure

1. Mix NaCl solutions in 4.5 g/l, 5.0 g/l, 5.5 g/l, 6.0g/l, and 6.5 g/l. Mix a liter of each solution. Check conductivity chart to assure correct range.

2. Check water quality at beginning and end of test. This includes pH, conductivity, alkalinity, DO, hardness, and temperature.

3. Set up 4 cups per dilution.

4. Insert 10 organism per cup. Assure transfer occurs under water .

5. Feed organisms 0.5 ml YCT per cup.

6. Measure temperature daily and count organisms.

7. Feed again on day 2.

8. Day 4 measure water quality and count organisms. Dispose of survivors. It is a thankless job.

9. Enter data from data sheets into TOXCALC computer system. Log results.

10. Recommended test conditions from EPA publication 600/R-94/024 (1994) are included as Appendix two.

Quality Assirance

1. Monthly reference toxicant tests are performed on a monthly basis.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates (1994).

Appendix C. Sediment Chemical Analysis

Metals/Organics	Indian Creek	Crummies Creek	Laurel Fork Control Site
	mg/kg	mg/kg	mg/kg
Oil and Grease (HEM)	236	746	267
Organic Carbon	3190	9730	4570
Aluminum	2510	7130	2830
Barium	44.9	91.7	42.1
Cadmium	0.253	**0.794**	**0.74**
Calcium	48,800	14,500	623
Chromium	5.33	10.5	7.19
Cobalt	5.35	15.6	6.83
Copper	5.71	**21.5**	5.13
Iron	7,510	**24,800**	9,770
Lead	6.34	13.2	8.42
Magnesium	2,930	3310	377
Manganese	290	**951**	409
Nickel	14.6	**24.7**	14.4
Potassium	471	974	372
Sodium	53.7	519	31.4
Strontium	81.9	63.9	3.46
Tin	ND	ND	1.82
Vanadium	4.61	13.1	7.15
Zinc	46.8	87.6	36.3
Arsenic	2.18	4.51	2.5
Mercury	0.0272	0.0726	0.0227
Selenium	ND	0.725	0.147
bis(2-Ethylhexyl)phthalate	ND	**63.2**	ND

Bold parameters have established toxic limits; highlighted values meet or exceed those limits.

Metals/Organics	Turkey Creek	Bark Camp Creek Control Site	Roaring Paunch
	mg/kg	mg/kg	mg/kg
Oil and Grease (HEM)	473	151	125
Organic Carbon	3610	2920	3,510
Aluminum	7680	2620	3,030
Barium	72.6	36.4	27.2
Cadmium	ND	ND	ND
Calcium	18900	449	431
Chromium	8.73	2.46	3.56
Cobalt	7.43	3.25	3.95
Copper	7.04	1.66	4.42
Iron	15,100	5,790	**23,300**
Lead	8.7	5.34	4.89
Magnesium	2,760	246	253
Manganese	**501**	103	264
Nickel	10.7	4.22	9.26
Potassium	818	175	172
Sodium	42.7	15	20.5
Strontium	33.5	2.16	3.26
Tin	ND	0.413	0.821
Vanadium	12.8	2.98	3.02
Zinc	40.7	17.3	29
Arsenic	3.93	1.05	2.5
Mercury	0.0554	.0 252	0.0342
Selenium	ND	0.224	0.229
bis(2-Ethylhexyl)phthalate	0.397	0.295	0.298

Bold parameters have established toxic limits; highlighted values meet or exceed those limits.

Metals/Organics	Little Clear Creek	Cranks Creek Reservoir	Cranks Creek
	mg/kg	mg/kg	mg/kg
Oil and Grease (HEM)	743	1,100	284
Organic Carbon	5490	10,400	9,020
Aluminum	5850	9810	9,810
Barium	58.4	85.2	86.8
Cadmium	ND	1.03	1.21
Calcium	14,200	1390	1430
Chromium	8	13.5	13.5
Cobalt	10.1	16.3	19.8
Copper	14.9	23.2	**21.8**
Iron	**20,400**	31,200	**36,100**
Lead	10.4	15.3	15.4
Magnesium	2560	2,960	2,650
Manganese	**524**	409	747
Nickel	15	26.6	**31**
Potassium	780	1,100	1,090
Sodium	51.4	72.3	94.3
Strontium	21.6	14.9	15.1
Tin	0.513	1.09	0.439
Vanadium	10.5	15.9	16.5
Zinc	57.8	106	117
Arsenic	2.86	4.42	4.89
Mercury	0.0455	0.487	0.0478
Selenium	0.354	0.378	0.398
bis(2-Ethylhexyl)phthalate	0.401	**45**	0.233

Bold parameters have established toxic limits; highlighted values meet or exceed those limits.

Appendix D. Toxicity Test Results

Toxicity Test Results	Indian Creek	Crummies Creek	Laurel Fork Control Site	Turkey Creek	Bark Camp Creek Control Site	Roaring Paunch	Little Clear Creek	Cranks Creek Reservoir	Cranks Creek
AVS/SEM Ratio	4.7	1.2	2.8	1.3	1.3	3.9	0.6	0.7	7.2
10 Day Toxicity	Yes	No		No		No	No	No	No
28 Day Toxicity	No	No		Yes		Yes	No	Yes	No

Appendix E. Macroinvertebrate Data

Indian Cr	Rep 1	Rep 2	Rep 3
TAXON			
Ephemera sp.	1	1	0
Dubiraphia sp.	3	2	2
Tanytarsus spp.	14	8	25
Parakiefferiella sp.	4	5	10
Polypedilum scalaenum gp.	1	1	0
UIW/OCS	11	12	0
UIWCS	1	6	21
Lumbriculidae	1	1	0
Corbicula fluminea	0	1	3
Pisidium sp.	0	2	0
Chrysops sp.	0	1	0
Cryptochironomus sp.	0	1	0
Stempellinella sp.	0	4	0
Aquatic acari	0	0	1
Nematoda	0	0	1
Baetidae (e.i.)	0	0	1
Leptoceridae (e.i.)	0	0	1
Caenis sp.	0	0	1
Bezzia/Palpomyia	0	0	3
Dero nivea	0	0	1

Crummies Cr	Rep 1	Rep 2	Rep 3
TAXON			
Chironomus sp.	1	0	0
Paratendipes albimanus	1	1	0
Stictochironomus devinctus	1	7	0
Tanytarsus spp.	1	0	2
UIW/OCS	35	22	43
Gomphidae (e.i.)	0	1	0
Polypedilum scalaenum gp.	0	1	1
Nematomorpha	0	0	1
Procladius sp.	0	0	1
Bezzia/Palpomyia	0	0	1
Eclipidrilus sp.	0	0	1

Laurel Fk	Rep 1	Rep 2	Rep 3
Pisidium sp	1	0	0
Corbicula fluminea	1	1	0
Heptageniidae (e.i.)	1	0	0
Tanytarsus spp.	1	0	0
Dero nivea	1	0	0
UIW/OCS	6	61	3
UIWCS	1	0	0
Dubiraphia sp.	0	1	2
Nematoda	0	1	1
Paralauterborniella sp.	0	1	0
Polypedilum scalaenum gp.	0	1	0
Bezzia/Palpomyia	0	3	0
Tipulidae	0	2	0
Centroptilum sp.	0	0	2
Ceratopsyche sp.	0	0	1
Cladotanytarsus sp.	0	0	3

*UIW/OCS = unidentified immature oligochaete without capilliform setae; UIWCS = unidentified immature oligochaete with capilliform setae

Roaring Paunch TAXON	Rep 1	Rep 2	Rep 3
Hydroptila sp.	2	14	0
Polypedilum halterale gp.	2	5	0
Tribelos jucundum	13	0	1
Procladius sp.	1	0	0
Polypedilum scalaenum gp.	13	1	1
Stictochironomus devinctus	6	2	0
Dubiraphia sp.	0	1	0
Atherix sp.	0	1	0
Djamabatista sp.	0	1	0
Tanytarsus spp.	0	1	0
Nilothauma sp.	0	1	0
UIW/OCS	0	1	0
Gomphidae	0	0	1

Bark Camp Cr	Rep 1	Rep 2	Rep 3
Ephemera sp.	1	0	1
Stictochironomus devinctus	15	2	27
Microtendipes pedellus gp.	2	0	0
UIW/OCS	4	0	0
Corynoneura sp.	0	1	0
Tanytarsus spp.	0	1	0
Polypedilum scalaenum gp.	0	0	1
Cladotanytarsus sp.	0	0	1

Turkey Cr	Rep 1	Rep 2	Rep 3
UIW/OCS	9	5	5
Bezzia/Palpomyia	1	2	0
UIWCS	0	1	0
Eclipidrilus sp.	0	1	0
Tanytarsus spp.	0	1	0
Parakiefferiella sp.	0	2	0

Little Clear Cr TAXON	Rep 1	Rep 2	Rep 3
Hexagenia sp.	1	0	0
Tricorythodes sp.	1	0	0
Hydra sp.	4	0	0
Dubiraphia sp.	17	3	5
Procladius sp.	8	1	6
Tanypus sp.	4	0	3
Ablabesmyia mallochi	3	0	0
Dicrotendipes sp.	1	0	0
Psectrocladius sp.	1	0	1
Stempellina sp.	1	0	0
Paralauterborniella sp.	7	0	1
Polypedilum halterale gp.	1	0	1
Tanytarsus spp.	3	0	1
Cladotanytarsus sp.	1	1	0
Labrundinea pillosella	1	0	0
UIW/OCS	22	2	43
UIWICS	12	1	0
Nais variabilis	3	0	0
Bezzia/Palpomyia	8	5	1
Bezzia sp.	3	0	0
Pristina sp.	1	0	0
Clinotanypus sp.	0	1	0
Aquatic acari	0	0	1
Sphaeriidae	0	0	1
Thienemannimyia gp.	0	0	1
Nematoda	0	0	1
Dero nivea	0	0	4

*UIW/OCS = unidentified immature oligochaete without capilliform setae; UIWCS = unidentified immature oligochaete with capilliform setae

Cranks Cr	Rep 1	Rep 2	Rep 3
TAXON			
Corbicul fluminea	44	29	24
Gyraulus parvus	1	0	1
Polypedilum halterale gp.	2	0	0
Tanytarsus spp.	10	16	4
Ablabesmyia mallochi	2	6	6
Dicrotendipes sp.	2	3	1
Cladotanytarsus sp.	1	0	0
Polypedilum scalaenum gp.	1	0	0
Cryptotendipes sp.	2	2	0
Psuedochironomus sp.	1	1	0
Bezzia/Palpomyia	1	0	0
Ablabesmyia annulata	1	0	0
Lumbriculidae	3	0	0
UIW/OCS	39	26	6
Branchiura sowerbyi	6	1	2
Hexagenia sp.	0	1	0
Sphaeriidae	0	7	0
Centroptilum sp.	0	2	3
Procladius sp.	0	1	1
Cryptochironomus sp.	0	2	1
Cricotopus bicinctus	0	1	0
Stictochironomus devinctus	0	1	0
Enchytraeidae	0	1	0
Progomphus obscurus	0	0	1
Caenis sp.	0	0	1
Paracladopelma sp.	0	0	4
Clinohelea sp.	0	0	1
Dasyhelea sp.	0	0	2
Parakiefferiella sp.	0	0	1
Dero trifida	0	0	1
Psectrocladius sp.	0	0	2

Cranks Cr Res	Rep 1	Rep 2	Rep 3
TAXON			
Utterbackia imbecilus	1	0	0
Epicordulia princeps	1	1	0
Ischnura sp.	1	0	0
Caenis sp.	5	7	3
Leptoceridae (e.i.)	1	0	1
Gyraulus parvus	2	0	0
Branchiura sowerbyi	24	14	13
Labrundinea neopilosella.	2	0	1
Ablabesmyia idei	2	1	2
Procladius sp.	1	1	1
Paratanytarsus sp.	1	1	2
Dicrotendipes sp.	2	3	0
Polypedilum halterale gp.	2	3	0
Tanytarsus spp.	2	8	4
Dero trifida	2	4	16
UIW/OCS	2	1	1
Nematoda	1	1	0
Corbicul fluminea	0	2	0
Hexagenia sp.	0	1	0
Sphaeriidae	0	20	3
Enallagma sp.	0	1	2
Leptophlebiidae	0	1	0
Dubiraphia sp.	0	3	0
Orthotrichia sp.	0	6	8
Clinotanypus sp.	0	1	1
Parachironomus hirtalatus	0	1	3
Aulodrilus pigueti	0	11	0
Bezzia sp.	0	1	0
Bezzia/Palpomyia	0	3	0
Labrundinea sp. 4 (Epler)	0	0	1
Dasyhelea sp.	0	0	1

*UIW/OCS = unidentified immature oligochaete without capilliform setae; UIWCS = unidentified immature oligochaete with capilliform setae

