Water **2014**, *6*, 1312-1338; doi:10.3390/w6051312

OPEN ACCESS

water

ISSN 2073-4441

www.mdpi.com/journal/water

Article

Benthic Communities of Low-Order Streams Affected by Acid Mine Drainages: A Case Study from Central Europe

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Received: 3 March 2014; in revised form: 16 April 2014 / Accepted: 25 April 2014 / Published: 14 May 2014

Abstract: Only little attention has been paid to the impact of acid mine drainages (AMD) on aquatic ecosystems in Central Europe. In this study, we investigate the physico-chemical properties of low-order streams and the response of benthic invertebrates to AMD pollution in the Banská Štiavnica mining region (Slovakia). The studied streams showed typical signs of mine drainage pollution: higher conductivity, elevated iron, aluminum, zinc and copper loads and accumulations of ferric precipitates. Electric conductivity correlated strongly with most of the investigated elements (weighted mean absolute correlation = 0.95) and, therefore, can be recommended as a good proxy indicator for rapid AMD pollution assessments. The diversity and composition of invertebrate assemblages was related to water chemistry. Taxa richness decreased significantly along an AMD-intensity gradient. While moderately affected sites supported relatively rich assemblages, the harshest environmental conditions (pH < 2.5) were typical for the presence of a limited number of very tolerant taxa, such as Oligochaeta and some Diptera

(*Limnophyes*, Forcipomyiinae). The trophic guild structure correlated significantly with AMD chemistry, whereby predators completely disappeared under the most severe AMD conditions. We also provide a brief review of the AMD literature and outline the needs for future detailed studies involving functional descriptors of the impact of AMD on aquatic ecosystems.

Keywords: water pollution; heavy metals; benthos; headwater streams; Banská Štiavnica; Slovakia

1. Introduction

The specific type of inorganic water pollution known as acid mine drainage (AMD) results from the biological and chemical oxidation of pyrite or other sulfide-containing minerals that are exposed to oxygenated water [1,2]. AMD is one of the major causes of environmental degradation throughout the world [3,4]. The nature of AMD (typically low pH, high metal concentrations and metal precipitations) makes it a particularly important factor, disturbing or even destroying river ecosystems. Various environmental pressures resulting from AMD affect all aquatic organisms. The effects of AMD may considerably reduce species richness and the abundance of primary producers [5–7] and significantly modify the composition and functional attributes of aquatic fauna [8].

Bottom-dwelling macroinvertebrates are generally seen as the most sensitive organisms in their response to AMD (for a brief summary, see Appendix Table A1). The relationship of AMD and benthic macroinvertebrate communities has been widely studied, and benthic invertebrate fauna is often used either as an indicator of the intensity of AMD effects or to differentiate reference and contaminated streams and rivers [9–13]. The intensity of the impact of AMD on bottom-dwelling invertebrates is related to concentrations of harmful substances or pH values and ranges from a hardly detectable influence to a nearly complete elimination of benthic fauna [14]. Generally, AMD is known to modify the species composition and diversity of benthic communities [15,16] and decreases overall or taxa-specific abundance and biomass [17,18]. In turn, it causes alterations in the trophic structure of assemblages [19,20] and stream food webs [21].

The specific responses of different groups of benthic invertebrates (at family, genera or even species levels) vary greatly [11], implying a need for detailed taxa-level resolution within AMD impact studies and more knowledge of the specific response of individual taxa to AMD effects, e.g., [12,22]. Despite this, a majority of the studies concerning the relationships of benthic invertebrates to AMD assess the types of response only at the family or even order levels [23].

In addition to the individual sensitivity of benthic animals, the overall effect of AMD also depends on the character of mining activities, the bedrock of the mining area, but also on the flow (dilution rate), pH, alkalinity or buffering capacity of the receiving stream, as well as geomorphic and biological conditions [24]. These environmental factors are strongly geographically variable, and therefore, it is expected that the characteristics of AMD vary from place to place [25]. Thus, studies from different parts of the world concerning various types of AMD acting at specific local conditions [22,26] could improve our understanding and allow for wider generalizations of the effects of AMD on benthic communities. In Central Europe, almost no attention has been paid to the impact of AMD on the environment, and case studies that would describe these effects on aquatic invertebrates are lacking.

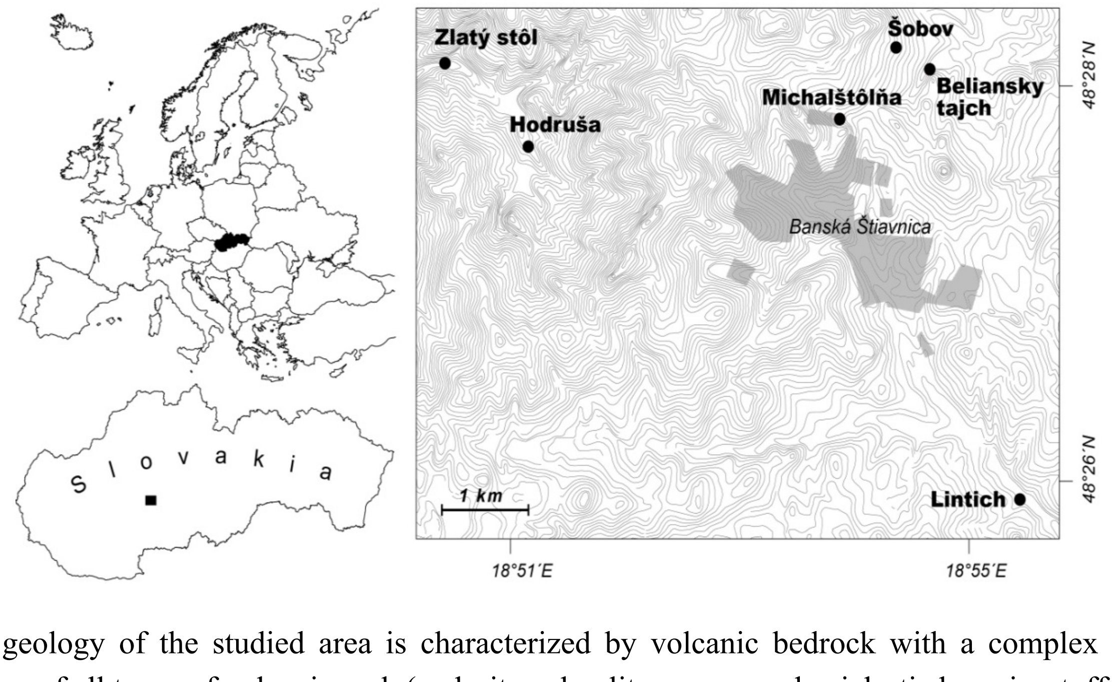
Here, we investigated the response of macroinvertebrate assemblages to AMD from locally specific conditions of abandoned mines and mine waste objects in the Banská Štiavnica mining region (Slovakia, Central Europe). We avoided rapid approaches of low taxonomic resolution and assessed the effect of AMD at the lowest possible taxonomic level. Our specific aims were: (i) to describe the physico-chemical properties of AMD-polluted streams in the studied area; (ii) to describe the assemblage composition of benthic invertebrates affected by AMD; and (iii) to assess the relationships between the AMD chemistry and benthic invertebrate assemblages.

