INTEGRATIVE BIOASSESSMENT TECHNIQUES TO PREDICT ECOTOXICOLOGICAL IMPAIRMENT BY ACID MINE DRAINAGE

by

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Abstract: A new integrative bioassessment technique, the Ecotoxicological Rating (ETR) is used to prioritize sites within watersheds for reclamation. The approach synthesizes ecological, toxicological, physical and chemical data into a single value that depicts the relative environmental integrity of a given site. The ETR has been used in two adjacent acid mine drainage (AMD) impacted tributaries of the Powell River watershed in southwest Virginia. The purpose of this study was to analyze the predictive power of the ETR parameters to create a more efficient means to bioassess AMD impacts, and seek more conclusive signals that can be designated as signs of AMD impact. Through step-wise Multiple Linear Regression Analysis (MRLA) and bivariate correlations between benthic macroinvertebrate indices (total richness, Ephemeroptera (E) abundance, percent E. abundance, Ephemeroptera-Plecoptera-Trichoptera (EPT) richness, EPT abundance, and percent EPT abundance) and independent variables (chemical and toxicological parameters), the most predictive parameters were determined to be Asian clam survival, mean conductivity and habitat assessment score (R-values = 0.4608 to 0.5541; 0.4079 to 0.5922; 0.4162 to 0.7049, respectively). Models utilizing all three of the most predictive ETR parameters were compared to models using the parameters selected by step-wise procedures, and two types of models tailored to the individual watersheds. Tailored models selected from all 18 parameters (n) which correlated with macroinvertebrate indices with R-values > 0.4999, (n=10 for Ely, n=12 for Puckett's Creeks). A second set of tailored models used only the parameters that occurred in both the tailored models for Ely and Puckett's Creeks (n=8). R^2-values for all these models were not significantly different (ANOVA p-value = 0.1883). These data suggest that Asian clam survival, mean conductivity, and habitat score should be emphasized in AMD bioassessment and could be used in a more streamlined bioassessment technique delineating AMD impacted watersheds.

Additional Key Words: integrative bioassessment, benthic macroinvertebrates.

Introduction

Acid mine drainage (AMD) impairs water quality and destroys habitat in over 13,000 miles of U.S. rivers and the US EPA has singled out AMD as the primary water quality problem in the Appalachian region (Banks, 1997; Office of Surface Mining, 1995). This degradation in water quality occurs when pyrite (FeS_2) is oxidized upon exposure to water and oxygen through mining activities. The process produces sulfuric acid, ferric hydroxides, and mobilizes other trace metals, depending on the surrounding mineralogy. These toxic metals flow to surface waters and precipitate, coating streambeds with metal oxides, destroying habitat, and adversely affecting water quality.

As a result of the damage to our freshwater natural resources, and the passage of the 1972 Federal Clean Water Act (US EPA, 1991 c), the Virginia Department of Mines, Minerals and Energy, Division of Mine Land Reclamation has made it a priority to remediate the AMD impacts of the Powell River watershed. However, reclaiming an entire watershed is a costly and time consuming process requiring an assessment system that can quantitatively evaluate environmentally impacted segments/tributaries relative to each other. As a result the Ecotoxicological Rating (ETR) was implemented to delineate the environmental damage in the Powell River watershed caused by AMD.

The ETR, first developed by Cherry et al. (1998), and modified by Soucek et al (2000a) for AMD, was modeled after the Sediment-Quality Triad (SQT). These techniques use integrative bioassessment to quantify environmental contamination such as AMD. The SQT uses bulk sediment chemistry, sediment bioassays, and *in situ* studies to derive three separate values grading the environmental state of the site (Long and Chapman 1985). Like the SQT, the ETR uses

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[1] Paper presented at the 2000 National Meeting of the American Society for Surface Mining and Reclamation, Tampa, Florida, June 11-15, 2000.