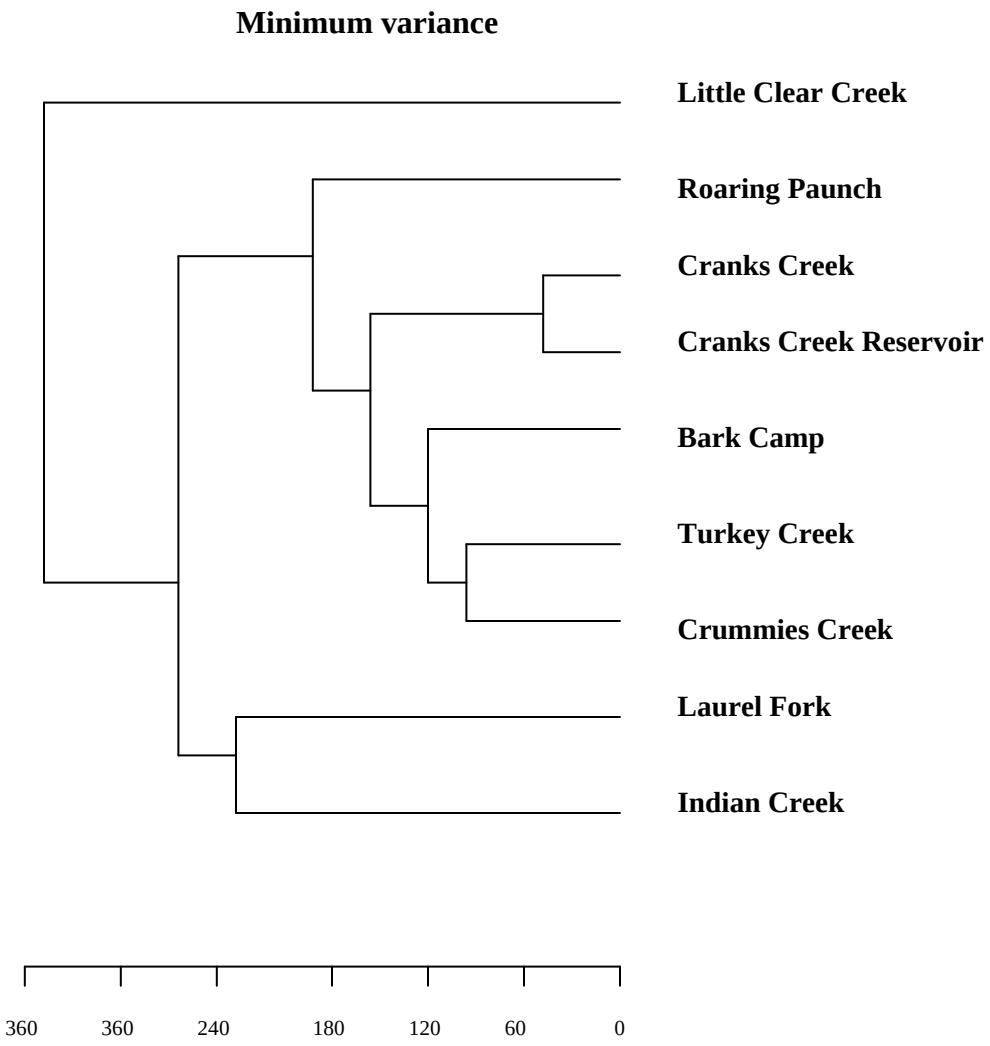

Figure 1: Results From URGMA Cluster Analysis

Project Final Report

Grant # C9994861

EVALUATION OF SEDIMENT TOXICITY AND MACROINVERTEBRATE COMMUNITIES AT SELECTED SITES IN THE UPPER CUMBERLAND RIVER (KENTUCKY, USA)

Workplan # 9908

Project Period September 2000-October 2001

This document is submitted by
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Funding for this project (EVALUATION OF SEDIMENT TOXICITY AND MACROINVERTEBRATE COMMUNITY AT SELECTED SITES IN THE UPPER CUMBERLAND RIVER, KENTUCKY, USA) was provided for in part by the U.S. Environmental Protection agency (USEPA) through the Kentucky Division of Water, Nonpoint Source Section, to the Bioassay Section within the Division of Water as authorized by the Clean Water Act Amendments of 1987, 319(h), Nonpoint Source Implementation Grant # C9994861-99. The contents of this document do not necessarily reflect the views and policy of the USEPA, KDOW nor does the mention of trade names or commercial products constitute endorsement. This document was printed on recycled paper.

ACKNOWLEDGMENTS

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EXECUTIVE SUMMARY

Sediment is an integral part of aquatic ecosystems. Resource extraction can adversely affect sediment quality through point and nonpoint source runoff. The objective of this study was to investigate the feasibility of utilizing the benthic macroinvertebrate community, sediment toxicity tests, and sediment chemistry in assessing selected aquatic systems in the Upper Cumberland River Basin that have drained areas of coal mining activity.

Sediment sampling was conducted in the fall of 2000 on seven test sites and two control sites. Quantitative benthic macroinvertebrate samples were collected concurrently with sediment used for chemical analysis and toxicity testing. The sediment was analyzed for metals, selected organic compounds, acid volatile sulfides, and total organic carbon. Both 10-day acute and 28-day chronic toxicity tests were performed using *Hyalella azteca*. Macroinvertebrate data were subjected to parametric and nonparametric analyses.

Six of the seven sites were found to have high levels of contaminants. Only one site exhibited acute toxicity and three showed evidence of chronic effects. Two sites had impaired benthic communities. However, when the results from the chemical analysis, toxicity tests and benthic investigation were considered in a Sediment Quality Triad (SQT) assessment methodology, only one site was determined to show evidence of contaminant induced degradation. Contaminants were considered unavailable at two sites and the benthic response at two sites was not attributable to contaminants. One site was determined to exhibit no contaminant degradation. The remaining site had contaminants available but the macroinvertebrate community response was unaffected by sediment chemistry.

The SQT assessment methodology was a good tool to investigate sediment contamination. More statistically robust and impact sensitive methods of performing chronic toxicity tests should be developed to increase assessment accuracy. Macroinvertebrate sampling strategies should consider ecoregion/subecoregion variability, as well as techniques for reducing within and between site sampling error.

The Bioassay Section gained valuable experience in toxicity testing from this project. The use of the triad approach as an assessment tool was very valuable. It enabled us to look at AVS/SEM (Model), benthic invertebrate data, chemistry and compare these data with our usual toxicity testing. This project led to the discovery of a better protocol for the 28-day toxicity test and it will be used in our next round of testing. We now realize we should have completed a particle size analysis. We gained valuable field experience and expanded our toxicity/chemical database.

INTRODUCTION

The Upper Cumberland River Basin in southeastern Kentucky contains some of the Commonwealths most valuable water resources. The most widespread water quality problem for the Upper Cumberland watershed is associated with the coal mining industry. Point and non-point sources of acid mine drainage from coal deposits, which contain large quantities of sulfides and sulfates, contribute to the problem. Oxidation of iron disulfide exposed by mining initiates the formation of water soluble acidic pollutants which cause the most objectionable characteristics of coal mine drainage. The most significant water quality problem associated with mining occurs when dissolved, suspended, or other solid mineral wastes and debris from mining or mine related operations enter streams, watercourses or ground water. Mine drainage includes not only water flowing by gravity or pumped from underground mines, but also runoff or seepage from surface and strip mines, excavated waste deposits and haul roads. (KDOW, 1975). Resource extraction is a major source of chemically altered sediments (Burton, 1992).

Sediment associated with aquatic systems play a vital ecological role. They serve as a potential reservoir for organic material as well as a sink for industrial contaminants (Call, et al. 2001). Contaminated sediment can be directly toxic to aquatic organisms. Additionally, non-lethal concentrations of chemicals sorbed to sediment can accumulate in benthic organisms and bioaccumulate up the food web. The goal of this study was to document sediment contaminant concentrations, measure sediment toxicity and evaluate negative impacts to the associated macroinvertebrate communities.

Seven potentially impacted sites were selected using data from the Kentucky Nonpoint Source Assessment Report (1999) and two KDOW reference reach sites were selected to provide control sediment. Sediment and macroinvertebrate samples were taken concurrently at each site in the Fall of 2000. Each site was analyzed for toxicity, total organic carbon content, and concentrations of metals, pesticides, and polychlorinated biphenyls (PCBs). Analysis of the benthic communities included identification of taxa to the lowest positive level, calculation of selected metrics, parametric, and nonparametric tests. The primary objective of the analysis was to identify possible toxic materials, evaluate the level of toxicity and relate these findings to the benthic biota.

METHODS AND MATERIALS

All sites were located within the Eastern Kentucky Coalfield Region (Table 1). Material taken from Cranks Creek was collected from two sites located in Cranks Creek Reservoir. The remaining sites were located in the free flowing portions of each stream. At each site, one composited sediment sample was collected and split for chemical analysis and toxicity testing. Three replicate samples were collected for macroinvertebrate monitoring.

Table 1. Site locations used in 10-day acute and 28-day chronic sediment toxicity test.

Water Body	GPS coordinates	Mile Point	Sample date
Turkey Creek Bell County	N 36d 46 m 06.3 s W 084d 02m 28.3s	**0.3**	**10/31/00**
Little Clear Creek Bell County	N36d 42m 31.8s W083d 44m 29.3s	**0.8**	**10/31/00**
Cranks Creek Harlan County	N36d 04 m 50.2s W 83d 12m 40.7s	**3.0**	**9/20/00**
Cranks Creek Reservoir Harlan County	N36d 44m 50.2s W083d 12m 55.5s	**3.2**	**9/20/00**
Indian Creek Jackson County	N 37d 22m 59.7s W084d 02m 28.3s	**1.5**	**9/19/00**
Crummies Creek Harlan County	N36d 47m 26.8s W083d 13m 02.2s	**1.6**	**9/20/00**
Roaring Paunch McCreary County	N36 d 40m 48.3s W084d 32m 28.0s	**0.1**	**10/30/00**
Laurel Fork* Jackson County	N84d 02m 28.9 s W37d 22m 11.5s	**1.0**	**9/19/00**
Bark Camp Creek* Laurel County	N36d 54m 14.8s W84d 16m 52.2s	**2.5**	**10/30/00**

* = Control site

DESCRIPTION OF MACROINVERTEBRATE ASSESSMENT

The macroinvertebrate samples were collected from the same depositional areas as the rest of the sediment used in the study. The benthic samples taken at Cranks Creek Reservoir were collected with a Petite Ponar dredge. Stream samples were taken by inverting a glass specimen jar into the sediment. All benthic samples were sieved through 300mNitex netting in the field. The samples were then preserved in 70 % ethanol and transported to the lab for identification. To reduce sample processing time, samples were subjected to sucrose flotation (Flannagan, 1973). Each sample was examined separately using a stereo-dissecting microscope with magnification to 75X.

Invertebrates were hand picked from samples, identified to the lowest positive taxonomic level and enumerated. Organisms requiring slide preparation for identification, such as Oligochaeta and Chironomidae, were mounted in CMC-10 and dried. Identifications were then made using a phase-contrast compound microscope with magnification to 1000X.

Counts of organisms were converted to number per square meter. Mathematical manipulations included calculation of diversity (Shannon 1948) and evenness (Pielou 1966) values for each sample. In lieu of standard protocols for determining negatively impacted sediment, best professional judgement was used on a site by site basis to designate impaired sites. Raw macroinvertebrate data are in Appendix E.

Macroinvertebrate communities at each site were analyzed by cluster analysis using the Bray-Curtis dissimilarity index and the unweighted pair-group arithmetic averaging (UPGMA) clustering algorithm. These two techniques have been used frequently together and are considered to be robust at classifying distinct objects. Prior to clustering, we transformed taxa densities using $\log_{10}$ (x+1). The technique arranges all clusters into a hierarchy so that the relationships between the different groups are apparent. Cluster analysis produces a tree-like diagram called a dendrogram.

SEDIMENT CHEMICAL ANALYSIS

Sediment was collected in the field for chemical analysis according to protocols established by USGS (1994). The Kentucky Department of Environmental Services (DES) analyzed each sample for metals and selected organics (see Appendix C) to identify suspected toxic material. Chemical contamination is a concept that is not always clearly defined relative to sediment. Traditionally, higher concentrations of potential toxicants are thought to produce higher risks to the biota. However, this assumption is not always true. Some evaluation must be made to estimate the potential risk to aquatic life that the compound may have. The EPA defines sediment criteria as a specific level of protection from the adverse effects of sediment associated pollutants, for beneficial uses of the environment, biota, and human health. Sediment quality criteria are the numerical concentrations of individual chemicals, which are intended to be predictive of biological effects, protective of the presence of benthic organisms and applicable to the range of natural sediments from lakes and streams. A sediment criterion must relate to the level of harm that the contaminant possesses by specifying an appropriate level of protection. Only if the contaminant concentration is less than all of the available criteria can exposure to the sediment, or to organisms that inhabit the sediment, be considered to be without significant risk.

Sediment contaminants primarily consist of heavy metals and persistent organic compounds (New York DEC 1998). Sediment criteria for non-polar organic compounds (e.g. PCBs and PAHs) are derived using equilibrium partitioning method (USEPA, 2000). Sediment criteria for metals are derived from empirically derived values establish by the National Oceanic and Atmospheric Agency (Long and Morgan, 1991). Total organic carbon (TOC) values are important to normalize the bioavailability of organic toxicants between sites (USEPA, 2000).

Simultaneously extracted metals/ acid volatile sulfides analysis was performed by EN CHEM, Incorporated Laboratory in Madison, Wisconsin. Simultaneously extracted metals (SEM) and acid volatile sulfide (AVS) are operationally defined methods for the analysis of sulfide and associated metals in aquatic sediments. The SEM to AVS ratio has been used to clarify the results of bioassay tests of metal toxicants. Sulfide production occurs in organically enriched sediment under anaerobic conditions. Typically, sulfides are restricted to a few centimeters beneath the sediment surface, depending on the influx of organic matter. The AVS-SEM model, developed by DiToro (1985), recognized that AVS is a reactive pool of solid phase sulfide that is available to bond with certain metals and reduce free metal ion concentrations. The AVS-SEM model has been verified for six divalent metals in anoxic sediment (i.e. cadmium, copper, lead, nickel, mercury and zinc). If the molar ratio of toxic metals measured by SEM to AVS exceeds one, the metals are potentially bioavailable. A ratio less than one suggests that the metals in the sediment are non-toxic (ENCHEM SOP WCM-63, April 2000).