2. Materials and Methods

2.1. Study Sites

The investigated sites are located in the vicinity of the Banská Štiavnica mining region (Slovakia, Central Europe) (Figure 1). Intensive historical ore mining and related activities have left a legacy of abandoned mines, sulfide-bearing waste rock piles and tailing ponds in this area. These abandoned mines and mine wastes are intensive sources of local environmental contamination, mostly in the form of acid mine drainages, which are typical in having a higher content of iron oxide, aluminum and other metals [27–29].

Figure 1. Map showing the location of the studied localities.



The geology of the studied area is characterized by volcanic bedrock with a complex structure consisting of all types of volcanic rock (andesites, rhyolites, pyro- and epiclastic breccias, tuffs and ash rocks, *etc.*; [30]). The climate is moderately cool, with a duration of snow cover of approximately 80 days. The mean annual precipitation totals ranges between 700 and 800 mm and mean annual specific runoff is about 10 L s^{-1} km^{-2} [31].

Six sites affected by effluents from the various types of AMD sources were investigated: abandoned mine adits (the sites Beliansky tajch, Hodruša, Zlatý stôl), an abandoned heap of metallurgical slag (Lintich) and spoil dumps (Šobov, Michalštôlňa) (see also Appendix Table A2). All sites were located in close proximity to a mine or mine waste objects and could be characterized as small, first order, permanently flowing streams with depths ranging from 0.06 to 0.17 m (average = 0.1 m) and a stream width of 0.7–2.1 m (1.3 m). Sites were located at elevations between 460 and 660 m a.s.l. Cobbles, coarse gravel, and accumulations of coarse particulate organic matter were the dominant substrate types at all the studied sites.

2.2. Water Chemistry and Benthic Invertebrates

Macroinvertebrates and water chemistry samples were taken during base-flow discharges from late August to September, 2010. Each site was sampled extensively during one sampling occasion. Physicochemical characteristics, such as pH and electrical conductivity (25 °C), were measured in the field using a Multi 3401i water parameter meter (WTW) with SenTix 41-3 and TetraCon®325 probes (Wissenschaftlig-Technische Werkstätten GmbH, Weilheim, Germany). At each site, water samples were taken from the stream current using 500-mL plastic bottles. The samples were immediately transported to the laboratory. Samples were filtered using Whatmann-grade 40 filter paper and maintained at 4 °C until analyzed. Chemical elements of mine waters were determined at Acme Analytical Laboratories, Ltd (Vancouver, Canada), by the ICP-ES/ICP-MS method. The sulfate concentration was determined by a titration method using $Pb(NO_3)_2$ [32].

Exhaustive kick-net (mesh size 250 μm) sampling [33] of benthic macroinvertebrates was performed at each site. All substrate types were sampled during a constant time interval (10 min). The collected material was preserved with 4% formaldehyde and stored in plastic bottles. Organisms were hand sorted and identified to the lowest possible taxonomic level using general and specialized keys to identify benthic macroinvertebrates [34–39].

2.3. Statistical Analysis

Data on chemical variables were summarized in a chemical data matrix, consisting of 6 sampling sites by 21 variables (Table 1). A list of invertebrate species was compiled to obtain a biological data set (Table 2). Moreover, taxa were classified into functional feeding groups (FFG) following Šporka [40]. Total species richness, the richness of sensitive groups (Ephemeroptera, Plecoptera and Trichoptera (EPT)), the numbers of FFG, the assemblage composition (species presence/absence matrix) and FFG composition (FFG presence/absence matrix) were used as response variables in the analyses.

Indirect ordination methods were employed to summarize and visualize major trends in the chemical and biological data sets. The chemical data matrix was submitted to principal component analysis (PCA). In order to equalize the weight of dimensionally heterogeneous variables in the analysis, data were standardized, and PCA was conducted on a correlation matrix. The broken stick model was used to determine the number of principal components (PCs) that represented non-trivial (significant) variation in the chemical data matrix [41]. PCA on the correlation matrix was also conducted on FFG composition. Data on species composition were summarized using principal coordinate analysis (PCoA, [42]) with the Sørensen index [43] as an ecologically meaningful measure of dissimilarity. Species scores were added into the PCoA ordination as weighted sums of the species matrix [44].

Table 1. Summary characteristics of environmental variables measured at sampling sites. The loadings of each variable on the first principal component (PC1.chem) derived from water-chemistry data are shown.

Variable	Unit	Mean	Minimum	Maximum	PC1.chem Loading
pH		5.63	2.45	7.20	−0.248
Conductivity	µS cm^{-1}	1971	363	6,960	0.256
SO_4^{2-}	mg L^{-1}	2129.3	186.0	8330.0	0.256
Al	mg L^{-1}	72.0	0.01	430.0	0.257
As	µg L^{-1}	0.6	0.5	1.2	−0.073
Ba	µg L^{-1}	41.9	6.3	135.0	0.251
Ca	mg L^{-1}	182.9	39.0	410.0	−0.019
Cd	µg L^{-1}	12.6	0.1	39.6	0.151
Co	µg L^{-1}	226.1	0.1	1082.0	0.256
Cr	µg L^{-1}	37.4	0.5	221.0	0.257
Cu	µg L^{-1}	420.9	4.2	2240.0	0.257
Fe	mg L^{-1}	232.2	0.01	1390.0	0.257
K	mg L^{-1}	2.9	0.5	4.1	−0.234
Mg	mg L^{-1}	68.7	9.5	221.0	0.251
Mn	mg L^{-1}	6.4	0.005	30.1	0.257
Na	mg L^{-1}	8.3	4.9	14.6	0.060
Ni	µg L^{-1}	7.9	2.4	20.6	0.077
S	mg L^{-1}	537.4	40.0	2256.0	0.257
Si	mg L^{-1}	14.5	3.1	40.2	0.243
Sr	µg L^{-1}	398.6	196.0	987.0	−0.058
Zn	µg L^{-1}	780.9	42.3	4050.0	0.255

In the next step, the relationships between chemical and biological characteristics of AMDs were examined. Unfortunately, our data possess several limitations that prevent the use of traditional modeling approaches: (1) the number of variables is much higher than the sample size, due to the intrinsically limited number of AMD sampling sites (the so-called "ill-posed problem"); and (2) potential predictors (chemical variables) are highly correlated ("multicollinearity problem"). Using classical regression-based approaches, in the first situation, there is an infinite number of solutions, and the second situation may lead to instable solutions. Therefore, we adopted indirect techniques to relate the chemical and biological properties of AMDs. In the analyses, significant principal components of chemical PCA were used instead of the originally measured water-chemistry data. The PCs are linear combinations of the original variables and uncorrelated to each other.

The relationship between diversity and environmental conditions were analyzed using generalized linear models (GLM; [45]). GLMs with Poisson distribution and the logarithmic link-function were used to relate invertebrate species richness, EPT richness and FFG richness with the significant PCs derived from the chemical data.

The Mantel test [46] was employed to explore how closely the multivariate data on chemistry and invertebrates (FFG and assemblage composition) match and to test these associations. In the first step, data sets were transformed to matrices of pair-wise dissimilarities among sampling sites. To be consistent with the previous analyses, dissimilarity matrices of chemical data and FFG were constructed using pair-wise Euclidean distances (a distance implicit in PCA), and the dissimilarity of species data was based on the Sørensen index (as used previously in PCoA). The Mantel statistic was calculated as the Spearman correlation coefficient, ρ, between the dissimilarity matrices. The statistical significance of the correlations was assessed using 95% confidence intervals obtained from 10,000 bootstrap replicates.