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Proceedings America Society of Mining and Reclamation, 2000 pp 162-168
DOI: 10.21000/JASMR00010162

https://doi.org/10.21000/JASMR00010162

chemical, toxicological, and ecological data, but synthesizes these data into one number summarizing several parameters. These parameters include: habitat assessment score, benthic macroinvertebrate indices, chemical parameters including metals analysis of both the water column and sediments, laboratory acute water column survivorship (*Ceriodaphnia dubia* survival), sediment chronic toxicity and impairment tests (*Daphnia magna* survival and reproduction, *Chironomus tentans* survival and post test weight), and *in situ* toxicity testing with the Asian clam, (*Corbicula fluminea* survival) (Soucek et al, 2000 a). Multiple Linear Regression Analysis (MLRA) and bivariate correlations determine a subset of 10 parameters that best describe the relative environmental state of a sampling station. A single number, summarizing this subset, is produced with a rating scale from 0-100, 0 being the most environmentally impacted and 100 as the most pristine (Cherry et al, 1998). This rating allows officials responsible for allocating ecological remediation funds to prioritize spending relative to the environmental condition of a particular segment of a tributary or watershed.

This study analyzed historical data from two tributaries of the Powell River watershed, Virginia; Ely and Puckett's Creeks, both previously analyzed by the ETR. The predictive power of each ETR parameter was tested by determining which parameters best-predicted macroinvertebrate responses to AMD in each watershed. The purpose of this study was to begin work creating an ETR less site specific by minimizing it to the most predictive but conclusive parameters. Also, minimizing the ETR to its most predictive parameters will render it more time and cost effective for large-scale restoration projects while maintaining sensitivity in delineating AMD impacts. Giving emphasis to the most predictive ETR parameters could help maximize our ability to restore these impacted areas to a more natural state.

Materials and Methods

Sampling stations/Sampling Schedule

Ely and Puckett's Creeks are tributaries of the North Fork of the Powell River, located in southwest Virginia. Ely Creek is a tributary of Stone Creek, which joins Straight Creek below its confluence with Puckett's Creek. Stone and Straight become Stone/Straight, which then contributes to the North Fork Powell River near Pennington Gap, Virginia. Thirty-seven sampling stations were selected, 20 stations in the Ely Creek study (Cherry et al, In Press), and 21 in the Puckett's Creek study (Soucek, 2000a), with 4 overlapping stations between the two studies. At stations where data overlapped, mean values were calculated for all parameters. Ely Creek was sampled from January 1997 to March 1997 while the Puckett's Creek's investigation was conducted from October 1997 to July 1998.

ETR Parameters Collected for Analysis

Eighteen ETR parameters (excluding macroinvertebrate indices) were used in this study. The sampling and collection methods can be found in Cherry et al. (In Press), or Soucek et al. (2000a). Macroinvertebrate community indices were determined as follows: total taxon richness, Ephemeroptera-Plecoptera-Trichoptera (EPT) richness, percent EPT abundance, and percent Ephemeroptera abundance. Chemical parameters were: pH, conductivity, aluminum (Al) in the water column and sediments, copper (Cu) in the sediment, iron (Fe) in water column and sediments, manganese (Mn) in the water column and sediments, nickel (Ni) and zinc (Zn) in the sediment. Toxicological end points used in this study were *C. dubia* survival, *D. magna* reproduction and survival, *C. tentans* weight and survival, and *in situ* Asian clam survival. A habitat assessment was conducted according to Plafkin et al (1989), as a measure of the physical condition of the stream.

Statistical Analysis

Means for all data were used where applicable. Transformations were conducted on all data that did not satisfy the normality assumption (JMP®, $p>0.05$). If data did not meet the normality assumption post transformation, then the data were not transformed. The following transformations were made: sin $(\text{Abundance})^{0.5}$, pH^3, sin (Fe in water column). Log + 1 transformations were made to: mean conductivity, Ni in sediment, and Al and Mn in water column. To avoid multicolinearity between variables, bounds on condition numbers were calculated using SAS® software, (1996).

The following procedure was conducted on both the Ely and Puckett's Creek data sets. Step-wise MLRA (SAS®) regressed the six macroinvertebrate indices (dependant variables) against the chemical, physical and toxicological parameters (independent variables listed above), selecting the model with the largest R^2 where all independent variables were significant to $p\leq0.05$. To determine which variables actually correlated with the macroinvertebrate indices and did not just add to the R^2 value of the models, bivariate correlations (JMP®) were performed using the independent variables selected in the step-wise procedure and the six dependent variables. Means of the correlation coefficients were then calculated for each independent variable that occurred in both data sets. For example, the mean r-value for correlations between conductivity and the six dependent variables was calculated. An ANOVA and post hoc comparisons (Least Significant Difference (LSD) t-test, α= 0.05) were used to determine which of the remaining parameters were the most predictive of the dependent variables. All the variables selected were used in another MLRA "most sensitive parameters". Bivariate correlations were then determined for all 18 independent variables and the six dependent variables, those with R values >0.4999 were selected and used in MLRA, "tailored models" for Ely and Puckett's Creeks. MLRA was conducted again using only those parameters occurring in both "tailored models" creating models "common parameters from tailored models". Another ANOVA and post hoc comparisons with LSD t-tests (α= 0.05) were then conducted.