DESCRIPTION OF THE 10 DAY TOXICITY TEST

The 10-day sediment toxicity test was performed using *Hyalella azteca* to determine acute effects. Conditions for conducting the test are fully outlined in USEPA(1994). Results were analyzed using ToxCalc, Tidepool Scientific Software. Toxicity was determined when the test site results were statistically different from control sites. Results of all toxicity tests are in Appendix D.

DESCRIPTION OF THE 28 DAY TOXICITY TEST

In order to determine the existence of chronic effects in sediment collected for this study, a 28-day test was conducted. In the absence of standardized protocols for such experiments, KDOW Bioassay Section personnel developed their own procedures. Initially, 400 ml of field sediment were placed in each 2.5-gallon aquarium. It was overlain by 2 liters of moderately hard-reconstituted water. The sediment was allowed to settle overnight. Twenty-five *Hyalella azteca* were introduced into each aquarium on the following day. Organisms were less than 14 days old. The organisms were fed 5 ml YCT daily after a one-liter water exchange. Prior to water exchange, the overlying water was measured daily for temperature, pH, and dissolved oxygen. At test end, the sediment was poured out gently into a Pyrex glass dish. Organisms were removed by pipette. If they could not be found, the sediment was sieved through a Standard US #30 screen. All test and control sites had organisms other than *Hyalella azteca*, presumably from latent egg deposition which occurred prior to collection. The sediment was considered to be toxic if mortality was greater than 20 % of the control results.

As with any bioassay technique, the results take careful consideration. *Hyalella azteca* is an organism that is used in sediment toxicity tests because they have contact with the sediment and are easily cultured in the laboratory. Their sensitivity can not be considered representative of all benthic organisms. Additionally, the test has daily water exchanges utilizing moderately hard reconstituted water. The expression of toxicity in the laboratory may not accurately reflect problems in the field. The chemical composition of the synthetic water can moderate the temperature, dissolved oxygen, and pH of the sediment in consideration.

INTEGRATIVE ASSESSMENT

The overall assessment of sediment quality is contingent upon many factors. Possible variables include toxic material concentrations, the synergistic/antagonistic properties associated with the pollutants, sediment depth and oxygen levels, particle size and physical chemistry of the sediment, and temperature. Compounding problems include genetic selection of benthic organisms in low level, chronically polluted streams. Organisms that are typically sensitive to pollution can appear to be quite tolerant (Burton, 1992). It has also been found that the testing process itself can release bound contaminants, yielding different results in the laboratory than are actually found in the field.

To increase the accuracy of identifying chemically altered sediments Chapman (1990), developed the Sediment Quality Triad (SQT). An SQT incorporates benthic community parameters, sediment chemistry and sediment toxicity into the overall assessment process. The

utilization of a multiple component system to evaluate sediment quality reduces the risk of misrepresenting actual conditions.

RESULTS AND DISCUSSION

Table 2 summarizes the results of the SQT and the possible conclusions that can be drawn. Results from sites located in Cranks Creek should be considered with caution. Since both sites were lentic in nature, the macroinvertebrate results are not directly comparable with the lotic control sites. The results from Cranks Creek were not included in the parametric analysis. However, the data were included in the nonparametric cluster analysis. The one way analysis of variance (ANOVA) followed by Tukey's Test was unable to differentiate sites by mean total organisms. However, several sites were significantly different at $p = 0.05$ level utilizing mean taxa richness (Table 3).

Table 2. Triad evaluation of Upper Cumberland sites; Y = evidence of degradation; N = no evidence of degradation

Site	Chemistry	10 day toxicity	28 day toxicity	Benthos	Possible Conclusions
Turkey Creek	Y	N	Y	Y	Evidence of contaminant induced degradation
Little Clear Creek	Y	N	N	N	Contaminants not bioavailable
Cranks Creek	Y	N	N	N	Contaminants not bioavailable
Cranks Creek Reservoir	Y	N	Y	N	Toxic pollutants may be stressing the system
Indian Creek	N	Y	N	N	No evidence of contaminant degradation or other conditions causing response
Crummies Creek	Y	N	N	Y	Contaminants not available or response not due to chemistry
Roaring Paunch	Y	N	Y	N	Toxic pollutants may be stressing the system

Table 3. Descriptors of the invertebrate community of sediments at The Upper Cumberland River Basin stream sites: dominant taxa densities, percent compositions, and selected measures of community quality.

Little Clear Creek	no/m^2	%
UIW/OCS*	701532	36.8
Dubiraphia sp.	261765	13.7
Procladius sp.	146589	7.7
Bezzia/Palpomyia *sp.*	146589	7.7
UIWICS*	136118	7.1
Paralauterborniella sp.	83765	4.4
Tanypus sp.	73294	3.8
Hydra sp.	41882	2.2
Tanytarsus spp.	41882	2.2
Ablabesmyia mallochi	31412	1.6
Nais variabilis	31412	1.6
Bezzia sp.	31412	1.6
Psectrocladius sp.	20941	1.1
Polypedilum halterale gp.	20941	1.1
Cladotanytarsus sp.	20941	1.1
Mean Taxa Richness	14[c]	
Mean Total Organisms/m^2	73294[b]	
Mean Sample Diversity	2.81	
Mean Sample Eveness	0.77	

Crummies Creek	no/m^2	%
UIW/OCS*	1047167	82.6
Stictochironomus sp.	83773	6.6
Tanytarsus spp.	31415	2.5
Paratendipes albimanus	20943	1.7
Polypedilum scalaenum gp	20943	1.7
Mean Taxa Richness	5.67[d]	
Mean Total Organisms/m^2	1267072[b]	
Mean Sample Diversity	0.98	
Mean Sample Eveness	0.39	

Cranks Creek	no/m^2	%
Corbicul fluminea	21292	36.7
UIW/OCS*	13824	23.8
Tanytarsus spp.	6593	11.4
Ablabesmyia mallochi	3067	5.3
Branchiura sowerbyi	1971	3.4
Sphaeriidae	1541	2.7
Dicrotendipes sp.	1319	2.3
Centroptilum sp.	1096	1.9
Cryptotendipes sp.	874	1.5
Paracladopelma sp.	874	1.5
Mean Taxa Richness	19.67	
Mean Total Organisms/m^2	58007	
Mean Sample Diversity	3.43	
Mean Sample Eveness	0.8	

Cranks Creek Reservoir	no/m^2	%
Branchiura sowerbyi	11201	25.1
Sphaeriidae	5052	11.3
Dero trifida.	4830	10.8
Caenis sp.	3289	7.4
Orthotrichia sp.	3067	6.9
Aulodrilus pigueti	2415	5.4
Tanytarsus spp.	1319	3.0
Ablabesmyia idei	1096	2.5
Dicrotendipes sp.	1096	2.5
Polypedilum halterale gp.	1096	2.5
Parachironomus hirtalatus	874	2.0
Paratanytarsus sp.	874	2.0
UIW/OCS*	874	2.0
Bezzia/Palpomyia sp.	667	1.5
Dubiraphia sp.	667	1.5
Enallagma sp.	667	1.5
Labrundinea neopillosella	667	1.5
Procladius sp.	667	1.5
Mean Taxa Richness	16.33	
Mean Total Organisms/m^2	44643	
Mean Sample Diversity	2.87	
Mean Sample Eveness	0.71	

Roaring Paunch	no/m^2	%
Polypedilum scalaenum gp.	157059	27.8
Tribelos jucundum	146589	25.9
Stictochironomus devinctus	83765	14.8
Polypedilum halterale gp.	73294	13.0
Hydroptila sp.	20941	3.7
Procladius sp.	10471	1.9
Dubiraphia sp.	10471	1.9
Atherix sp.	10471	1.9
Djamabatista sp.	10471	1.9
Tanytarsus spp.	10471	1.9
Nilothauma sp.	10471	1.9
UIW/OCS*	10471	1.9
Gomphidae	10471	1.9
Mean Taxa Richness	6[d]	
Mean Total Organisms/m^2	565413[b]	
Mean Sample Diversity	2.17	
Mean Sample Eveness	0.9	

Bark Camp Creek	no/m^2	%
Stictochironomus devinctus	460707	78.6
UIW/OCS*	41882	7.1
Ephemera sp	20941	3.6
Microtendipes pedellus gp..	20941	3.6
Corynoneura sp.	10471	1.8
Tanytarsus spp.	10471	1.8
Polypedilum scalaenum gp.	10471	1.8
Cladotanytarsus sp.	10471	1.8
Mean Taxa Richness	3.67[a]	
Mean Total Organisms/m^2	586355[b]	
Mean Sample Diversity	1.16	
Mean Sample Eveness	0.64	

Turkey Creek	no/m^2	%
UIW/OCS*	198942	61.3
UIWCS*	52353	16.1
Mean Taxa Richness	3[a]	
Mean Total Organisms/m^2	324589[b]	
Mean Sample Diversity	0.99	
Mean Sample Eveness	0.45	

Indian Creek	no/m^2	%
Tanytarsus spp.	492168	30.9
UIWCS*	293207	18.4
UIW/OCS*	240848	15.1
Parakiefferiella sp.	198962	12.5
Dubiraphia sp.	73302	4.6
Corbicula fluminea	41887	2.6
Stempellinella sp.	41887	2.6
Bezzia/Palpomyia sp.	31415	2.0
Mean Taxa Richness	11.33[c]	
Mean Total Organisms/m^2	75795[b]	
Mean Sample Diversity	2.67	
Mean Sample Eveness	0.76	

Laurel Fork	no/m^2	%
UIW/OCS*	733017	73.7
Dubiraphia sp.	31415	3.2
Bezzia/Palpomyia sp.	31415	3.2
Cladotanytarsus sp.	31415	3.2
Corbicula fluminea	20943	2.1
Nematoda	20943	2.1
Tipulidae	20943	2.1
Centroptilum sp.	20943	2.1
Pisidium sp.	10472	1.1
Heptageniidae	10472	1.1
Tanytarsus spp.	10472	1.1
Dero nivea	10472	1.1
UIWCS*	10472	1.1
Paralauterborniella sp.	10472	1.1
Polypedilum scalenum gp.	10472	1.1
Ceratopsyche sp.	10472	1.1
Mean Taxa Richness	7[d]	
Mean Total Organisms/m^2	994808[b]	
Mean Sample Diversity	1.9	
Mean Sample Eveness	0.7	

Means with same letter are not significantly different (oneway ANOVA followed by Tukey's test; p = 0.05)

*UIW/OCS = unidentified immature oligochaete without capilliform setae; UIWCS = unidentified immature oligochaete with capilliform setae

TURKEY CREEK

The chemical analysis of sediment at Turkey Creek indicated high levels of manganese (see Appendix C). Based on sediment quality criteria, impact from manganese may be considered to be moderate. Sediment toxicity test results indicated no acute effects, but chronic effects were found. The SEM/AVS molar ratio at Turkey Creek was 1.3. Since none of the metals found in high concentration have been verified in the AVS-SEM model, their bioavailability is in question. Macroinvertebrate data (Table 3) indicate Turkey Creek was dominated by immature worms (UIW/OCS, probably *Limnodrilus* sp.). Mean taxa richness was 3.0. These results are typical of streams with high nutrient loads, although the absence of tolerant chironomids (e.g. *Chironomus* and *Dicrotendipes*) would suggest impairment other than organic enrichment. It is important to note that Turkey Creek had no other habitat available in the stream channel other than soft mud. The lack of a sand or sand/gravel constituent in the sediment could limit the number of organisms that can inhabit it. Nonetheless, we agree with the conclusion of the SQT that the impairment is due to (a) sediment contaminant(s).

LITTLE CLEAR CREEK

The chemical analysis of sediment at Little Clear Creek indicated high levels of magnesium, aluminum and iron (see Appendix C). Based on sediment quality criteria, impact from manganese and iron may be considered to be moderate. Sediment toxicity test results indicated that no acute or chronic effects were found. The SEM/AVS molar ratio at Little Clear Creek was 0.6. Therefore toxicity due to metals in the AVS/SEM model is unlikely. Like Turkey Creek, none of the metals found in high concentration have been verified in the AVS-SEM model. Macroinvertebrate data (Table 3) indicated Little Clear Creek was dominated by immature oligochaetes (UIW/OCS, probably *Limnodrilus* sp.). Mean taxa richness was 14.0, this value is statistically similar to Indian Creek. Cluster analysis (Figure 1), however, indicates that the community is unlike any of the rest of the study sites. We agree with the SQT conclusion that the sediment contaminants are not available.

CRANKS CREEK

The chemical analysis of sediment at Cranks Creek indicated high levels of copper, nickel and iron (see Appendix C). Based on sediment quality criteria, impact from copper, nickel and iron may be considered to be moderate. Sediment toxicity test results indicated that no acute or chronic effects were found. The SEM/AVS molar ratio at Cranks Creek was 7.2. The AVS-SEM model predicts that metal toxicity is likely, at least from copper and nickel. Macroinvertebrate data (Table 3) indicated Cranks Creek was dominated by the Asian clam, *Corbicula fluminea*, immature oligochaetes (UIW/OCS, probably *Limnodrilus* sp.) and midges of the genus *Tanytarsus*. Mean taxa richness was 19.67. We agree in part with the SQT assessment. Although sediment contaminants are available, they are not causing an impact. Unsurprisingly, the Cranks Creek sites were grouped together in the cluster analysis.