To further investigate the relationships between biological and environmental variables, the significant PCs of the chemical data matrix were overlain on the PCoA (assemblage composition) and PCA (FFG composition) ordinations as a smooth environmental surface using the function, ordisurf [44]. The function fits an environmental surface using a generalized additive model (GAM) with thin plate splines [47] and then uses the resulting GAM to predict and plot the surface on an ordination space. The smoothing parameter was selected by restricted maximum likelihood, and the complexity of the smoothing was restricted using five knots. The resulting indirect ordinations with a smoothed environmental surface provide a visual reference to those environmental conditions in which each taxa and FFG, respectively, are located.

All analyses were performed in R [48] using the packages, ecodist [49] and vegan [44].

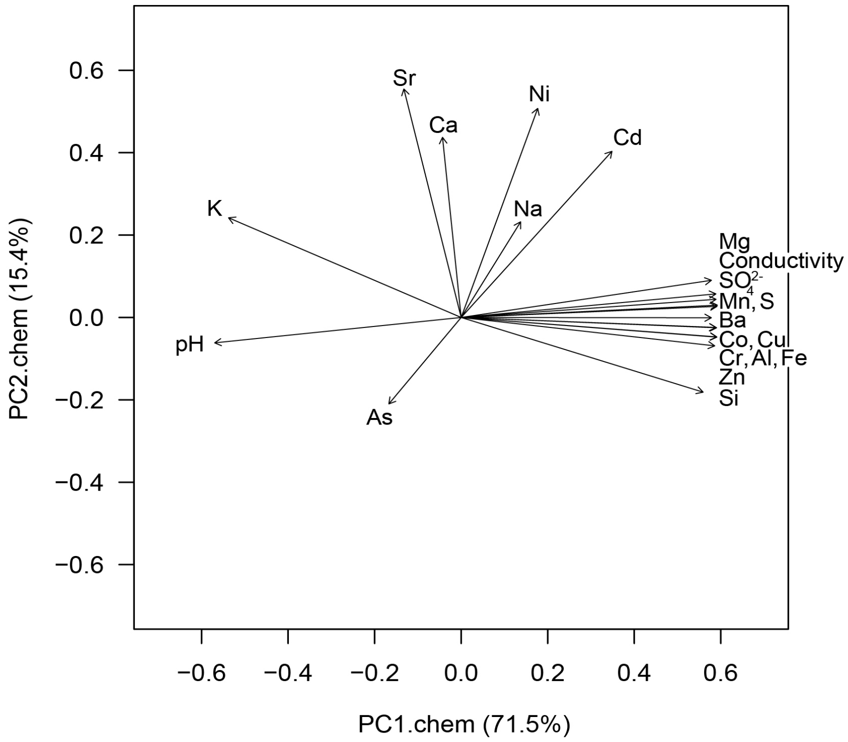
3. Results

3.1. Water Chemistry

The investigated AMD-affected sites showed variable chemical properties (Table 1, Appendix Table A2). Water pH ranged from strongly acidic to circum-neutral. Most of the sites showed typical signs of mine drainage pollution: higher conductivity, elevated iron, aluminum, zinc, sulfates and copper concentrations and accumulations of ferric hydroxide precipitations. In general, the measured chemical parameters were strongly intercorrelated (weighted mean absolute correlation (95% CL) = 0.86 (0.84, 0.88)). For details, see Appendix Table A3.

In particular, electric conductivity was highly correlated with the other variables (0.95 (0.91, 0.97)). The high degree of correlation between variables is evident from the principal component analysis (Figure 2). According to the broken stick model, the first principal component (PC1.chem) by itself accounted for a significant amount (71.5%) of the total variability in the chemical data. PC1.chem represents an AMD-intensity gradient, since the concentration of metals, conductivity and sulfur compounds showed relatively high positive loadings, while pH had a negative loading on this component (Table 1). In other words, the left side of the ordination space is characterized by higher pH and lower concentrations of dissolved metals, and towards the right side, the acidity and concentrations of metals sharply increase.

Figure 2. PCA on the correlation matrix of water-chemistry data. The variance explained by a particular component is displayed in parentheses.



3.2. Diversity

In total, 26 taxa were identified at the AMD-affected sites investigated (Table 2, Appendix Table A4). The local diversity of benthic invertebrates was low, with richness ranging from three to 12 taxa. Species composition was typical for small rivulets, with the dominance of Oligochaeta and small Dipteran larvae of the families, Chironomidae and Ceratopogonidae. The highly sensitive mayflies were absent from all the studied sites. The dominance of shredders, collector gatherers and predators was typical for these headwater streams.

The results of the GLM showed that aquatic invertebrate diversity was significantly related to the water chemistry data ($\chi^2_{(1)} = 4.32$, $p = 0.038$). The number of invertebrate taxa (S) was negatively related to the first principal component of the chemical data (PC1.chem) according to the GLM: $\ln(S) = 1.98 - 0.30\text{PC1.chem}$. That is, invertebrate diversity decreased along a gradient of decreasing pH and increasing concentrations of dissolved metals. The model explained 67.2% of the deviance in invertebrate taxa richness. Similarly, EPT richness was significantly related to PC1.chem ($\chi^2_{(1)} = 4.46$, $p = 0.035$). The GLM model ($\ln(\text{EPT}) = -0.19 - 1.64\text{PC1.chem}$) explained 73.4% of the deviance in EPT richness. The number of functional feeding groups (FFG) did not show a significant relationship to the water-chemistry data ($\chi^2_{(1)} = 0.56$, $p = 0.453$).

More detailed relationships between invertebrate diversity and measured environmental data are given in Appendix Figure A1.

Table 2. Checklist of aquatic invertebrates found at acid mine drainages (AMD) sites. Abbreviations of taxa names, the classification to the functional feeding group (F, filtering collector, G, gathering collector, P, predator, Sc, scraper, Sh, shredder) and the relative frequency of occurrence are displayed. FFG, functional feeding groups.