Results

Equations created by the initial step-wise selection can be found in Tables 1a-1b. R^2 values ranged from 0.1696 to 0.8317 for Ely Creek, and 0.3687 to 0.9444 for Puckett's Creek (Tables 1a-1b). Seven independent variables occurred in both data sets; clam survival, *D. magna* reproduction, *C. tentans* survival, conductivity, *C. dubia* survival, habitat score, and Fe in the sediment, all selected as sensitive parameters (Table 2). Water column Fe, mean pH, Zn in the sediment, and *C. tentans* weight were the only parameters not selected in the step-wise procedure. An ANOVA and LSD t-test determined that three of the seven important parameters selected were significantly more sensitive (denoted as A in Table 3) than the other

Table 1a. Equations created through step-wise procedures for Ely Creek.

Richness $R^2 = 0.4734$, p = 0.0001.	Y = 0.1617 + 0.1080(*C. dubia* survival).
%E. abundance $R^2 = 0.4760$, p =0.0008.	Y = 1.2968 + 0.2927(Habitat).
E. abundance $R^2 = 0.7354$, p = 0.0001.	Y =171.2121 0.3006(Clam survival) - 38.3737(conductivity) - 0.8428(Habitat).
EPT richness $R^2 = 0.7384$, p = 0.0003.	Y = 3.8004 + 0.0591(*C. dubia* survival) - 0.0544(*D. magna* reproduction) - 0.0022(Al in sediment) - 0.8163(Cu in sediment).
%EPT abundance $R^2 = 0.1696$, p = 0.0712.	Y = 150.1635 - 43.5437(conductivity).
EPT abundance $R^2 = 0.8317$, p = 0.0001.	Y = - 4.1927 + 0.6262(Clam survival) - 0.6384(*D.magna* reproduction) + 0.8090(*C. tentans* survival) - 0.0102(Al in sediment).

Table 1b. Equations created through step-wise procedures for Puckett's Creek.

Richness $R^2 = 0.4260$, p = 0.0013.	Y = 44.0599 - 12.1154(conductivity).
%E. abundance $R^2 = 0.7054$, p = 0.0126.	Y = 159.1275 - 22.0073(conductivity) - 67.8595(Ni in sediments) - 0.7308(Al in water column).
E. abundance $R^2 = 0.9444$, p = 0.0001.	Y = 68.0360 + 0.3290(clam survival) - 0.0002(Fe in sediments) - 0.6959(habitat).
EPT richness $R^2 =0.5064$, p = 0.0003.	Y = 34.5299 - 10.5543(conductivity)
%EPT abundance $R^2 = 0.6681$, p = 0.0046.	Y = 146.8641 - 48.9602(conductivity) + 0.6561(*C. tentans* survival)
EPT abundance $R^2 = 0.3687$ p = 0.0035.	Y = 252.3097 - 84.2216(conductivity)

Table 2. Mean R-values for the six sensitive parameters chosen in the bivariate analysis.

ETR Parameter	Ely Creek	Puckett's Creek
Clam survival	0.5761	0.5446
C. dubia survival	0.5223	0.4355
D. magna reproduction	0.1439	0.1181
C. tentans survival	0.2489	0.2755
Conductivity	0.5504	0.6329
Habitat	0.7780	0.4053
Fe in the Sediment	0.0869	0.3348

Table 3. Results of LSD t-test and mean R-values of the six most sensitive parameters.

ETR Parameter	Mean R-value	Significance
Clam survival	0.5604	A
C. dubia survival	0.4789	AB
D. magna reproduction	0.1310	C
C. tentans survival	0.2622	BC
Conductivity	0.5917	A
Habitat	0.5917	A
Fe in the Sediment	0.2109	BC

Table 4. Parameters used in respective model development.