Figure 1 Page 9

CRANKS CREEK RESERVOIR

The chemical analysis of sediment at Cranks Creek Reservoir indicated high levels of copper, nickel, mercury and iron (see Appendix C). Based on sediment quality criteria, impact from copper, nickel, mercury and iron may be considered to be moderate. The SEM/ AVS molar ratio at Cranks Creek Reservoir was 0.6. The AVS-SEM model predicts that metal toxicity is unlikely, at least from mercury, copper and nickel. The ten day sediment toxicity test results indicated no acute effects, but chronic effects were found in the 28 day test. A high level of bis (2-Ethylhexyl) phthalate (45 mg/kg) was detected in the sediment chemistry analysis. Macroinvertebrate data (Table 3) indicated Cranks Creek Reservoir was dominated by the tubificid, *Branchiura sowerbyi*, sphaeriid clams and the naidid worm *Dero nivea*. Mean taxa richness was 16.33. It should be noted that this site was covered with a mat of the musk-grass *Chara* sp. and the depth of the site was 3 to 4 meters. The musk-grass supported many taxa not associated with sediment and inflated the overall taxa richness. The water depth probably lowered sediment temperature and created anoxic conditions. Both of these factors reduce sediment toxicity *in situ.* Conditions in the laboratory likely promoted sediment toxicity through increased temperature and aerobic conditions. We agree with the SQT assessment that the benthos response is not due to sediment contaminants.

INDIAN CREEK

The chemical analysis of sediment at Indian Creek indicated no high levels of metals or organics (see Appendix C). Sediment toxicity test results indicated acute effects were present but chronic toxicity was not found. The SEM/AVS molar ratio at Indian Creek was 4.7. The AVS-SEM model predicts that metal toxicity is likely, however no elevated levels of the model metals were found. Macroinvertebrate data (Table 3) indicated Indian Creek was dominated by midges of the genus *Tanytarsus*, aquatic worms (UIWCS and UIW/OCS) and the midge, *Parakiefferiella* sp. Mean taxa richness was 11.33. The cluster analysis indicated that this site was most similar in macroinvertebrate composition to the control site at Laurel Fork. We believe the result of the toxicity test is most likely a false positive. The SQT method of sediment assessment uses multiparameters in anticipation of these results. We agree with the SQT assessment that there is no evidence of contaminant degradation.

CRUMMIES CREEK

The chemical analysis of sediment at Crummies Creek indicated high levels of cadmium, copper, manganese, nickel and iron (see Appendix C). Based on sediment quality criteria, impacts from cadmium, copper, manganese, nickel and iron may be considered to be moderate. Sediment toxicity test results indicated that no acute or chronic toxicity was evident. The SEM/AVS molar ratio at Crummies Creek was 1.2. The AVS-SEM model predicts that metal toxicity is likely but the value is still low, at least from cadmium, copper and nickel. Macroinvertebrate data (Table 3) indicated Crummies Creek was dominated by the immature oligochaetes (UIW/OCS, probably *Limnodrilus* sp.). Mean taxa richness was 5.67. Cluster analysis indicated that this site was most similar to Turkey Creek, these results are typical of systems with high nutrient loading. Straight pipes are a suspected problem on Crummies Creek

(Dave Harmon, pers. com.). This site also supported other organisms that are tolerant of organically enriched streams (e.g. *Chironomus* sp. and *Poypedilum scalaenum* gp.). We agree with the SQT assessment that the benthos response is due to factors other than sediment toxicity.

ROARING PAUNCH

The chemical analysis of sediment at Roaring Paunch indicated high levels of iron (see Appendix C). Sediment toxicity test results indicated no acute toxicity, but chronic toxicity was evident. The SEM/AVS molar ratio at Roaring Paunch was 3.9. The AVS-SEM model predicts that metal toxicity is likely. Macroinvertebrate data (Table 3) indicated Roaring Paunch was dominated by midges, *Polypedilum scalaenum* group, *Tribelos jucundum*, *Stictochironomus devinctus* and *Polypedilum halterale* group. Mean taxa richness was 6.00. Statistically, this site is no different from Crummies Creek or Laurel Fork, although the cluster analysis indicates the community structure is unlike the other sites in the study. Since this site was within 50 meters of the confluence of the study stream and the Big South Fork Cumberland River, the community may be influenced by the latter more than the former. We agree with the conclusion of the SQT that pollutants may be stressing the system.

CONCLUSIONS

In retrospect, the Cranks Creek Reservoir sites should not have been included in this study. The lack of comparable controls leaves the macroinvertebrate data open to speculation. Otherwise, the SQT methodology is a viable tool for assessing sediment impairment. There is need for improvement. It is apparent that more robust methods need to be developed to integrate sediment chemistry, sediment toxicity and the sediment biotic components. The chronic 28-day test should include multiple replicates to examine statistical significance with controls, as well as utilizing an end point other than mortality (e.g. growth) to detect more subtle chronic effects.

Other descriptors of the macroinvertebrate community need to be investigated or developed to differentiate between ecoregion and subecoregion variability. Similarity Indices or an index involving tolerance values may prove useful in this regard. Larger sample sizes may reduce sample variance. Moreover, a larger database restricted to sediment dwelling organisms, could greatly improve diagnostic efficiency. Clean, least impacted sediment should be vigorously examined in order to produce baseline data to provide a benchmark. Also, a particle analysis of the sediment collected at each site could shed light on between site variance. Another issue of concern was the occurrence of other organisms in the 28 day tests. It is presumed that these indigenous taxa hatched from eggs deposited in the sediment before it was collected. This pool of potential competitors with test organisms could alter results.

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Appendix A. Financial and Administrative Close-out

1. List all outputs

Milestone	Result
Sample 8 sites	Samples 9 sites
Run Toxicity Tests	Run Toxicity Tests
Run Chemical Analysis	Run Chemical Analysis
Annual and Final Close Out Report	Report submitted

Additional outputs included macroinvertebrate identification.

2. Summarization of Budget expenditures

Project Budget

Budget Summary

	BMP Imp	Project Man.	Public Ed	Monitoring	Tech-ass	Other	Total
Personnel				$23,614.00			
Supplies			,				
Equipment							
Travel							
Contractual							
Operating Costs				$9,719.00			
Other							
TOTAL	$ 0	$0	$0	$33,333.00	$0	$0	$0

3. There was no equipment purchased with project funds.

4. There was one special condition.

The following project must have an approved quality assurance plan before water quality monitoring begins. The quality assurance plan is attached in Appendix B.

Appendix B. Quality Assurance / Quality Control Plan for Sediment Toxicity Testing To Monitor Sediments in the Upper Cumberland Watershed

Methods for Culturing and Conducting Toxicity Tests with *Hyalella azteca*

(Third Edition)

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THE MENTION OF BRAND NAMES OR COMMERCIAL PRODUCTS DOES NOT CONSTITUTE ENDORSEMENT OR RECOMMENDATION FOR USE OF THE PRODUCT.

PREFACE

This document is a product of the educational pilot project sponsored by the Natural Resources and Environmental Protection Agency. The project provides incentive to employees to obtain additional education that will directly enhance their job duties. The graduate level course entitled “Special Problems in Biology” was specifically tailored to assist the Bioassay Section establish the basis for a sediment toxicity program. The main objectives of the course included culturing, sampling, testing, quality assurance, and statistical analysis. This document has been prepared as part of the quality assurance portion of the course work.

MODERATELY HARD RECONSTITUTED WATER

Purpose

The purpose of preparing moderately hard reconstituted water is to supply chemically defined water for maintaining test cultures and for conducting toxicity tests. This water is a standard synthetic dilution water prepared with deionized water and reagent grade chemicals specified in the EPA Methods Manual 600/R-94/024 (1994).

Equipment and Reagents

120 Liter Carboy
Aerator
Plastic tubing
Stir boxes (2)
2 liter jugs (2)
Scale
Meters (pH, DO, Temp., Conductivity)
Titration for Hardness and Alkalinity

NaHCO
$CaSO_4$
$MgSO_4$
$CaCl_2$
KCl
Deionized Water

Procedure

1. To prepare 100 liters of reconstituted water, place 75 liters of deionized water in the carboy.

2. Add 5 grams of $CaSO_4$ and 5 grams of $CaCl_2$ to a 2 liter aliquot of deionized water and mix on a stir plate until salts dissolve. Overnight stirring is recommended.

3. Add 3 grams of $MgSO_4$, 9.6 grams of $NaHCO_3$, and 0.4 grams of KCl to a second two liter aliquot of deionized water. And mix on a stir plate for at least 30 minutes. Assure all salts are dissolved prior to use.

4. Pour the two 2-liter aliquot containing the dissolved salts into the 75 liters of deionized water and fill the carboy to 100 liters with DI water.

5. Aerate the mixture for at least 24 hours prior to chemical analysis and use. Aerate the mixture for the duration of use not to exceed 14 days.

Quality Assurance

1. Temperature, conductivity, dissolved oxygen, hardness, alkalinity, and pH should be taken on each water batch. Each batch should be numbered and logged into the culture water log book along with meter readings.

Chemistry	**Acceptable Range**
Temperature	**23 + or - 1 degree**
Conductivity	**330 to 360**
Dissolved Oxygen	**below 40% unacceptable**
Hardness	**90-100 mg CaCO3/L**
Alkalinity	**50-70 mg CaCO3/L**
pH	**7.8 - 8.2**

2. If chemistries fail to meet the acceptable range, the water must be discarded and the procedure redone.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates (1994).

Section: SOP #2
Revision: 01
Date: 10/98

HYALELLA AZTECA CULTURE MAINTENANCE

Purpose

The quality of the health and reproduction of cultured organisms is essential to the quality of toxicity testing data. *Hyalella azteca* are cultured in-house to assure this quality and provide ease in organism availability.

Equipment and Reagents

Hyalella azteca
Plastic containers 2-8 liter capacity
Nitex screening
Aerators and air stones
Temperature control
Light timer
Screens size 35,50, and 60
Culture log book
Dechlorinated tap water
Culture Water (SOP #1)
Food (SOP #3)

Procedure

1. A mass culture is maintained in a 10 gallon aquarium using dechlorinated tap water. They are also kept in one section of the recirculating water system used for culturing fish. The Hyalella azteca cultured in the fish system are maintained as backup. They are fed the same as the fish (flakes and brine shrimp). The volume of water in the static aquarium is dependent on the number of organisms. The water level is maintained at about four to six inches deep.The system is a static system and is constantly aerated. Nitex screening is used as substrate.

2. Feed all Hyalella cultures daily. Static mass culture receives 15 ml of spirallina algae. YCT is fed every other day (15 ml). Feed on weekends as schedule allows.

3. Prior to feeding on Mon.,Wed., and Fri., do a half water change on the static cultures. Perform water changes by siphoning at least half the water from the aquarium while allowing the organisms to remain under water. Replenish with dechlorinated tap water.

4. Restart cultures with known age young every five to six months or as needed.

5. When young of a specific age range is needed for testing, collect adults and separated 24 hours prior to collecting the young. Collect young by placing a #60 screen under the #35 screen.

Maintain adults if needed, and culture young not longer than 14 days. Try to keep organisms underwater as much as possible during the separation process.

Quality Assurance

1. The organisms should appear healthy, behave normally, feed well, and have low mortality in cultures.

2.All feeding and water change activities are logged daily in the daily maintenance log book.

3. Monthly reference toxicant tests are performed to assure organism health.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates (1994).

Section: SOP#3
Revision: 01
Date: 10/98

HYALELLA AZTECA FOOD PREPARATION

Purpose

The preparation of quality food for the cultured organism is essential for the overall success of any testing procedure. This procedure assures quality food for culturing as well as toxicity testing.

Equipment and Reagents

Blender
Plastic Storage Containers
Scale
Weigh boats
Refrigerator
Yeast
Alfalfa loose or pelleted
Trout chow
DI water
Spirallina

Procedure

1. Combine five grams of trout chow in one liter of DI water. Aerate for seven days. Put in the refrigerator and allow to settle over night.

2. Combine 5 grams of alfalfa with one liter of DI water. Grind and allow to settle overnight.

3. Decant or siphon the supernatant from the alfalfa and the trout chow. Set aside.

4. Combine 5 grams of yeast and one liter of DI water. Stir.

5. Combine equal volumes of the supernatants and the yeast.

6. Store in small screw top plastic containers and freeze until needed.

Quality Assurance

1. Log date of food and batch number in log book to assure age.

SEDIMENT COLLECTION, HANDLING, AND STORAGE

Purpose

Sediment collection, handling and storage may change the physical, chemical, or biological characteristics of the sediment. This procedure has been established to maintain the sediment integrity of field collected samples.

Equipment and Reagents

Wildco hand core sediment sampler
Plastic ziplock baggies
Stainless steel bucket
Stainless steel spoon
Cooler
Plastic gloves
Hip boots or Chest waders
Ice
#10 screen

Procedure

1. Assess stream for acquiring a representative sample. Also assess where the sediment may be located, depth of sediment, flow patterns, older deposits, organic silts, different depositional areas.

3. Collect a representative sample. If collecting by boat, use dredge or core sampler. If wading the stream, collect sample with spoon. The total volume needed for testing is 800 ml. Collect at least 1 liter of sediment. Sediment should be collected with as little disruption as possible.

4. If the sediment is not collectable by the core sampler due to shallow depth or sandy texture, collect with stainless steel spoon and annotate collection description on the chain of custody.

5. Composite the samples in the stainless steel bucket and stir.

6. Screen sediment through a #10 screen to remove other organisms and large rocks.

7. Pour sediment into plastic Nalge low density polyethelene bottles. Keep overlying water to a minimum. Double bag sample for transport.

8. Store sample in cooler with ice for transport. Sample should be stored at 4 degrees C and should never be frozen.

9. Samples can be stored for up to 8 weeks at this temperature.

Quality Assurance

1. Once samples are collected, general safety precautions should be taken. Dispose of plastic and disposable gear, -wash hands and exposed areas with soap, and all equipment should be properly cleaned with soap, 10% nitric acid, and acetone.

2. Complete chain of custody for files.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates, and USGS. 1994 Guidelines for the Collecting and Processing of Samples of Stream Bed Sediment for the Analysis of of Trace elementsand Organic Contaminants for the National Water Quality Assessment Program. Open File Report # 94-458.