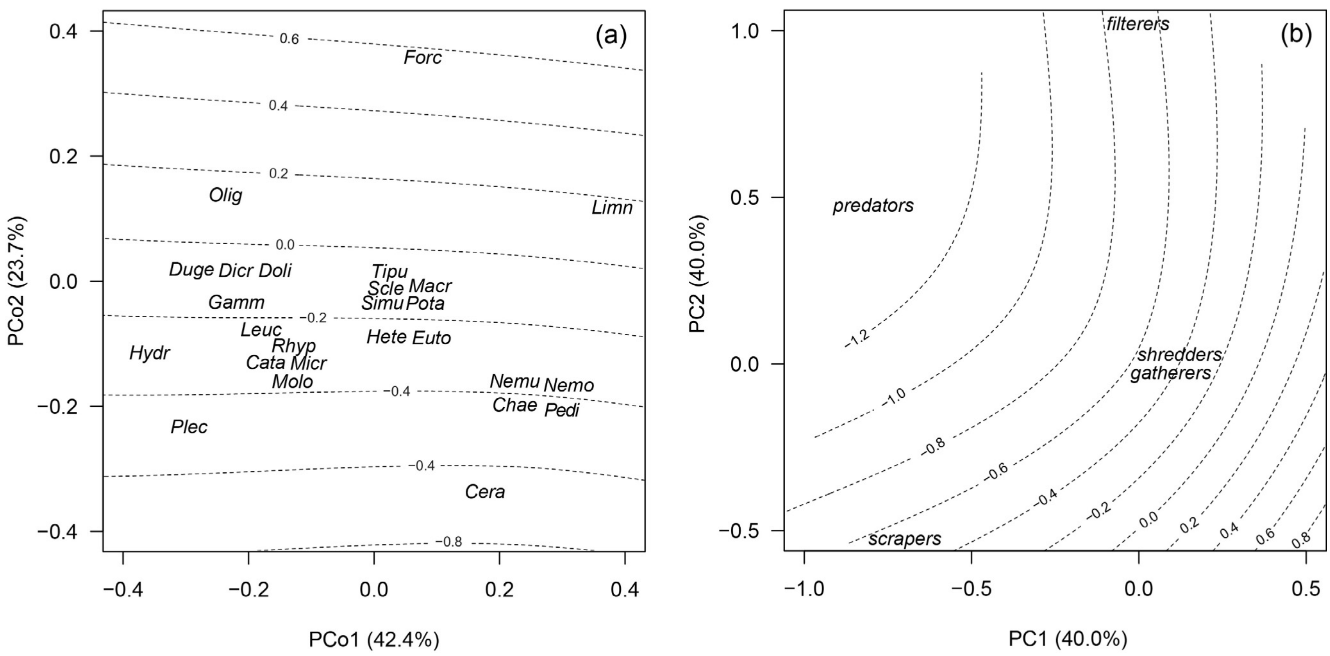
Taxon	Abbreviation	FFG	Frequency (%)
Turbellaria			
Dugesia polychroa (Schmidt, 1861)	Duge	P	14
Oligochaeta			
Oligochaeta indet.	Olig	G	71
Amphipoda			
Gammarus fossarum (Koch, 1836)	Gamm	Sh	29
Plecoptera			
Nemoura spp.	Nemo	Sh	29
Nemurella pictetii (Klapálek, 1900)	Nemu	G	29
Leuctra spp.	Leuc	G/Sc/Sh	29
Coleoptera			
Hydroporus sp.	Hydr	P	29
Trichoptera			
Plectrocnemia conspersa (Curtis, 1834)	Plec	P	57
Micropterna sp.	Micr	Sh	14
Potamophylax nigricornis (Pictet, 1834)	Pota	Sh	14
Lepidoptera			
Cataclysta lemnata (Linnaeus, 1758)	Cata	Sh	14
Diptera			
Tipulini indet.	Tipu	Sh	14
Molophilus sp.	Molo	G	29
Rhypholophus haemorrhoidalis (Zetterstedt, 1838)	Rhyp	P	14
Scleroprocta sp.	Scle	G	14
Eutonia sp.	Euto	P	14
Dicranota sp.	Dicr	P	14
Pedicia sp.	Pedi	P	29
Macropelopia sp.	Macr	P	14
Chaetocladius sp.	Chae	G	29
Heterotrissocladius marcidus (Walker, 1856)	Hete	G	14
Limnophyes sp.	Limn	G	57
Ceratopogoninae indet.	Cera	P	43
Forcipomyiinae indet.	Forc	Sh	14
Simuliidae indet.	Simu	F	14
Dolichopodidae indet.	Doli	P	14

3.3. Assemblage Composition

The site-to-site variation in water-chemistry data was significantly correlated with the variation in invertebrate assemblages (Mantel ρ (95% CL) = 0.38 (0.16, 0.57)), meaning that environmentally similar sites also have similar assemblage compositions, and different sites have dissimilar assemblage compositions. A significant correlation was also found between FFG composition and water-chemistry (0.46 (0.29, 0.78)).

The PCoA ordination of invertebrates was overlain with the fitted smooth surface of PC1.chem (Figure 3a). The GAM showed that invertebrate assemblage composition was significantly related to water chemistry (equivalent degrees of freedom of smoothness = 2.5, approximately $p = 0.026$). Taxa typical for milder AMD conditions, such as stoneflies (*Nemoura*, *Nemurella*) and some Diptera from the families, Ceratopogonidae (Ceratopogoninae), Chironomidae (*Chaetocladius*) and Pediciidae (*Pedicia*), are clustered in the lower right side of the ordination plot. As evident from the contour lines of fitted PC1.chem, environmental conditions change towards the upper part of the ordination, where low pH and a high concentration of dissolved metals dominate. The presence of Oligochaeta, *Limnophyes* (Chironomidae) and the subfamily, Forcipomyiinae (Ceratopogonidae), was typical for these extreme AMDs. Furthermore, FFG composition was significantly related to water chemistry (GAM, estimated degrees of freedom = 2.0, approximate $p = 0.009$). Predators occurred in milder AMD conditions, while shredders and gathering collectors dominated at extreme AMD sites (Figure 3b).

Figure 3. (**a**) Principal coordinate plot based on the Sørensen dissimilarity of invertebrate assemblage compositions; (**b**) principal component plot of the functional feeding group composition. Contour lines represent the smooth surface of chemical PC1.chem (see Figure 2) fitted using a GAM. Variation explained by a particular axis is displayed in parentheses. Abbreviations of taxa names are given in Table 2.



4. Discussion

4.1. Water Chemistry

The chemical properties of AMD-affected streams in the Banská Štiavnica mining region vary substantially among sites. Exceptionally low pH (<2.5), associated with extremely high metal (particularly iron, aluminum, zinc and copper) and sulfate concentrations, was detected in the effluents from the spoil dump of the pyritized hydroquarzite mine, Šobov. On the other hand, slightly acid or even neutral conditions were found in waters draining long-abandoned sites. The water chemistry of the drainages from the old mine adit, Zlatý stôl, and metallurgic slag heap, Lintich, was similar to unaffected streams in the region [50] or showed only slightly elevated concentrations of some elements (e.g., copper, strontium). Finally, effluents from mine adits with intermediate pH values (5.0–6.0) were characterized by massive precipitations of ferric compounds consisting of goethite, ferrihydrite and schwertmannite [51,52]. In general, the chemical composition of AMD-affected streams changes considerably in response to climatic, hydrogeological, geological and mineralogical factors and may even vary seasonally [53]. In the Banská Štiavnica mining region, the studied sites are located within the same geological and climatic region, and thus, the variability of chemical properties can be mainly attributed to differences in the local properties of AMD sources. For example, the low pH and high metal concentrations of the spoil dump, Šobov, are related to the ongoing intensive microbiological-chemical degradation of pyrite. In contrast to the other sites, Šobov is an open system directly exposed to surface precipitation and air, which are the main conditions required for the biochemical activity of mineral decomposing microorganisms (at this site, *Acidithiobacillus*; [29]). This supports the findings that local AMD chemistry usually depends on the character of the mine waste deposit [53,54].

Unsurprisingly, there was a near absolute correlation between electric conductivity and concentrations of the majority of the elements. This confirms conductivity as a good and relatively simply measured proxy indicator of the degree of stream contamination by AMD [53].

4.2. Diversity

The diversity of benthic invertebrate assemblages was quite low in the investigated streams. Total taxa richness ranged from three taxa at the site with an extremely low pH to moderate numbers (maximum 13 taxa) at sites with a higher pH. Such a low diversity at AMD-affected streams has also been reported elsewhere [17,55].