ETR Parameters	Most Sensitive Parameters	Common Parameters from Step-wise	Common Parameters from Tailored Model	Tailored Models	
Watershed	Both	Both	Both	Ely	Puckett's
Asian clam survival	X	X	X	X	X
C. dubia survival		X	X	X	X
D.magna reproduction		X			
D.magna survival					X
C. tentans survival		X			X
C. tentants weight					X
Conductivity	X	X	X	X	X
pH			X	X	X
Al in water column			X	X	X
Fe in water column			X	X	X
Mn in water column				X	
Cu in sediment				X	
Fe in sediment		X			X
Mn in sediment			X	X	X
Habitat	X	X	X	X	X

variables (denoted as B,C) (mean R-values): clam survival (0.5225), conductivity (0.5085), and habitat score (0.5192).

Table 4 describes which parameters each ETR model utilized. The MLRA models consisting only of those three "most sensitive parameters" resulted in the lowest mean R^2 value = 0.5395 as compared to the "common parameters from step-wise" resulting in the second highest mean R^2 value = 0.6716 (Table 5). The "tailored models" created the largest mean R^2 value = 0.7858; however, they were not significantly higher than the models "common parameters from tailored models", mean R^2 value = 0.6680. ANOVA of mean R^2 values was not significant with a p-value = 0.1883, however; LSD comparisons were still made with p-value > 0.05.

Table 5. Mean R^2 values for the model created by step-wise procedures and the modified model.

Mean Macroinvertebrate Indices	Most Sensitive Parameters	Common Parameters from Step-wise	Common Parameters from Tailored Model	Tailored Model
Means for Ely Creek	0.5473	0.7943	0.6554	0.8010
St. Dev.	0.1846	0.1142	0.2093	0.1690
Means for Puckett's Creek	0.5317	0.5488	0.6805	0.7706
St. Dev.	0.1997	0.1068	0.1481	0.1441
Combined Mean values	0.5395	0.6716	0.6680	0.7858
Combined St. Dev.	0.1836	0.1660	0.1734	0.1506
ANOVA p-value=0.1883	B	AB	AB	A

Discussion

The purpose of this study was to investigate the predictive power of the ETR parameters, find parameters that might occur in any watershed and signal AMD impact, and in these processes create a time and cost efficient ETR. It was found that a modified model using the three most sensitive parameters: conductivity, clam and habitat score, to describe the variability of the macroinvertebrate indices was not significantly different from models tailored for individual watersheds or any combination of the ETR parameters. Conductivity, a water quality measure, and habitat score, a measure of the ability of the physical environment to support life, were both found to be stronger predictors of AMD impacts than clam survival. However, the latter parameter is a direct measure of impact to aquatic organisms that along with conductivity and a habitat assessment create an integrative bioassessment model capable of delineating AMD impacts. Conversely, these three parameters alone, do not adequately describe the benthic assemblages found in AMD impacted streams, as evidenced by the 30% increase in the "tailored models" ability to depict the benthic community structure compared to the "most sensitive models" (Table 5). As a result, ETR's delineating AMD impacted watersheds should include a measure of conductivity, Asian clam studies, and habitat score, but should not be limited to just these three parameters.

Conductivity and habitat score are strongly supported as sensitive ETR parameters, occurring in all the MLRA models and resulting in the two largest mean R-values 0.5085, and 0.5192, respectively (Table 3). Conductivity has been used as a water column parameter to describe macroinvertebrate community responses to anthropogenic impacts, specifically mining impacts (Garcia-Criado, 1999). When assessing mining impacts high conductivity often is found with low pH and high levels of dissolved metals and water column toxicity (Soucek et al, 2000 a, b; Latimer, 1999). Habitat assessments are integral to bioassessments because the prediction of aquatic community distributions; thus they characterize the streams' physical ability to support life (EPA, 1989). Fine sedimentation is often a cohort of low pH and high levels of dissolved metals found in AMD and AML impacted watersheds. However, excessive sedimentation in the absence of low pH and high levels of dissolved metals has been shown to depress taxa richness and abundances of macroinvertebrates, particularly the most sensitive orders, by limiting the habitat diversity (Latimer, 1999; Anderson, 1984; McClelland, 1980).