Section: SOP # 5
Revision: 01
Date: 04/97

HYALELLA AZTECA: WHOLE SEDIMENT TOXICITY TEST

Purpose

The purpose of this test is to detect through the use of cultured organisms, toxicity that may be found in whole sediments. This is a 10 day renewal test using *Hyalella azteca*. Overlying water is changed daily and organisms are fed daily. The endpoint monitored at the end of the test is survival.

Equipment and Reagents

Water flow through system
pH meter
DO meter
Conductivity meter
hardness and alkalinity titration
stir boxes
hardness and alkalinity buffers
thermometer
light board
light/magnifier
stainless steel spoon
disposable pipette
temperature controlled room with light timer
Data sheets
Glass dishes for organism recovery
Moderately hard reconstituted water (SOP # 1)
Hyponex potting soil
YCT
sediment samples
Hyalella azteca

Procedure

1. The test chamber allows for one set of controls and two testing sites (24 test chambers). This is a 10 day test, however the preparation starts at **day -1.** It is called this because 1 day prior to inserting the organisms the sediment must have time to reach optimum temperature as well as have time to settle. So with the sediment in front of you, probably straight from the cooler, put the sediment in a clean stainless steel pan. Any pore water that has separated from the sediment should be reincorporated back into the sediment. Allow for as little sediment disruption as possible.

2. 100 ml of sediment should be added to each cup. There will be 8 cups per test. Prepare control dilutions by using potting soil (100 ml also).

3. 175 ml of moderately hard reconstituted water (SOP#1) should be added to the test cups. This includes controls.

4. **Day 0**. Measure water quality by removing with a syringe enough water to measure pH, conductivity, alkalinity, hardness, DO, and temperature. Take an equal volume from each replicate. This will be approximately 10 ml for a total volume of 80 ml. This should be enough volume for meters.

5. Do water exchange by pouring 2100 ml of moderately hard reconstituted water into each pan of the flow through system. This allows for a complete water exchange in each test chamber. 6 test chambers x 175 ml x 2 = 2100 ml. Water exchange should cause minimal sediment disruption. Assure all syringes and cups are lined up.

6. Transfer organisms. Put 10 organisms in each test chamber. Transfer by keeping under water at all times.

7. Feed 1.5 ml of yct daily after each water exchange.

8. **Day 1-9.** Measure DO and temperature daily by technique described in step 4. Renew overlying water daily with a two volume water exchange and feed 1.5 ml of YCT. Log measurements on log sheets and note any observations in regard to organism activity.

9. **Day 10.** Measure temperature and DO. End the test by collecting organisms. Count and mark data sheet. Try to spend the same amount of time collecting organisms on each cup. If the hyalella are dead there will little to no trace. This can lead to excessive amounts of time being spend on retrieval.

10. Test conditions, general activity schedule, and test acceptability requirements from EPA publication 600/R-94/024 (1994) are included as Appendix # 1.

11. After test has ended, dispose of test chambers and test organisms.

12. Results from data sheets can be entered on TOXCALC computer program for statistical analysis and results.

Quality Assurance

1. 96 hour reference toxicant tests are performed on a monthly basis.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates (1994).

Section: SOP # 6
Revision: 02
Date: 10/01

96 HOUR REFERENCE TOXICANT TEST USING *HYALELLA AZTECA*

Purpose

Reference toxicity test are performed to assure the quality of the organisms used in toxicity testing. These tests help evaluate the sensitivity and health of the organisms by exposing them to a known toxicant. This is a 96 hour static test.

Equipment and Reagents

Hyalella azteca
30 ml test cups
Brood boards
scales
pH meter
conductivity meter
DO meter
thermometer
data sheets
alkalinity and hardness titrator and buffers
volumetric flasks
gallon jugs

Sodium chloride
moderately hard reconstituted water
YCT

Procedure

1. Mix NaCl solutions in 4.5 g/l, 5.0 g/l, 5.5 g/l, 6.0g/l, and 6.5 g/l. Mix a liter of each solution. Check conductivity chart to assure correct range.

2. Check water quality at beginning and end of test. This includes pH, conductivity, alkalinity, DO, hardness, and temperature.

3. Set up 4 cups per dilution.

4. Insert 10 organism per cup. Assure transfer occurs under water .

5. Feed organisms 0.5 ml YCT per cup.

6. Measure temperature daily and count organisms.

7. Feed again on day 2.

8. Day 4 measure water quality and count organisms. Dispose of survivors. It is a thankless job.

9. Enter data from data sheets into TOXCALC computer system. Log results.

10. Recommended test conditions from EPA publication 600/R-94/024 (1994) are included as Appendix two.

Quality Assirance

1. Monthly reference toxicant tests are performed on a monthly basis.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates (1994).

Appendix C. Sediment Chemical Analysis

Metals/Organics	Indian Creek	Crummies Creek	Laurel Fork
			Control Site
	mg/kg	mg/kg	mg/kg
Oil and Grease (HEM)	236	746	267
Organic Carbon	3190	9730	4570
Aluminum	2510	7130	2830
Barium	44.9	91.7	42.1
Cadmium	0.253	**0.794**	**0.74**
Calcium	48,800	14,500	623
Chromium	5.33	10.5	7.19
Cobalt	5.35	15.6	6.83
Copper	5.71	**21.5**	5.13
Iron	7,510	**24,800**	9,770
Lead	6.34	13.2	8.42
Magnesium	2,930	3310	377
Manganese	290	**951**	409
Nickel	14.6	**24.7**	14.4
Potassium	471	974	372
Sodium	53.7	519	31.4
Strontium	81.9	63.9	3.46
Tin	ND	ND	1.82
Vanadium	4.61	13.1	7.15
Zinc	46.8	87.6	36.3
Arsenic	2.18	4.51	2.5
Mercury	0.0272	0.0726	0.0227
Selenium	ND	0.725	0.147
bis(2-Ethylhexyl)phthalate	ND	**63.2**	ND

Bold parameters have established toxic limits; highlighted values meet or exceed those limits.

Metals/Organics	Turkey Creek	Bark Camp Creek Control Site	Roaring Paunch
	mg/kg	mg/kg	mg/kg
Oil and Grease (HEM)	473	151	125
Organic Carbon	3610	2920	3,510
Aluminum	7680	2620	3,030
Barium	72.6	36.4	27.2
Cadmium	ND	ND	ND
Calcium	18900	449	431
Chromium	8.73	2.46	3.56
Cobalt	7.43	3.25	3.95
Copper	7.04	1.66	4.42
Iron	15,100	5,790	**23,300**
Lead	8.7	5.34	4.89
Magnesium	2,760	246	253
Manganese	**501**	103	264
Nickel	10.7	4.22	9.26
Potassium	818	175	172
Sodium	42.7	15	20.5
Strontium	33.5	2.16	3.26
Tin	ND	0.413	0.821
Vanadium	12.8	2.98	3.02
Zinc	40.7	17.3	29
Arsenic	3.93	1.05	2.5
Mercury	0.0554	.0 252	0.0342
Selenium	ND	0.224	0.229
bis(2-Ethylhexyl)phthalate	0.397	0.295	0.298

Bold parameters have established toxic limits; highlighted values meet or exceed those limits.

Metals/Organics	Little Clear Creek	Cranks Creek Reservoir	Cranks Creek
	mg/kg	mg/kg	mg/kg
Oil and Grease (HEM)	743	1,100	284
Organic Carbon	5490	10,400	9,020
Aluminum	5850	9810	9,810
Barium	58.4	85.2	86.8
Cadmium	ND	1.03	1.21
Calcium	14,200	1390	1430
Chromium	8	13.5	13.5
Cobalt	10.1	16.3	19.8
Copper	14.9	23.2	**21.8**
Iron	**20,400**	31,200	**36,100**
Lead	10.4	15.3	15.4
Magnesium	2560	2,960	2,650
Manganese	**524**	409	747
Nickel	15	26.6	**31**
Potassium	780	1,100	1,090
Sodium	51.4	72.3	94.3
Strontium	21.6	14.9	15.1
Tin	0.513	1.09	0.439
Vanadium	10.5	15.9	16.5
Zinc	57.8	106	117
Arsenic	2.86	4.42	4.89
Mercury	0.0455	0.487	0.0478
Selenium	0.354	0.378	0.398
bis(2-Ethylhexyl)phthalate	0.401	**45**	0.233

Bold parameters have established toxic limits; highlighted values meet or exceed those limits.

Appendix D. Toxicity Test Results

Toxicity Test Results	Indian Creek	Crummies Creek	Laurel Fork Control Site	Turkey Creek	Bark Camp Creek Control Site	Roaring Paunch	Little Clear Creek	Cranks Creek Reservoir	Cranks Creek
AVS/SEM Ratio	4.7	1.2	2.8	1.3	1.3	3.9	0.6	0.7	7.2
10 Day Toxicity	Yes	No		No		No	No	No	No
28 Day Toxicity	No	No		Yes		Yes	No	Yes	No

Appendix E. Macroinvertebrate Data

Indian Cr	Rep 1	Rep 2	Rep 3
TAXON			
Ephemera sp.	1	1	0
Dubiraphia sp.	3	2	2
Tanytarsus spp.	14	8	25
Parakiefferiella sp.	4	5	10
Polypedilum scalaenum gp.	1	1	0
UIW/OCS	11	12	0
UIWCS	1	6	21
Lumbriculidae	1	1	0
Corbicula fluminea	0	1	3
Pisidium sp.	0	2	0
Chrysops sp.	0	1	0
Cryptochironomus sp.	0	1	0
Stempellinella sp.	0	4	0
Aquatic acari	0	0	1
Nematoda	0	0	1
Baetidae (e.i.)	0	0	1
Leptoceridae (e.i.)	0	0	1
Caenis sp.	0	0	1
Bezzia/Palpomyia	0	0	3
Dero nivea	0	0	1

Crummies Cr	Rep 1	Rep 2	Rep 3
TAXON			
Chironomus sp.	1	0	0
Paratendipes albimanus	1	1	0
Stictochironomus devinctus	1	7	0
Tanytarsus spp.	1	0	2
UIW/OCS	35	22	43
Gomphidae (e.i.)	0	1	0
Polypedilum scalaenum gp.	0	1	1
Nematomorpha	0	0	1
Procladius sp.	0	0	1
Bezzia/Palpomyia	0	0	1
Eclipidrilus sp.	0	0	1

Laurel Fk	Rep 1	Rep 2	Rep 3
Pisidium sp	1	0	0
Corbicula fluminea	1	1	0
Heptageniidae (e.i.)	1	0	0
Tanytarsus spp.	1	0	0
Dero nivea	1	0	0
UIW/OCS	6	61	3
UIWCS	1	0	0
Dubiraphia sp.	0	1	2
Nematoda	0	1	1
Paralauterborniella sp.	0	1	0
Polypedilum scalaenum gp.	0	1	0
Bezzia/Palpomyia	0	3	0
Tipulidae	0	2	0
Centroptilum sp.	0	0	2
Ceratopsyche sp.	0	0	1
Cladotanytarsus sp.	0	0	3

*UIW/OCS = unidentified immature oligochaete without capilliform setae; UIWCS = unidentified immature oligochaete with capilliform setae

Roaring Paunch TAXON	Rep 1	Rep 2	Rep 3
Hydroptila sp.	2	14	0
Polypedilum halterale gp.	2	5	0
Tribelos jucundum	13	0	1
Procladius sp.	1	0	0
Polypedilum scalaenum gp.	13	1	1
Stictochironomus devinctus	6	2	0
Dubiraphia sp.	0	1	0
Atherix sp.	0	1	0
Djamabatista sp.	0	1	0
Tanytarsus spp.	0	1	0
Nilothauma sp.	0	1	0
UIW/OCS	0	1	0
Gomphidae	0	0	1

Bark Camp Cr	Rep 1	Rep 2	Rep 3
Ephemera sp.	1	0	1
Stictochironomus devinctus	15	2	27
Microtendipes pedellus gp.	2	0	0
UIW/OCS	4	0	0
Corynoneura sp.	0	1	0
Tanytarsus spp.	0	1	0
Polypedilum scalaenum gp.	0	0	1
Cladotanytarsus sp.	0	0	1

Turkey Cr	Rep 1	Rep 2	Rep 3
UIW/OCS	9	5	5
Bezzia/Palpomyia	1	2	0
UIWCS	0	1	0
Eclipidrilus sp.	0	1	0
Tanytarsus spp.	0	1	0
Parakiefferiella sp.	0	2	0

Little Clear Cr TAXON	Rep 1	Rep 2	Rep 3
Hexagenia sp.	1	0	0
Tricorythodes sp.	1	0	0
Hydra sp.	4	0	0
Dubiraphia sp.	17	3	5
Procladius sp.	8	1	6
Tanypus sp.	4	0	3
Ablabesmyia mallochi	3	0	0
Dicrotendipes sp.	1	0	0
Psectrocladius sp.	1	0	1
Stempellina sp.	1	0	0
Paralauterborniella sp.	7	0	1
Polypedilum halterale gp.	1	0	1
Tanytarsus spp.	3	0	1
Cladotanytarsus sp.	1	1	0
Labrundinea pillosella	1	0	0
UIW/OCS	22	2	43
UIWICS	12	1	0
Nais variabilis	3	0	0
Bezzia/Palpomyia	8	5	1
Bezzia sp.	3	0	0
Pristina sp.	1	0	0
Clinotanypus sp.	0	1	0
Aquatic acari	0	0	1
Sphaeriidae	0	0	1
Thienemannimyia gp.	0	0	1
Nematoda	0	0	1
Dero nivea	0	0	4

*UIW/OCS = unidentified immature oligochaete without capilliform setae; UIWCS = unidentified immature oligochaete with capilliform setae