In the present study, invertebrate diversity decreased significantly along the gradient of increased AMD-intensity (*i.e.*, the gradient of increasing acidity and metal loads). In general, most studies concerning AMD and its effect on benthic invertebrate communities show clear reductions in the number of taxa following low pH or metal pollution (Appendix Table A1). Acidity is a well-known factor with a strong impact on macroinvertebrate diversity, as has been demonstrated by numerous AMD studies (e.g., [12,14]). Among various invertebrate groups, Ephemeroptera are particularly sensitive to low pH [17,56,57]. This is in accordance with our results from the Banská Štiavnica mining region, where mayflies were totally absent from AMD sites, and the overall reduction in diversity could be, at least partially, attributable to the loss of mayflies.

As has been shown by Gray and Delaney [23], the effects of low pH and high metal loads on aquatic invertebrate diversity may be similar, and they usually cannot be separated (but, see Soucek *et al.*, Gerhardt *et al.* [58,59]). Similar to the effect of acidity, streams contaminated by metals show reduced richness of many invertebrate groups (Appendix Table A1). For example, Malmqvist and Hoffsten [12] indicated that zinc along with copper might be responsible for the reductions in taxonomic richness they observed in Swedish streams. Clements *et al.* [60] showed that even intermediate contamination of streams by heavy metals may significantly reduce the diversity of sensitive groups. In some of the streams investigated here, we found very high loads of dissolved metals, such as zinc (4050 μg L^{-1}), copper (2240 μg L^{-1}) and cadmium (40 μg L^{-1}), to name a few, which are far beyond the tolerance limits of most aquatic invertebrates.

Indeed, the effect of AMD on surface waters is a combination of acidity and metal toxicity. Moreover, various metals often occur in high concentrations simultaneously, and the possible synergistic effects of these elements on aquatic invertebrates are largely unknown. Thus, it is difficult or even impossible to identify one single factor that has the greatest effects on invertebrate diversity in AMD-impacted streams.

4.3. Assemblage Composition

Both pH and metal loads are important drivers of benthic community structure at AMD-affected sites. Community composition usually shifts towards the dominance of the most tolerant taxa, such as some chironomids and oligochaetes [22,23,61]. On the other hand, taxonomic groups that are sensitive to the negative effects of AMD are reduced or completely disappear. This is especially pronounced in the case of Ephemeroptera, Plecoptera and Trichoptera (EPT taxa). EPT taxa are generally known to be particularly sensitive to AMD-related pollution and, therefore, are widely used in monitoring its impact on stream ecosystems (e.g., [13,20,62]). Impoverished benthic communities with a shifted composition are especially evident in heavily affected sites, like those in the Banská Štiavnica mining region. Here, mayflies were totally absent, and stonefly and caddisfly assemblages consisted of just a few tolerant taxa. As stated above, mayflies have been recognized as being particularly sensitive to metal pollution in streams and quickly disappear with decreasing pH [63]. Nevertheless, some EPT taxa may be largely tolerant and even dominate at AMD-polluted sites [12,22,59,64]. Stoneflies and caddisflies found in the Banská Štiavnica mining region have frequently been reported from acidified water bodies in Central Europe [65,66]. Some of these taxa (*Nemurella pictetii*, *Plectrocnemia conspersa*) are even referred to as acid-tolerant generalists, extremely acid-tolerant or have been found to dominate in strongly acidified stream communities [67].

An extreme example of an altered assemblage composition is the spoil dump site, Šobov, where the metal loads greatly exceed the values reported to have significant effects on macroinvertebrate communities (e.g., [55,68,69]). This site supported a species-poor assemblage of only three taxa—oligochaetes (Oligochaeta indet.), midges of the genus, *Limnophyes* (Diptera, Chironomidae), and the subfamily, Forcipomyiinae (Diptera, Ceratopogonidae). Such an assemblage composition is in a good agreement with previous mine drainage studies, where those taxa were proven to be very tolerant. Oligochaetes, especially tubificid worms, are known to be resistant to AMD-related pollution [59,70].

Chironomids, particularly Orthocladiinae, have also been reported to be relatively tolerant to metal pollution [12,69,71]. Orthocladiinae midges of the genus, *Limnophyes*, found in the present study, are considered to be strongly acidophilic [72]. Larvae of *L. asquamatus* (Andersen, 1937) were, together with the larvae of *Agabus* sp., the only benthic invertebrates found in an extremely acid brook polluted by drainage waters from waste deposits of abandoned sulfuric mines in Vígľašská Huta, Kalinka (Central Slovakia) [73]. Finally, representatives of the subfamily, Forcipomyiinae, are also able to tolerate extreme environmental conditions. For example, *Forcipomyia* sp. is frequently found in water-filled tree holes, an extreme anoxic habitat characterized by a high level of dissolved solids and low pH [74].

It should be noted that identification to the lowest possible taxonomic level provided important information in our analysis. The family, Ceratopogonidae, was represented by two subfamilies, which showed strikingly different responses to AMD. Representatives of the subfamily, Ceratopogoninae, were sensitive to AMD, while those of Forcipomyiinae appeared to be extremely tolerant. Thus, rapid assessment protocols involving identification to a higher taxonomic level (e.g., [23]) would not have been useful here.

The indirect effects of AMDs are usually associated with massive precipitations of ferric compounds. Ferric hydroxide precipitations may change the structure and quality of benthic habitats and alter the availability of food resources. Precipitate plaques restrict the diversity and biomass of periphyton [69] or make it inaccessible to many invertebrates as a food resource [21]. Coupled with toxic metals, they are also known to impact the organic matter breakdown process by hindering microbial colonization and/or by altering the function and reproduction of aquatic hyphomycetes [75,76]. Consequently, this may change the availability and palatability of the food supply and lead to a decrease or even complete elimination of shredding and scraping macroinvertebrates (e.g., [20,70,77]). The complex study of Hogsden and Harding [78] documented that the combined effect of anthropogenic acidity and metal loads may even change the size and simplify whole benthic food webs, due to substantial changes in multiple trophic levels [21,78]. In the present study, the composition of functional feeding groups changed significantly along the AMD-intensity gradient. Predators were completely missing in the assemblage of AMD sites with extremely low pH, while the other trophic guilds did not markedly differ in their composition. This discrepancy between a general tendency toward the elimination of scrapers and shredders and our results perhaps reflects two facts. Firstly, scrapers are naturally under-represented, while shredders dominate in the assemblages of low-order streams [79], such as those studied in the Banská Štiavnica mining region. Secondly, precipitations of ferric compounds did not develop compactly at the investigated sites, and thus, the blanketing effect of ferric hydroxides was restricted to particular areas only. Thus, detritivorous invertebrates (shredders and collector gatherers) were likely able to find enough food to maintain their populations. The complete elimination of predators in extremely metal-polluted streams was also observed by Clements *et al.* [60]. From this field study alone, we can only speculate on the factors that limit predators under strong AMD pollution. Possible explanations include higher sensitivity and biomagnification. However, there is little support for either hypothesis in the literature, and the latter is still a poorly understood phenomenon in complex aquatic food webs [80,81].

5. Conclusions

Understanding the locally-specific effects of AMDs on benthic organisms is critical to the successful remediation of impacted streams and for the general planning of stream restoration guidelines. The results from this study conducted in Central Europe indicate that water chemistry varies substantially among AMD sites and depends on the character of the mine waste deposit. Most of the studied sites showed typical signs of mine drainage pollution: elevated iron, aluminum, zinc and copper loads and accumulations of ferric precipitates. Electric conductivity correlated strongly with most of the elements and could thus be used as a good proxy indicator for AMD pollution.