Clam survival (mean R-value 0.5225) has great potential as a bioassessment tool because of its predictive power of instream conditions. Bivalves have long been used to detect pollution in lotic systems (Doherty, 1990). Asian clams are capable of ingesting and concentrating pollutants from both the water column and sediment, giving *in situ* Asian clam studies great power as a test of real-time stream conditions. Preliminary data in the North Fork of the Powell River has shown that *in situ* clam studies are sensitive to AMD impacts (Soucek, unpublished data). The Ely Creek study (Cherry et al, In Press) also found clam survival to be an important factor when assessing AMD; however, it was not used in the final Puckett's Creek ETR (Soucek et al, 2000 a). This might be a factor of the statistical procedures followed in the different studies. Preliminary data from an additional study in Puckett's Creek has found that in fact, clam survival and growth are sensitive to both AMD and dilute nutrient impacts, further supporting the significance of this technique.

C. dubia survival, a measure of water column toxicity, became an important measure of water quality as a result of the creation of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) passed in 1946 and reauthorized in 1972 (Cooney, 1995), along with the Toxic Substance Control Act (TSCA) and Clean Water Act (CWA) of 1972. Since the standardization of toxicity tests using cladocerans in 1984, aquatic toxicity tests have been established as a cheap and time efficient bioassessment tool (EPA, 1991 b; Mount 1984). This testing technique has been included in both ETRs developed created in the Powell River watershed (Cherry, In Press; Soucek 2000 a) and again was found in all the models created through these analyses, exclusive of the "most sensitive parameters" models. This technique should be considered when assessing headwater streams that may not be ideal for *in situ* studies with Asian clams. Growth rates and survival of the Asian clam are only predictable when stream temperatures exceed 15°C, and do not exceed 25-30°C, temperature easily exceeded in headwaters during mid summer and winter (McMahon, 1983 and 1986: Belanger et al, 1991). The *C. dubia* survival test could allow for bioassessment to be conducted during these seasons and still produce accurate descriptions of the impacted sites. However, caution must be used when extrapolating these data because of the limited time period sampled with a 48-hour acute *C. dubia* test. More replication is desirable with this technique to gain a more accurate idea of the state of the water column toxicity at any given site.

The ability to test the toxicity of water flowing in small systems further supports the inclusion of *C. dubia* survival as a parameters for delineating ephemeral streams. The North Fork Powell in particular and Ely Creek, to a degree, are larger (flow) systems than Puckett's Creek and may provide more hospitable conditions for successful use of an *in situ* clam study. Reed's Creek, a smaller (flow) watershed near Puckett's Creek was reconnaissanced recently using the *in situ* clam technique where mean growth at all stations was negative, suggesting that ephemeral flow rates in small streams like Reed's Creek might impact the *in situ* test results (Schmidt, unpublished data). However, because flow is not a factor in acute toxicity testing, becomes an even more important parameter for consideration.

All the parameters discussed above have qualities that make them effective and sensitive measures of AMD impact, but they also have qualities that are time and cost effective. Removing the need to collect macroinvertebrates for bioassessment reduces the time (~78% decrease) and the cost (~15% decrease) required for successful delineation. The three parameter model does not require sediment collection and testing, resulting in an even more efficient system. The relative cost of the three-parameter model is approximately 2.3 times less than the 10 or 12 parameter "tailored models". This cost benefit may not offset the increase in the quality of the ETR derived from the "tailored models". Although these data suggest that clam survival, conductivity, and habitat score may provide direct comparisons of AMD impacted sites from different sub-watersheds of the Powell River, models including these parameters exclusively result in inadequate depictions of the ecological communities of these streams. Further research will be conducted by expanding this work to 4 new sub-watersheds of the North Fork of the Powell River and the addition of more historical analysis.

Conclusions

Eighteen different bioassessment parameters were evaluated for their predictive power of macroinvertebrate community responses to AMD impacts in a proposed ETR. Data from two adjacent watersheds of the Powell River, Southern VA, were reevaluated and three parameters: Asian clam survival, conductivity, and habitat were found to be most predictive across sub-watersheds. A series of other models depicting the macroinvertebrate community structure (models including up to 12 different bioassessment measures) failed to produce statistically more predictive models. This study suggests that measuring these three parameters alone can provide cheaper and quick integrative bioassessment of AMD impacts throughout the Powell River watershed, however, they may not be predictive enough of the aquatic communities when used alone. Further research will allow for construction of a cheap universal means to bioassess AMD impacted watersheds.

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