Cranks Cr	Rep 1	Rep 2	Rep 3	Cranks Cr Res	Rep 1	Rep 2	Rep 3
TAXON				TAXON			
Corbicul fluminea	44	29	24	*Utterbackia imbecilus*	1	0	0
Gyraulus parvus	1	0	1	*Epicordulia princeps*	1	1	0
Polypedilum halterale gp.	2	0	0	*Ischnura* sp.	1	0	0
Tanytarsus spp.	10	16	4	*Caenis sp.*	5	7	3
Ablabesmyia mallochi	2	6	6	Leptoceridae (e.i.)	1	0	1
Dicrotendipes sp.	2	3	1	Gyraulus parvus	2	0	0
Cladotanytarsus sp.	1	0	0	*Branchiura sowerbyi*	24	14	13
Polypedilum scalaenum gp.	1	0	0	*Labrundinea neopilosella.*	2	0	1
Cryptotendipes sp.	2	2	0	*Ablabesmyia idei*	2	1	2
Psuedochironomus sp.	1	1	0	*Procladius* sp.	1	1	1
Bezzia/Palpomyia	1	0	0	*Paratanytarsus* sp.	1	1	2
Ablabesmyia annulata	1	0	0	*Dicrotendipes* sp.	2	3	0
Lumbriculidae	3	0	0	*Polypedilum halterale* gp.	2	3	0
UIW/OCS	39	26	6	*Tanytarsus* spp.	2	8	4
Branchiura sowerbyi	6	1	2	*Dero trifida*	2	4	16
Hexagenia sp.	0	1	0	UIW/OCS	2	1	1
Sphaeriidae	0	7	0	Nematoda	1	1	0
Centroptilum sp.	0	2	3	*Corbicul fluminea*	0	2	0
Procladius sp.	0	1	1	*Hexagenia* sp.	0	1	0
Cryptochironomus sp.	0	2	1	Sphaeriidae	0	20	3
Cricotopus bicinctus	0	1	0	*Enallagma* sp.	0	1	2
Stictochironomus devinctus	0	1	0	Leptophlebiidae	0	1	0
Enchytraeidae	0	1	0	*Dubiraphia* sp.	0	3	0
Progomphus obscurus	0	0	1	*Orthotrichia* sp.	0	6	8
Caenis sp.	0	0	1	*Clinotanypus* sp.	0	1	1
Paracladopelma sp.	0	0	4	*Parachironomus hirtalatus*	0	1	3
Clinohelea sp.	0	0	1	*Aulodrilus pigueti*	0	11	0
Dasyhelea sp.	0	0	2	*Bezzia* sp.	0	1	0
Parakiefferiella sp.	0	0	1	*Bezzia/Palpomyia*	0	3	0
Dero trifida	0	0	1	*Labrundinea* sp. 4 (Epler)	0	0	1
Psectrocladius sp.	0	0	2	*Dasyhelea* sp.	0	0	1

*UIW/OCS = unidentified immature oligochaete without capilliform setae; UIWCS = unidentified immature oligochaete with capilliform setae

Methods for Culturing and Conducting Toxicity Tests With *Hyalella Azteca*

(Third Edition)

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Natural Resources
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PREFACE

This document is a product of the educational pilot project sponsored by the Natural Resources and Environmental Protection Agency. The project provides incentive to employees to obtain additional education that will directly enhance their job duties. The graduate level course entitled “Special Problems in Biology” was specifically tailored to assist the Bioassay Section establish the basis for a sediment toxicity program. The main objectives of the course included culturing, sampling, testing, quality assurance, and statistical analysis. This document has been prepared as part of the quality assurance portion of the course work.

TABLE OF CONTENTS

MODERATELY HARD RECONSTITUTED WATER

Purpose

The purpose of preparing moderately hard reconstituted water is to supply chemically defined water for maintaining test cultures and for conducting toxicity tests. This water is a standard synthetic dilution water prepared with deionized water and reagent grade chemicals specified in the EPA Methods Manual 600/R-94/024.

Equipment and Reagents

120 Liter Carboy
Aerator
Plastic tubing
Stir boxes (2)
2 liter jugs (2)
Scale
Meters (pH, DO, Temp., Conductivity)
Titration for Hardness and Alkalinity

NaHCO
$CaSO_4$
$MgSO_4$
$CaCl_2$
KCl
Deionized Water

Procedure

1. To prepare 100 liters of reconstituted water, place 75 liters of deionized water in the carboy.

2. Add 5 grams of $CaSO_4$ and 5 grams of $CaCl_2$ to a 2 liter aliquot of deionized water and mix on a stir plate until salts dissolve. Overnight stirring is recommended.

3. Add 3 grams of $MgSO_4$, 9.6 grams of $NaHCO_3$, and 0.4 grams of KCl to a second two liter aliquot of deionized water. And mix on a stir plate for at least 30 minutes. Assure all salts are dissolved prior to use.

4. Pour the two 2-liter aliquot containing the dissolved salts into the 75 liters of deionized water and fill the carboy to 100 liters with DI water.

5. Aerate the mixture for at least 24 hours prior to chemical analysis and use. Aerate the mixture for the duration of use not to exceed 14 days.

Quality Assurance

1. Temperature, conductivity, dissolved oxygen, hardness, alkalinity, and pH should be taken on each water batch. Each batch should be number and logged into the culture water log book along with meter readings.

Chemistry	**Acceptable Range**
Temperature	**23 + or - 1 degree**
Conductivity	**330 to 360**
Dissolved Oxygen	**below 40% unacceptable**
Hardness	**90-100 mg CaCO3/L**
Alkalinity	**50-70 mg CaCO3/L**
pH	**7.8 - 8.2**

2. If chemistries fail to meet the acceptable range, the water must be discarded and the procedure redone.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates.

HYALELLA AZTECA CULTURE MAINTENANCE

Purpose

The quality of the health and reproduction of cultured organisms is essential to the quality of toxicity testing data. *Hyalella Azteca* are cultured in-house to assure this quality and provide ease in organism availability.

Equipment and Reagents

Hyalella azteca
Plastic containers 2-8 liter capacity
Nitex screening
Aerators and air stones
Temperature control
Light timer
Screens size 35,50, and 60
Culture log book
Dechlorinated tap water
Culture Water (SOP #1)
Food (SOP #3)

Procedure

1. A mass culture is maintained in a 10 gallon aquarium using dechlorinated tap water. They are also kept in one section of the recirculating water system used for culturing fish. The hyalella azteca cultured in the fish system are maintained as backup. They are fed the same as the fish (flakes and brine shrimp). The volume of water in the static aquarium is dependent on the number of organisms. The water level is maintained at about four to six inches deep.The system is a static system and is constantly aerated. Nitex screening is used as substrate.

2. Feed all Hyalella cultures daily. Static mass culture receives 15 ml of spirallina algae. YCT is fed every other day (15 ml). Feed on weekends as schedule allows.

3. Prior to feeding on Mon.,Wed., and Fri., do a half water change on the static cultures. Perform water changes by siphoning at least half the water from the aquarium while allowing the organisms to remain under water. Replenish with dechlorinated tap water.

4. Restart cultures with known age young every five to six months or as needed.

5. When young of a specific age range is needed for testing, collect adults and separated 24 hours prior to collecting the young. Collect young by placing a #60 screen under the #35 screen.

Maintain adults if needed, and culture young not longer than 14 days. Try to keep organisms underwater as much as possible during the separation process.

Quality Assurance

1. The organisms should appear healthy, behave normally, feed well, and have low mortality in cultures.

2.All feeding and water change activities are logged daily in the daily maintenance log book.

3. Monthly reference toxicant tests are performed to assure organism health.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates.

HYALELLA AZTECA FOOD PREPARATION

Purpose

The preparation of quality food for the cultured organism is essential for the overall success of any testing procedure. This procedure assures quality food for Culturing as well as toxicity testing.

Equipment and Reagents

Blender
Plastic Storage Containers
Scale
Weigh boats
Refrigerator
Yeast
Alfalfa loose or pelleted
Trout chow
DI water
Spirallina

Procedure

1. Combine five grams of trout chow in one liter of DI water. Aerate for seven days. Put in the refrigerator and allow to settle over night.

2. Combine 5 grams of alfalfa with one liter of DI water. Grind and allow to settle overnight.

3. Decant or siphon the supernatant from the alfalfa and the trout chow. Set aside.

4. Combine 5 grams of yeast and one liter of DI water. Stir.

5. Combine equal volumes of the supernatants and the yeast.

6. Store in small screw top plastic containers and freeze until needed.

7. To prepare spirallina, mix one gram of algae to 150 ml's of DI water. Refrigerate in small plastic cotainers.

Quality Assurance

1. Log date of food and batch number in log book to assure age.

SEDIMENT COLLECTION, HANDLING, AND STORAGE

Purpose

Sediment collection, handling and storage may change the physical, chemical, or biological characteristics of the sediment. This procedure has been established to maintain the sediment integrity of field collected samples.

Equipment and Reagents

Wildco hand core sediment sampler
Plastic ziplock baggies
Stainless steel bucket
Stainless steel spoon
Cooler
Plastic gloves
Hip boots or Chest waders
Ice
#10 screen

Procedure

1. Assess stream for acquiring a representative sample. Also assess where the sediment may be located, depth of sediment, flow patterns, older deposits, organic silts, different depositional areas.

3. Collect a representative sample. If collecting by boat, use dredge or core sampler. If wading the stream, collect sample with spoon. The total volume needed for testing is 800 ml. Collect at least 1 liter of sediment. Sediment should be collected with as little disruption as possible.

4. If the sediment is not collectable by the core sampler due to shallow depth or sandy texture, collect with stainless steel spoon and annotate collection description on the chain of custody.

5. Composite the samples in the stainless steel bucket and stir.

6. Screen sediment through a #10 screen to remove other organisms and large rocks.

7. Pour sediment into plastic Nalge low density polyethelene bottles. Keep overlying water to a minimum. Double bag sample for transport.

8. Store sample in cooler with ice for transport. Sample should be stored at 4 degrees C and should never be frozen.

9. Samples can be stored for up to 8 weeks at this temperature.

Quality Assurance

1. Once samples are collected, general safety precautions should be taken. Dispose of plastic and disposable gear, -wash hands and exposed areas with soap, and all equipment should be properly cleaned with soap, 10% nitric acid, and acetone.

2. Complete chain of custody for files.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates, and USGS. 1994 Guidelines for the Collecting and Processing of Samples of Stream Bed Sediment for the Analysis of of Trace elementsand Organic Contaminants for the National Water Quality Assessment Program. Open File Report # 94-458.

Revision: 01
Date: 04/97

HYALELLA AZTECA: WHOLE SEDIMENT TOXICITY TEST

Purpose

The purpose of this test is to detect through the use of cultured organisms, toxicity that may be found in whole sediments. This is a 10 day renewal test using *hyalella azteca*. Overlying water is changed daily and organisms are fed daily. The endpoint monitored at the end of the test is survival.

Equipment and Reagents

Water flow through system
pH meter
DO meter
Conductivity meter
hardness and alkalinity titration
stir boxes
hardness and alkalinity buffers
thermometer
light board
light/magnifier
stainless steel spoon
disposable pipette
temperature controlled room with light timer
Data sheets
Glass dishes for organism recovery
Moderately hard reconstituted water (SOP # 1)
Hyponex potting soil
YCT
sediment samples
Hyalella azteca

Procedure

1. The test chamber allows for one set of controls and two testing sites (24 test chambers). This is a 10 day test, however the preparation starts at **day -1.** It is called this because 1 day prior to inserting the organisms the sediment must have time to reach optimum temperature as well as have time to settle. So with the sediment in front of you, probably straight from the cooler, put the sediment in a clean stainless steel pan. Any pore water that has separated from the sediment

should be reincorporated back into the sediment. Allow for as little sediment disruption as possible.

2. 100 ml of sediment should be added to each cup. There will be 8 cups per test. Prepare control dilutions by using potting soil (100 ml also).

3. 175 ml of moderately hard reconstituted water (SOP#1) should be added to the test cups. This includes controls.

4. **Day 0**. Measure water quality by removing with a syringe enough water to measure pH, conductivity, alkalinity, hardness, DO, and temperature. Take an equal volume from each replicate. This will be approximately 10 ml for a total volume of 80 ml. This should be enough volume for meters.

5. Do water exchange by pouring 2100 ml of moderately hard reconstituted water into each pan of the flow through system. This allows for a complete water exchange in each test chamber. 6 test chambers x 175 ml x 2 = 2100 ml. Water exchange should cause minimal sediment disruption. Assure all syringes and cups are lined up.

6. Transfer organisms. Put 10 organisms in each test chamber. Transfer by keeping under water at all times.

7. Feed 1.5 ml of yct daily after each water exchange.

8. **Day 1-9.** Measure DO and temperature daily by technique described in step 4. Renew overlying water daily with a two volume water exchange and feed 1.5 ml of YCT. Log measurements on log sheets and note any observations in regard to organism activity.

9. **Day 10.** Measure temperature and DO. End the test by collecting organisms. Count and mark data sheet. Try to spend the same amount of time collecting organisms on each cup. If the hyalella are dead there will little to no trace. This can lead to excessive amounts of time being spend on retrieval.

10. Test conditions, general activity schedule, and test acceptability requirements from EPA publication 600/R-94/024 are included as Appendix # 1.

11. After test has ended, dispose of test chambers and test organisms.

12. Results from data sheets can be entered on TOXCALC computer program for statistical analysis and results.

Quality Assurance

1. 96 hour reference toxicant tests are performed on a monthly basis.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates.