The invertebrate assemblages found generally confirm the observations made by other authors working with AMDs in other parts of the world: a decrease in total invertebrate diversity, a tolerance of some Oligochaeta and Diptera and a high sensitivity of Ephemeroptera. Assemblage compositions and the composition of trophic guilds changed significantly along the gradient of AMD intensity. In contrast to approaches based on coarse taxonomic resolution, the identification of invertebrates to the lowest possible taxonomic level provided important additional details for the analyses.

From the brief literature review provided here (see Appendix Table A1), it is apparent that studies of AMD effects on the biomass and functional structure of benthic communities are generally lacking. Moreover, we are not aware of any study considering the impact of AMD on the production of benthic invertebrates. Indeed, studies of biomass and secondary production are more laborious and time consuming, but provide information of high value, because they integrate a number of components of ecological relevance, such as density, biomass, individual growth rate, reproduction, survivorship and development time [82]. Furthermore, research on species traits, such as functional feeding groups, holds much potential for predicting the functional consequences of human activities on aquatic ecosystems [83]. We believe that future studies of AMD impacts on aquatic ecosystems would be greatly enhanced if they involved secondary production, species traits or other functional descriptors that provide ecologically informative insights into ecosystem functioning.

Acknowledgments

This study was co-founded by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Academy of Sciences, Grant No. 1/0176/12, and the Slovak Research and Development Agency under the contract, No. SK-CZ-0187-11. We are grateful for language correction by David Hardekopf. Thanks are due to Lívia Olahová for providing the map and Andrea Zapriháčová for her help during field and laboratory works.

Author Contributions

Eva Michalková designed the study. Invertebrate and water samples were collected by Eva Michalková, Miroslav Očadlík, Milan Novikmec and Marek Svitok. Eva Michalková and Branislav Máša performed chemical analyses, Peter Bitušík, Milan Novikmec, Jozef Oboňa and Miroslav Očadlík determined macroinvertebrates and Marek Svitok conducted data analyses. The manuscript was conceptually proposed and largely written by Marek Svitok and Milan Novikmec. All authors contributed to the review of the manuscript.

Appendix

Table A1. A brief literature summary of the impact of AMD on benthic macroinvertebrates. The plus signs denote a response of total invertebrate richness (Richness), total density (Density) and biomass (Biomass), functional feeding groups (FFG), Ephemeroptera (Eph), Plecoptera (Ple), Trichoptera (Tri) and Diptera (Dip) to the effects of low pH (pH), high iron concentration (Fe) and metal pollution (MP), respectively.

Source	Richness			Density			Biomass			FFG			Eph			Ple			Tri			Dip		
	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP
[11]			+			+									+						+			
[12]			+			+			+						+			+			+			
[13]													+		+	+		+				+		+
[14]	+	+	+																					
[17]			+			+																		
[18]	+			+			+																	
[19]											+	+												
[20]	+			+						+			+			+			+			+		
[22]	+		+																					
[23]	+		+	+		+																		
[26]				+	+																		+	
[59]				+		+							+		+				+		+	+		+
[60]			+									+			+			+			+			
[62]													+		+	+		+	+		+	+		+
[70]																						+		
[84]															+			+						
[85]		+			+																			
[86]												+			+		+	+			+			
[87]																					+			
[88]				+															+					
[89]															+			+						
[90]			+									+			+			+						
[91]																+								

Table A1. *Cont.*

Source	Richness			Density			Biomass			FFG			Eph			Ple			Tri			Dip		
	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP	pH	Fe	MP
[92]			+																					
[93]																				+	+			
[94]															+									
[95]				+		+																		
[96]														+	+									
[97]														+										
[98]			+																					
[99]	+			+									+			+			+					
[100]	+		+	+		+																+		+
[101]																						+		+
[102]			+																					
[103]	+		+	+		+																		
[104]			+			+									+			+			+			
[105]												+			+									
[106]															+									
[107]			+			+						+			+			+			+			
[108]													+		+									
[109]			+			+									+			+			+			+
[110]	+	+	+	+	+	+																		
[111]			+																					+
[112]	+		+																+		+			
[113]			+			+									+			+			+			+
[114]			+						+						+						+			+

Table A2. Basic characteristics and physico-chemical parameters of the studied sites.

Characteristic	**Hodruša**	**Beliansky tajch**	**Lintich**	**Zlatý stôl**	**Michalštôlňa**	**Šobov**
	48°27'39.10" N 18°51'12.64" E	48°28'14.23" N 18°54'37.67" E	48°26'1.18" N 18°55'40.07" E	48°28'3.18" N 18°50'26.36" E	48°27'56.17" N 18°53'52.92" E	48°28'20.23" N 18°54'19.36" E
Altitude (m)	559	582	460	520	660	650
Type of mining activity	Abandoned mine adit for polymetallic and silver ores mining	Abandoned drainage mine adit	Abandoned slag heap	Abandoned drainage mine adit	Abandoned spoil dump	Abandoned spoil dump
Source of contamination	outflow	outflow	drainage	outflow	drainage	drainage
Average stream width (m)	2.1	1.5	1.3	1.0	1.1	0.9
Average stream depth (m)	0.17	0.12	0.14	0.09	0.09	0.06
Proportion of substrates (% Co/Pe/CG/CPOM) *	30/15/35/20	40/0/20/40	30/10/30/30	20/10/40/30	30/10/30/30	50/10/20/20
Presence of metal hydroxides precipitates **	3	3	2	1	1 $^{+}$	2
pH	5.80	5.90	6.50	7.20	5.92	2.45
Conductivity (μS cm^{-1})	831	1740	363	464	1465	6960
SO_4^{2-}(mg L^{-1})	860	1840	210	186	1350	8330
Al (mg L^{-1})	0.645	0.594	0.006	0.022	0.570	430.0
As (μg L^{-1})	1.15	0.50	0.60	0.50	0.50	0.50
Ba (μg L^{-1})	13.6	41.9	34.2	6.3	20.5	135.0
Ca (mg L^{-1})	169.5	410.0	57.7	39.0	286.0	135.0
Cd (μg L^{-1})	0.27	0.32	1.40	0.05	39.60	33.80
Co (μg L^{-1})	60.0	200.0	0.2	0.1	14.1	1082.0
Cr (μg L^{-1})	0.7	0.5	0.7	0.6	1.1	221.0
Cu (μg L^{-1})	39.5	4.2	55.0	34.4	152.0	2240.0
Fe (mg L^{-1})	0.01	3.02	0.02	0.03	0.01	1390.00
K (mg L^{-1})	3.2	3.6	3.3	2.8	4.1	0.5
Mg (mg L^{-1})	14.1	57.4	9.5	43.3	66.7	221.0
Mn (mg L^{-1})	0.426	4.900	0.022	0.005	3.195	30.100
Na (mg L^{-1})	6.2	14.6	7.1	4.9	7.5	9.3
Ni (μg L^{-1})	4.9	4.7	3.4	2.4	20.6	11.5
S (mg L^{-1})	168.5	430.0	43.0	40.0	287.0	2256.0

Table A2. *Cont.*

Characteristic	Hodruša	Beliansky tajch	Lintich	Zlatý stôl	Michalštôlňa	Šobov
Si (mg L^{-1})	8.8	9.7	14.5	10.5	3.1	40.2
Sr (μg L^{-1})	309.0	398.6	273.0	196.0	987.0	228.0
Zn (μg L^{-1})	42.3	50.0	44.3	424.0	74.8	4050.0

Notes: * Co, cobbles; Pe, pebbles; CG, coarse gravel; CPOM, coarse organic matter; **metal hydroxides precipitates: 1, light; 2, moderate; 3, intensive; + aluminum hydroxide.

Table A3. Correlations among environmental variables. Pearson correlation coefficients are displayed above the table diagonal and corresponding probabilities based on 9999 permutations are below the diagonal.

Variable	pH	Conductivity	SO_4^{2-}	Al	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	S	Si	Sr	Zn
pH	−	−0.97	−0.98	−0.95	0.09	−0.95	−0.08	−0.61	−0.96	−0.95	−0.95	−0.95	0.81	−0.92	−0.97	−0.30	−0.38	−0.97	−0.87	0.11	−0.92
Conductivity	0.016	-	1.00	0.98	−0.28	0.97	0.05	0.62	0.99	0.98	0.98	0.98	−0.86	0.98	1.00	0.31	0.36	1.00	0.90	−0.15	0.96
SO_4^{2-}	0.010	0.003	-	0.98	−0.25	0.97	0.04	0.60	0.99	0.98	0.98	0.98	−0.87	0.97	1.00	0.31	0.34	1.00	0.91	−0.17	0.96
Al	0.010	0.013	0.013	-	−0.23	0.96	−0.16	0.55	0.98	1.00	1.00	1.00	−0.94	0.96	0.99	0.15	0.25	0.98	0.96	−0.28	1.00
As	0.832	0.472	0.505	0.598	-	−0.31	−0.11	−0.37	−0.24	−0.24	−0.24	−0.24	0.12	−0.41	−0.30	−0.33	−0.27	−0.26	−0.22	−0.18	−0.26
Ba	0.042	0.028	0.028	0.064	0.405	-	−0.01	0.51	0.98	0.96	0.96	0.96	−0.86	0.93	0.98	0.37	0.23	0.97	0.94	−0.25	0.94
Ca	0.878	0.915	0.931	0.822	0.830	0.994	-	0.18	−0.02	−0.16	−0.16	−0.16	0.40	0.03	0.00	0.81	0.36	0.01	−0.30	0.54	−0.21
Cd	0.205	0.154	0.224	0.257	0.598	0.295	0.743	-	0.50	0.56	0.60	0.55	−0.28	0.68	0.60	0.02	0.94	0.59	0.34	0.62	0.54
Co	0.012	0.013	0.010	0.009	0.635	0.026	0.967	0.253	-	0.98	0.98	0.98	−0.91	0.96	0.99	0.31	0.22	0.99	0.94	−0.29	0.97
Cr	0.052	0.072	0.073	0.080	0.634	0.095	0.765	0.177	0.143	-	1.00	1.00	−0.94	0.96	0.99	0.15	0.25	0.98	0.96	−0.28	1.00
Cu	0.075	0.066	0.070	0.088	0.499	0.095	0.765	0.176	0.148	0.003	-	1.00	−0.92	0.96	0.98	0.13	0.30	0.98	0.95	−0.23	0.99
Fe	0.066	0.028	0.030	0.062	0.302	0.023	0.798	0.279	0.033	0.170	0.166	-	−0.94	0.96	0.99	0.15	0.25	0.98	0.96	−0.28	1.00
K	0.152	0.151	0.156	0.137	0.766	0.137	0.436	0.684	0.123	0.153	0.144	0.101	-	−0.84	−0.88	0.02	0.05	−0.88	−0.97	0.57	−0.95
Mg	0.081	0.014	0.026	0.034	0.226	0.073	0.968	0.128	0.057	0.056	0.051	0.051	0.143	-	0.98	0.27	0.41	0.98	0.86	−0.08	0.96
Mn	0.022	0.003	0.002	0.018	0.372	0.021	1.000	0.214	0.009	0.065	0.060	0.037	0.149	0.012	-	0.29	0.32	1.00	0.92	−0.19	0.98
Na	0.465	0.429	0.425	0.699	0.400	0.352	0.052	0.942	0.434	0.791	0.791	0.696	0.959	0.536	0.435	-	0.03	0.29	0.13	0.08	0.11
Ni	0.381	0.396	0.442	0.524	0.594	0.648	0.466	0.020	0.738	0.520	0.484	0.594	0.911	0.342	0.446	0.944	-	0.32	0.01	0.85	0.23
S	0.010	0.002	0.002	0.013	0.503	0.026	0.996	0.217	0.009	0.071	0.075	0.036	0.150	0.022	0.002	0.438	0.456	-	0.92	−0.19	0.97
Si	0.140	0.142	0.137	0.152	0.534	0.062	0.585	0.529	0.101	0.143	0.138	0.077	0.018	0.158	0.132	0.730	0.979	0.133	-	−0.50	0.96
Sr	0.737	0.697	0.645	0.482	0.700	0.483	0.246	0.149	0.419	0.485	0.480	0.435	0.192	0.835	0.639	0.843	0.096	0.621	0.209	-	−0.31
Zn	0.149	0.106	0.119	0.120	0.203	0.156	0.697	0.229	0.139	0.109	0.080	0.071	0.032	0.073	0.116	0.877	0.818	0.125	0.069	0.328	-

Table A4. Checklist of the aquatic invertebrates found at the AMD sites. The plus signs denote the presence of taxa.

Taxon	Hodruša	Beliansky tajch	Lintich	Zlatý stôl	Michalštôlňa	Šobov
Turbellaria						
Dugesia polychroa (Schmidt, 1861)		+				
Oligochaeta						
Oligochaeta indet	+	+	+		+	+
Amphipoda						
Gammarus fossarum (Koch, 1836)		+	+			
Plecoptera						
Nemoura spp.			+	+		
Nemurella pictetii （Klapálek, 1900）			+	+		
Leuctra spp.	+	+				
Coleoptera						
Hydroporus sp.		+			+	
Trichoptera						
Plectrocnemia conspersa (Curtis, 1834)	+	+	+		+	
Micropterna sp.					+	
Potamophylax nigricornis (Pictet, 1834)			+			
Lepidoptera						
Cataclysta lemnata (Linnaeus, 1758)					+	
Diptera						
Tipulini indet			+			
Molophilus sp.			+		+	
Rhypholophus haemorrhoidalis (Zetterstedt, 1838)					+	
Scleroprocta sp.			+			
Eutonia sp.	+					
Dicranota sp.		+				
Pedicia sp.	+			+		
Macropelopia sp.			+			
Chaetocladius sp.	+			+		
Heterotrissocladius marcidus (Walker, 1856)	+					
Limnophyes sp.	+		+	+		+
Ceratopogoninae indet.	+			+	+	
Forcipomyiinae indet.						+
Simuliidae indet.			+			
Dolichopodidae indet.		+				

Figure A1. Relationships between measured environmental variables, species richness and EPT richness. Pearson correlation coefficients and corresponding probabilities based on 9999 permutations are displayed in each scatterplot.