96 HOUR REFERENCE TOXICANT TEST USING *HYALELLA AZTECA*

Purpose

Reference toxicity test are performed to assure the quality of the organisms used in toxicity testing. These tests help evaluate the sensitivity and health of the organisms by exposing them to a known toxicant. This is a 96 hour static test.

Equipment and Reagents

Hyalella azteca
30 ml test cups
Brood boards
scales
pH meter
conductivity meter
DO meter
thermometer
data sheets
alkalinity and hardness titrator and buffers
volumetric flasks
gallon jugs

Sodium chloride
moderately hard reconstituted water
YCT

Procedure

1. Mix NaCl solutions in 4.5 g/l, 5.0 g/l, 5.5 g/l, 6.0g/l, and 6.5 g/l. Mix a liter of each solution. Check conductivity chart to assure correct range.

2. Check water quality at beginning and end of test. This includes pH, conductivity, alkalinity, DO, hardness, and temperature.

3. Set up 4 cups per dilution.

4. Insert 10 organism per cup. Assure transfer occurs under water .

5. Feed organisms 0.5 ml YCT per cup.

6. Measure temperature daily and count organisms.

7. Feed again on day 2.

8. Day 4 measure water quality and count organisms. Dispose of survivors. It is a thankless job.

9. Enter data from data sheets into TOXCALC computer system. Log results.

10. Recommended test conditions from EPA publication 600/R-94/024 are included as Appendix two.

Quality Assirance

1. Monthly reference toxicant tests are performed on a monthly basis.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates.

APPENDIX 1 TEST CONDITIONS

APPENDIX 2 FORMS

Project Final Report

Grant # C9994861

EVALUATION OF SEDIMENT TOXICITY AND MACROINVERTEBRATE COMMUNITIES AT SELECTED SITES IN THE UPPER CUMBERLAND RIVER (KENTUCKY, USA)

Workplan # 9908

Project Period September 2000-October 2001

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Funding for this project (EVALUATION OF SEDIMENT TOXICITY AND MACROINVERTEBRATE COMMUNITY AT SELECTED SITES IN THE UPPER CUMBERLAND RIVER, KENTUCKY, USA) was provided for in part by the U.S. Environmental Protection agency (USEPA) through the Kentucky Division of Water, Nonpoint Source Section, to the Bioassay Section within the Division of Water as authorized by the Clean Water Act Amendments of 1987, 319(h), Nonpoint Source Implementation Grant # C9994861-99. The contents of this document do not necessarily reflect the views and policy of the USEPA, KDOW nor does the mention of trade names or commercial products constitute endorsement. This document was printed on recycled paper.

ACKNOWLEDGMENTS

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EXECUTIVE SUMMARY

Sediment is an integral part of aquatic ecosystems. Resource extraction can adversely affect sediment quality through point and nonpoint source runoff. The objective of this study was to investigate the feasibility of utilizing the benthic macroinvertebrate community, sediment toxicity tests, and sediment chemistry in assessing selected aquatic systems in the Upper Cumberland River Basin that have drained areas of coal mining activity.

Sediment sampling was conducted in the fall of 2000 on seven test sites and two control sites. Quantitative benthic macroinvertebrate samples were collected concurrently with sediment used for chemical analysis and toxicity testing. The sediment was analyzed for metals, selected organic compounds, acid volatile sulfides, and total organic carbon. Both 10-day acute and 28-day chronic toxicity tests were performed using Hyalella azteca. Macroinvertebrate data were subjected to parametric and nonparametric analyses.

Six of the seven sites were found to have high levels of contaminants. Only one site exhibited acute toxicity and three showed evidence of chronic effects. Two sites had impaired benthic communities. However, when the results from the chemical analysis, toxicity tests and benthic investigation were considered in a Sediment Quality Triad (SQT) assessment methodology, only one site was determined to show evidence of contaminant induced degradation. Contaminants were considered unavailable at two sites and the benthic response at two sites was not attributable to contaminants. One site was determined to exhibit no contaminant degradation. The remaining site had contaminants available but the macroinvertebrate community response was unaffected by sediment chemistry.

The SQT assessment methodology was a good tool to investigate sediment contamination. More statistically robust and impact sensitive methods of performing chronic toxicity tests should be developed to increase assessment accuracy. Macroinvertebrate sampling strategies should consider ecoregion/subecoregion variability, as well as techniques for reducing within and between site sampling error.

The Bioassay Section gained valuable experience in toxicity testing from this project. The use of the triad approach as an assessment tool was very valuable. It enabled us to look at AVS/SEM (Model), benthic invertebrate data, chemistry and compare these data with our usual toxicity testing. This project led to the discovery of a better protocol for the 28-day toxicity test and it will be used in our next round of testing. We now realize we should have completed a particle size analysis. We gained valuable field experience and expanded our toxicity/chemical database.

INTRODUCTION

The Upper Cumberland River Basin in southeastern Kentucky contains some of the Commonwealths most valuable water resources. The most widespread water quality problem for the Upper Cumberland watershed is associated with the coal mining industry. Point and non-point sources of acid mine drainage from coal deposits, which contain large quantities of sulfides and sulfates, contribute to the problem. Oxidation of iron disulfide exposed by mining initiates the formation of water soluble acidic pollutants which cause the most objectionable characteristics of coal mine drainage. The most significant water quality problem associated with mining occurs when dissolved, suspended, or other solid mineral wastes and debris from mining or mine related operations enter streams, watercourses or ground water. Mine drainage includes not only water flowing by gravity or pumped from underground mines, but also runoff or seepage from surface and strip mines, excavated waste deposits and haul roads. (KDOW, 1975). Resource extraction is a major source of chemically altered sediments (Burton, 1992).

Sediment associated with aquatic systems play a vital ecological role. They serve as a potential reservoir for organic material as well as a sink for industrial contaminants (Call, et al. 2001). Contaminated sediment can be directly toxic to aquatic organisms. Additionally, non-lethal concentrations of chemicals sorbed to sediment can accumulate in benthic organisms and bioaccumulate up the food web. The goal of this study was to document sediment contaminant concentrations, measure sediment toxicity and evaluate negative impacts to the associated macroinvertebrate communities.

Seven potentially impacted sites were selected using data from the Kentucky Nonpoint Source Assessment Report (1999) and two KDOW reference reach sites were selected to provide control sediment. Sediment and macroinvertebrate samples were taken concurrently at each site in the Fall of 2000. Each site was analyzed for toxicity, total organic carbon content, and concentrations of metals, pesticides, and polychlorinated biphenyls (PCBs). Analysis of the benthic communities included identification of taxa to the lowest positive level, calculation of selected metrics, parametric, and nonparametric tests. The primary objective of the analysis was to identify possible toxic materials, evaluate the level of toxicity and relate these findings to the benthic biota.

METHODS AND MATERIALS

All sites were located within the Eastern Kentucky Coalfield Region (Table 1). Material taken from Cranks Creek was collected from two sites located in Cranks Creek Reservoir. The remaining sites were located in the free flowing portions of each stream. At each site, one composited sediment sample was collected and split for chemical analysis and toxicity testing. Three replicate samples were collected for macroinvertebrate monitoring.

Table 1. Site locations used in 10-day acute and 28-day chronic sediment toxicity test.

Water Body	GPS coordinates	Mile Point	Sample date
Turkey Creek			

Bell County
N 36d 46 m 06.3 s
W 084d 02m 28.3s
0.3
10/31/00
Little Clear Creek
Bell County
N36d 42m 31.8s
W083d 44m 29.3s
0.8
10/31/00
Cranks Creek
Harlan County
N36d 04 m 50.2s
W 83d 12m 40.7s
3.0
9/20/00
Cranks Creek Reservoir
Harlan County
N36d 44m 50.2s
W083d 12m 55.5s
3.2
9/20/00
Indian Creek
Jackson County
N 37d 22m 59.7s
W084d 02m 28.3s
1.5
9/19/00
Crummies Creek
Harlan County
N36d 47m 26.8s
W083d 13m 02.2s
1.6
9/20/00
Roaring Paunch
McCreary County
N36 d 40m 48.3s
W084d 32m 28.0s
0.1
10/30/00
Laurel Fork*
Jackson County
N84d 02m 28.9 s
W37d 22m 11.5s
1.0
9/19/00
Bark Camp Creek*
Laurel County
N36d 54m 14.8s
W84d 16m 52.2s
2.5
10/30/00
* = Control site

DESCRIPTION OF MACROINVERTEBRATE ASSESSMENT

The macroinvertebrate samples were collected from the same depositional areas as the rest of the sediment used in the study. The benthic samples taken at Cranks Creek Reservoir were collected with a Petite Ponar dredge. Stream samples were taken by inverting a glass specimen jar into the sediment. All benthic samples were sieved through 300(Nitex netting in the field. The samples were then preserved in 70 % ethanol and transported to the lab for identification. To reduce sample processing time, samples were subjected to sucrose flotation (Flannagan, 1973). Each sample was examined separately using a stereo-dissecting microscope with magnification to 75X.

Invertebrates were hand picked from samples, identified to the lowest positive taxonomic level and enumerated. Organisms requiring slide preparation for identification, such as Oligochaeta and Chironomidae, were mounted in CMC-10 and dried. Identifications were then made using a phase-contrast compound microscope with magnification to 1000X.

Counts of organisms were converted to number per square meter. Mathematical manipulations included calculation of diversity (Shannon 1948) and evenness (Pielou 1966) values for each sample. In lieu of standard protocols for determining negatively impacted sediment, best professional judgement was used on a site by site basis to designate impaired sites. Raw macroinvertebrate data are in Appendix E.

Macroinvertebrate communities at each site were analyzed by cluster analysis using the Bray-Curtis dissimilarity index and the unweighted pair-group arithmetic averaging (UPGMA) clustering algorithm. These two techniques have been used frequently together and are considered to be robust at classifying distinct objects. Prior to clustering, we transformed taxa densities using log10 (x+1). The technique arranges all clusters into a hierarchy so that the relationships between the different groups are apparent. Cluster analysis produces a tree-like diagram called a dendrogram.

SEDIMENT CHEMICAL ANALYSIS

Sediment was collected in the field for chemical analysis according to protocols established by USGS (1994). The Kentucky Department of Environmental Services (DES) analyzed each sample for metals and selected organics (see Appendix C) to identify suspected toxic material. Chemical contamination is a concept that is not always clearly defined relative to sediment. Traditionally, higher concentrations of potential toxicants are thought to produce higher risks to the biota. However, this assumption is not always true. Some evaluation must be made to estimate the potential risk to aquatic life that the compound may have. The EPA defines sediment criteria as a specific level of protection from the adverse effects of sediment associated pollutants, for beneficial uses of the environment, biota, and human health. Sediment quality criteria are the numerical concentrations of individual chemicals, which are intended to be predictive of biological effects, protective of the presence of benthic organisms and applicable to the range of natural sediments from lakes and streams. A sediment criterion must relate to the level of harm that the contaminant possesses by specifying an appropriate level of protection. Only if the contaminant concentration is less than all of the available criteria can exposure to the sediment, or to organisms that inhabit the sediment, be considered to be without significant risk.

Sediment contaminants primarily consist of heavy metals and persistent organic compounds (New York DEC 1998). Sediment criteria for non-polar organic compounds (e.g. PCBs and PAHs) are derived using equilibrium partitioning method (USEPA, 2000). Sediment criteria for metals are derived from empirically derived values establish by the National Oceanic and Atmospheric Agency (Long and Morgan, 1991). Total organic carbon (TOC) values are important to normalize the bioavailability of organic toxicants between sites (USEPA, 2000).

Simultaneously extracted metals/ acid volatile sulfides analysis was performed by EN CHEM, Incorporated Laboratory in Madison, Wisconsin. Simultaneously extracted metals (SEM) and acid volatile sulfide (AVS) are operationally defined methods for the analysis of sulfide and associated metals in aquatic sediments. The SEM to AVS ratio has been used to clarify the results of bioassay tests of metal toxicants. Sulfide production occurs in organically enriched sediment under anaerobic conditions. Typically, sulfides are restricted to a few centimeters beneath the sediment

surface, depending on the influx of organic matter. The AVS-SEM model, developed by DiToro (1985), recognized that AVS is a reactive pool of solid phase sulfide that is available to bond with certain metals and reduce free metal ion concentrations. The AVS-SEM model has been verified for six divalent metals in anoxic sediment (i.e. cadmium, copper, lead, nickel, mercury and zinc). If the molar ratio of toxic metals measured by SEM to AVS exceeds one, the metals are potentially bioavailable. A ratio less than one suggests that the metals in the sediment are non-toxic (ENCHEM SOP WCM-63, April 2000).

DESCRIPTION OF THE 10 DAY TOXICITY TEST

The 10-day sediment toxicity test was performed using Hyalella azteca to determine acute effects. Conditions for conducting the test are fully outlined in USEPA(1994). Results were analyzed using ToxCalc, Tidepool Scientific Software. Toxicity was determined when the test site results were statistically different from control sites. Results of all toxicity tests are in Appendix D.

DESCRIPTION OF THE 28 DAY TOXICITY TEST

In order to determine the existence of chronic effects in sediment collected for this study, a 28-day test was conducted. In the absence of standardized protocols for such experiments, KDOW Bioassay Section personnel developed their own procedures. Initially, 400 ml of field sediment were placed in each 2.5-gallon aquarium. It was overlain by 2 liters of moderately hard-reconstituted water. The sediment was allowed to settle overnight. Twenty-five Hyalella azteca were introduced into each aquarium on the following day. Organisms were less than 14 days old. The organisms were fed 5 ml YCT daily after a one-liter water exchange. Prior to water exchange, the overlying water was measured daily for temperature, pH, and dissolved oxygen. At test end, the sediment was poured out gently into a Pyrex glass dish. Organisms were removed by pipette. If they could not be found, the sediment was sieved through a Standard US #30 screen. All test and control sites had organisms other than Hyalella azteca, presumably from latent egg deposition which occurred prior to collection. The sediment was considered to be toxic if mortality was greater than 20 % of the control results.