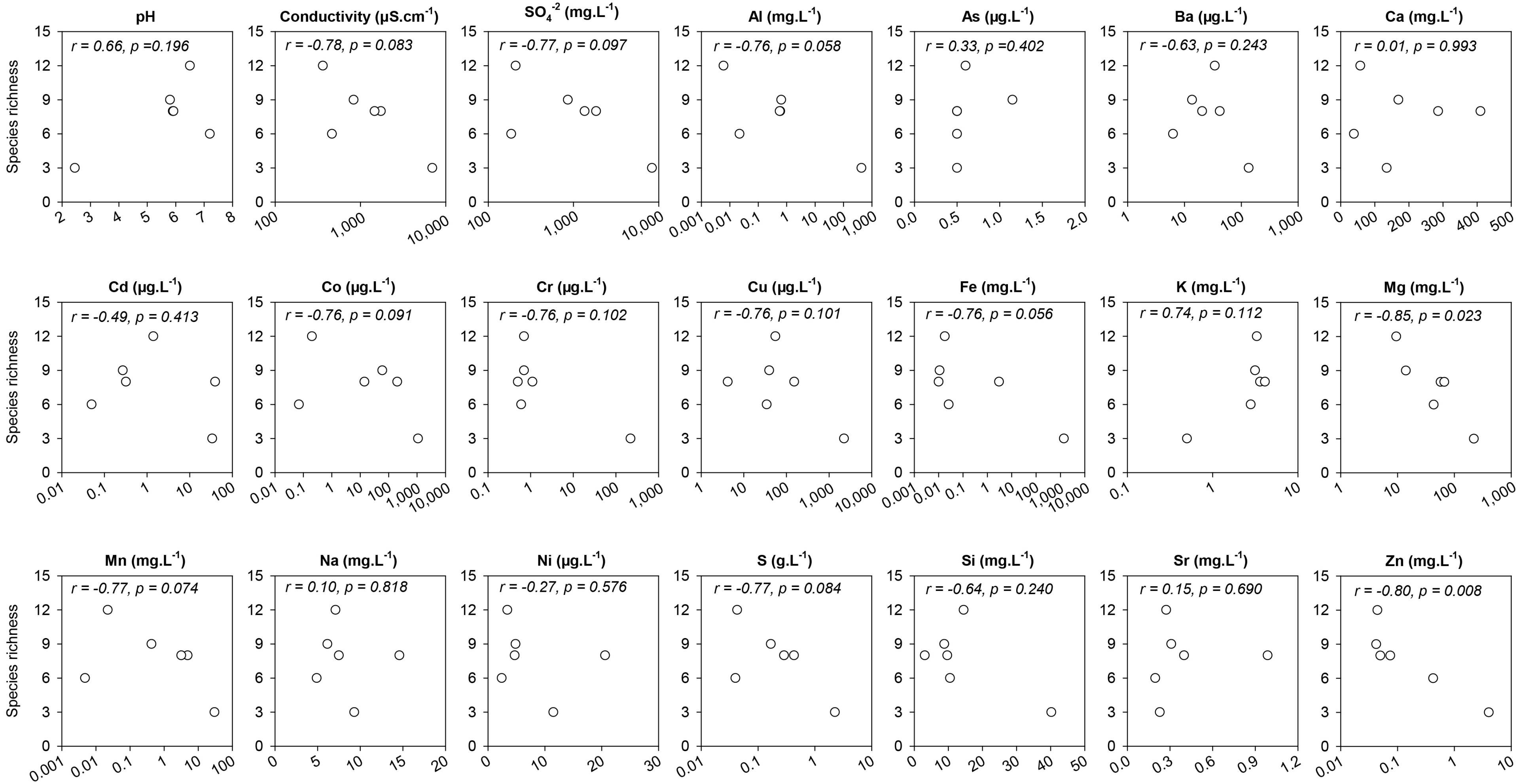
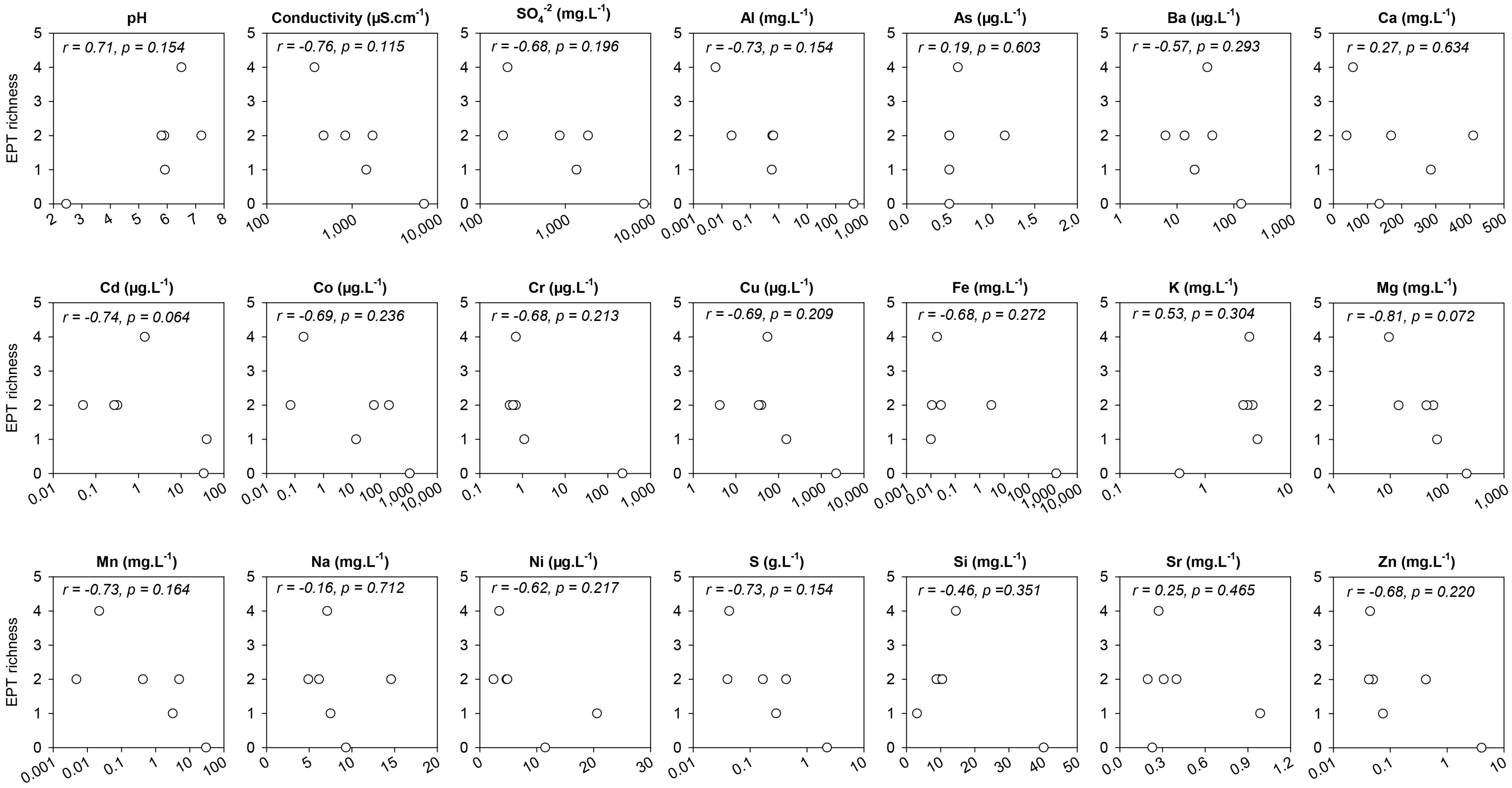


Figure A1. *Cont.*



Conflicts of Interest

The authors declare no conflict of interest.

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