As with any bioassay technique, the results take careful consideration. Hyalella azteca is an organism that is used in sediment toxicity tests because they have contact with the sediment and are easily cultured in the laboratory. Their sensitivity can not be considered representative of all benthic organisms. Additionally, the test has daily water exchanges utilizing moderately hard reconstituted water. The expression of toxicity in the laboratory may not accurately reflect problems in the field. The chemical composition of the synthetic water can moderate the temperature, dissolved oxygen, and pH of the sediment in consideration.

INTEGRATIVE ASSESSMENT

The overall assessment of sediment quality is contingent upon many factors. Possible variables include toxic material concentrations, the synergistic/antagonistic properties associated with the pollutants, sediment depth and oxygen levels, particle size and physical chemistry of

[illegible]sites in the Upper Cumberland River (Kentucky, USA)

Workplan # 9908

Project Period September 2000-October 2001

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Methods for Culturing and Conducting Toxicity Tests With Hyalella Azteca

(Third Edition)

By
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Natural Resources
and Environmental Protection Cabinet

DISCLAIMER

THE MENTION OF BRAND NAMES OR COMMERCIAL PRODUCTS DOES NOT CONSTITUTE ENDORSEMENT OR RECOMMENDATION FOR USE OF THE PRODUCT.

PREFACE

This document is a product of the educational pilot project sponsored by the Natural Resources and Environmental Protection Agency. The project provides incentive to employees to obtain additional education that will directly enhance their job duties. The graduate level course entitled "Special Problems in Biology" was specifically tailored to assist the Bioassay Section establish the basis for a sediment toxicity program. The main objectives of the course included culturing, sampling, testing, quality assurance, and statistical analysis. This document has been prepared as part of the quality assurance portion of the course work.

TABLE OF CONTENTS

Section: SOP#1
Revision: 01
Date: 04/97

MODERATELY HARD RECONSTITUTED WATER

Purpose

The purpose of preparing moderately hard reconstituted water is to supply chemically defined water for maintaining test cultures and for conducting toxicity tests. This water is a standard synthetic dilution water prepared with deionized water and reagent grade chemicals specified in the EPA Methods Manual 600/R-94/024.

Equipment and Reagents

120 Liter Carboy
Aerator
Plastic tubing
Stir boxes (2)
2 liter jugs (2)
Scale
Meters (pH, DO, Temp., Conductivity)
Titration for Hardness and Alkalinity

NaHCO
CaSO4
MgSO4
CaCl2
KCl
Deionized Water

Procedure

1. To prepare 100 liters of reconstituted water, place 75 liters of deionized water in the carboy.

2. Add 5 grams of $CaSO_4$ and 5 grams of $CaCl_2$ to a 2 liter aliquot of deionized water and mix on a stir plate until salts dissolve. Overnight stirring is recommended.

3. Add 3 grams of $MgSO_4$, 9.6 grams of $NaHCO_3$, and 0.4 grams of KCl to a second two liter aliquot of deionized water. And mix on a stir plate for at least 30 minutes. Assure all salts are dissolved prior to use.

4. Pour the two 2-liter aliquot containing the dissolved salts into the 75 liters of deionized water and fill the carboy to 100 liters with DI water.

5. Aerate the mixture for at least 24 hours prior to chemical analysis and use. Aerate the mixture for the duration of use not to exceed 14 days.

Quality Assurance

1. Temperature, conductivity, dissolved oxygen, hardness, alkalinity, and pH should be taken on each water batch. Each batch should be number and logged into the culture water log book along with meter readings.

Chemistry	Acceptable Range
Temperature	23 + or - 1 degree
Conductivity	330 to 360
Dissolved Oxygen	below 40% unacceptable
Hardness	90-100 mg $CaCO_3$/L
Alkalinity	50-70 mg $CaCO_3$/L
pH	7.8 - 8.2

2. If chemistries fail to meet the acceptable range, the water must be discarded and the procedure redone.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates.

HYALELLA AZTECA CULTURE MAINTENANCE

Purpose

The quality of the health and reproduction of cultured organisms is essential to the quality of toxicity testing data. Hyalella Azteca are cultured in-house to assure this quality and provide ease in organism availability.

Equipment and Reagents

Hyalella azteca
Plastic containers 2-8 liter capacity
Nitex screening
Aerators and air stones
Temperature control
Light timer
Screens size 35,50, and 60
Culture log book
Dechlorinated tap water
Culture Water (SOP #1)
Food (SOP #3)

Procedure

1. A mass culture is maintained in a 10 gallon aquarium using dechlorinated tap water. They are also kept in one section of the recirculating water system used for culturing fish. The hyalella azteca cultured in the fish system are maintained as backup. They are fed the same as the fish (flakes and brine shrimp). The volume of water in the static aquarium is dependent on the number of organisms. The water level is maintained at about four to six inches deep.The system is a static system and is constantly aerated. Nitex screening is used as substrate.

2. Feed all Hyalella cultures daily. Static mass culture receives 15 ml of spirallina algae. YCT is fed every other day (15 ml). Feed on weekends as schedule allows.

3. Prior to feeding on Mon.,Wed., and Fri., do a half water change on the static cultures. Perform water changes by siphoning at least half the water from the aquarium while allowing the organisms to remain under water. Replenish with dechlorinated tap water.

4. Restart cultures with known age young every five to six months or as needed.

5. When young of a specific age range is needed for testing, collect adults and separated 24 hours prior to collecting the young. Collect young by placing a #60 screen under the #35 screen. Maintain adults if needed, and culture young not longer than 14 days. Try to keep organisms underwater as much as possible during the separation process.

Quality Assurance

1. The organisms should appear healthy, behave normally, feed well, and have low mortality in cultures.

2.All feeding and water change activities are logged daily in the daily maintenance log book.

3. Monthly reference toxicant tests are performed to assure organism health.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates.

Section: SOP#3
Revision: 01
Date: 10/98

HYALELLA AZTECA FOOD PREPARATION

Purpose

The preparation of quality food for the cultured organism is essential for the overall success of any testing procedure. This procedure assures quality food for Culturing as well as toxicity testing.

Equipment and Reagents

Blender
Plastic Storage Containers
Scale
Weigh boats
Refrigerator
Yeast
Alfalfa loose or pelleted
Trout chow
DI water
Spirallina

Procedure

1. Combine five grams of trout chow in one liter of DI water. Aerate for seven days. Put in the refrigerator and allow to settle over night.

2. Combine 5 grams of alfalfa with one liter of DI water. Grind and allow to settle overnight.

3. Decant or siphon the supernatant from the alfalfa and the trout chow. Set aside.

4. Combine 5 grams of yeast and one liter of DI water. Stir.

5. Combine equal volumes of the supernatants and the yeast.

6. Store in small screw top plastic containers and freeze until needed.

7. To prepare spirallina, mix one gram of algae to 150 ml's of DI water. Refrigerate in small plastic cotainers.

Quality Assurance

1. Log date of food and batch number in log book to assure age.

Section: SOP #4
Revision: 02
Date: 10/01

SEDIMENT COLLECTION, HANDLING, AND STORAGE

Purpose

Sediment collection, handling and storage may change the physical, chemical, or biological characteristics of the sediment. This procedure has been established to maintain the sediment integrity of field collected samples.

Equipment and Reagents

Wildco hand core sediment sampler
Plastic ziplock baggies
Stainless steel bucket
Stainless steel spoon
Cooler
Plastic gloves
Hip boots or Chest waders
Ice
#10 screen

Procedure

1. Assess stream for acquiring a representative sample. Also assess where the sediment may be located, depth of sediment, flow patterns, older deposits, organic silts, different depositional areas.

3. Collect a representative sample. If collecting by boat, use dredge or core sampler. If wading the stream, collect sample with spoon. The total volume needed for testing is 800 ml. Collect at least 1 liter of sediment. Sediment should be collected with as little disruption as possible.

4. If the sediment is not collectable by the core sampler due to shallow depth or sandy texture, collect with stainless steel spoon and annotate collection description on the chain of custody.

5. Composite the samples in the stainless steel bucket and stir.

6. Screen sediment through a #10 screen to remove other organisms and large rocks.

7. Pour sediment into plastic Nalge low density polyethelene bottles. Keep overlying water to a minimum. Double bag sample for transport.

8. Store sample in cooler with ice for transport. Sample should be stored at 4 degrees C and should never be frozen.

9. Samples can be stored for up to 8 weeks at this temperature.

Quality Assurance

1. Once samples are collected, general safety precautions should be taken. Dispose of plastic and disposable gear, -wash hands and exposed areas with soap, and all equipment should be properly cleaned with soap, 10% nitric acid, and acetone.

2. Complete chain of custody for files.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates, and USGS. 1994 Guidelines for the Collecting and Processing of Samples of Stream Bed Sediment for the Analysis of of Trace elementsand Organic Contaminants for the National Water Quality Assessment Program. Open File Report # 94-458.

Section: SOP # 5
Revision: 01
Date: 04/97

HYALELLA AZTECA: WHOLE SEDIMENT TOXICITY TEST

Purpose

The purpose of this test is to detect through the use of cultured organisms, toxicity that may be found in whole sediments. This is a 10 day renewal test using hyalella azteca. Overlying water is changed daily and organisms are fed

daily. The endpoint monitored at the end of the test is survival.

Equipment and Reagents

Water flow through system
pH meter
DO meter
Conductivity meter
hardness and alkalinity titration
stir boxes
hardness and alkalinity buffers
thermometer
light board
light/magnifier
stainless steel spoon
disposable pipette
temperature controlled room with light timer
Data sheets
Glass dishes for organism recovery
Moderately hard reconstituted water (SOP # 1)
Hyponex potting soil
YCT
sediment samples
Hyalella azteca

Procedure

1. The test chamber allows for one set of controls and two testing sites (24 test chambers). This is a 10 day test, however the preparation starts at day -1. It is called this because 1 day prior to inserting the organisms the sediment must have time to reach optimum temperature as well as have time to settle. So with the sediment in front of you, probably straight from the cooler, put the sediment in a clean stainless steel pan. Any pore water that has separated from the sediment should be reincorporated back into the sediment. Allow for as little sediment disruption as possible.

2. 100 ml of sediment should be added to each cup. There will be 8 cups per test. Prepare control dilutions by using potting soil (100 ml also).

3. 175 ml of moderately hard reconstituted water (SOP#1) should be added to the test cups. This includes controls.

4. Day 0. Measure water quality by removing with a syringe enough water to measure pH, conductivity, alkalinity, hardness, DO, and temperature. Take an equal volume from each replicate. This will be approximately 10 ml for a total volume of 80 ml. This should be enough volume for meters.

5. Do water exchange by pouring 2100 ml of moderately hard reconstituted water into each pan of the flow through system. This allows for a complete water exchange in each test chamber. 6 test chambers x 175 ml x 2 = 2100 ml. Water exchange should cause minimal sediment disruption. Assure all syringes and cups are lined up.

6. Transfer organisms. Put 10 organisms in each test chamber. Transfer by keeping under water at all times.

7. Feed 1.5 ml of yct daily after each water exchange.

8. Day 1-9. Measure DO and temperature daily by technique described in step 4. Renew overlying water daily with a two volume water exchange and feed 1.5 ml of YCT. Log measurements on log sheets and note any observations in

regard to organism activity.

9. Day 10. Measure temperature and DO. End the test by collecting organisms. Count and mark data sheet. Try to spend the same amount of time collecting organisms on each cup. If the hyalella are dead there will little to no trace. This can lead to excessive amounts of time being spend on retrieval.

10. Test conditions, general activity schedule, and test acceptability requirements from EPA publication 600/R-94/024 are included as Appendix # 1.

11. After test has ended, dispose of test chambers and test organisms.

12. Results from data sheets can be entered on TOXCALC computer program for statistical analysis and results.

Quality Assurance

1. 96 hour reference toxicant tests are performed on a monthly basis.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates.

Section: SOP # 6
Revision: 02
Date: 10/01

96 HOUR REFERENCE TOXICANT TEST USING HYALELLA AZTECA

Purpose

Reference toxicity test are performed to assure the quality of the organisms used in toxicity testing. These tests help evaluate the sensitivity and health of the organisms by exposing them to a known toxicant. This is a 96 hour static test.

Equipment and Reagents

Hyalella azteca
30 ml test cups
Brood boards
scales
pH meter
conductivity meter
DO meter
thermometer
data sheets
alkalinity and hardness titrator and buffers
volumetric flasks

gallon jugs

Sodium chloride
moderately hard reconstituted water
YCT

Procedure

1. Mix NaCl solutions in 4.5 g/l, 5.0 g/l, 5.5 g/l, 6.0g/l, and 6.5 g/l. Mix a liter of each solution. Check conductivity chart to assure correct range.

2. Check water quality at beginning and end of test. This includes pH, conductivity, alkalinity, DO, hardness, and temperature.

3. Set up 4 cups per dilution.

4. Insert 10 organism per cup. Assure transfer occurs under water .

5. Feed organisms 0.5 ml YCT per cup.

6. Measure temperature daily and count organisms.

7. Feed again on day 2.

8. Day 4 measure water quality and count organisms. Dispose of survivors. It is a thankless job.

9. Enter data from data sheets into TOXCALC computer system. Log results.

10. Recommended test conditions from EPA publication 600/R-94/024 are included as Appendix two.

Quality Assirance

1. Monthly reference toxicant tests are performed on a monthly basis.

References

EPA Publication 600/R-94/024 Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants and Freshwater Invertebrates.

APPENDIX 1 TEST CONDITIONS

APPENDIX 2 FORMS