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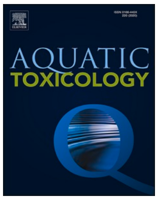

Proposal of integrative scores and biomonitor selection for metal bioaccumulation risk assessment in mine-impacted rivers

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ABSTRACT

Development of sound criteria for metal and metalloid bioaccumulation risk assessment in river basins affected by mining activities is a necessary tool to protect the aquatic communities. The aim of this study is to propose integrative scores for tissue residues that are suitable for surveillance programs and readily interpreted in terms of risk assessment in mining impacted rivers. Tissue residues of 7 trace metals and 2 metalloids were measured in ten macroinvertebrate taxa from the Nalón River basin (Spain), affected by Hg, Cu and Au mining activities. Compared with reference sites, biomonitor taxa from Hg and Au mining districts showed the highest bioaccumulation. However, low or non-significant bioaccumulation was found in sites influenced by historical Cu mining. Multivariate analyses (ANOSIM) performed on individual taxa revealed significant differences in tissue residues between sites classified according to their ecological status. The bioaccumulation risk assessment was based on the average ratio of the actual metal tissue residues in each macroinvertebrate taxon to the corresponding Ecological Threshold tissue concentration (Tissue residue Ratio to Threshold, TRT). The suitability of the biomonitors was evaluated using linear regression models fitted to the relationships between TRT scores and site sediment pollution or ecological status scores. Biomonitor selection also considered differences in invertebrate functional traits, which can influence metal and metalloid bioavailability. Site bioaccumulation risk was assessed on an Integrated Tissue concentration score (INTISS), calculated over a selection of the most relevant chemicals (As, Cu and Hg) and 3 biomonitor taxa (Baetidae, Hydropsychidae, Microdrile oligochaetes) comprising a set of feeding styles. Based on INTISS, it was possible to predict community alteration scores, using linear regression models. A comparison of site bioaccumulation and ecological status assessments based on the departure from reference conditions showed that operational monitoring programs in basins impaired by mining can be optimized by combining both approaches.

1. Introduction

The body concentration of a chemical is an integrated measure of its bioavailability, and includes the uptake, storage and elimination processes over the preceding time period. Biomonitor organisms has been shown to be a promising tool to identify and assess those sites having chemical tissue residues higher than background or reference levels (Adams et al., 2011). Moreover, they provide an assessment of the relationships between tissue residues and benthic macroinvertebrate community's impairment over space and time in a particular area (Luoma et al., 2010; Bervoets et al., 2016).

In rivers affected by mining activities, metal body burden measured in selected macroinvertebrates provides evidence of metal bioavailability, and has been used to estimate critical levels to protect the aquatic invertebrate communities (De Jonge et al., 2013; Meador et al., 2014). To accomplish this goal, the relationships between the community-level effect of concern and the body metal concentration in biomonitors occurring across the range of metal exposure in a specified ecoregion must be modeled (Adams et al., 2011). These models can then be used to provide warning signals to protect freshwater biodiversity.

Several approaches used for predicting biological effects due to bioaccumulation were reviewed by Simpson & Batley (2007), e.g. the

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Biotic Ligand model (BLM) and the Simultaneously Extracted Metals / Acid-Volatile Sulfide (SEM/AVS) model. That review pointed out some limitations of the models used to predict alterations in field communities. Thus, the BLM assesses the bioavailability of dissolved metals, but it can be objected that diet is one of the main uptake routes for benthic macroinvertebrates (Luoma and Rainbow, 2005; Simpson and Batley, 2007). On the other hand, the SEM/AVS model does not always describe satisfactorily the metal availability from contaminated sediments (e.g. in oligochaetes and chironomids in polluted sediments: De Jonge et al., 2009; Méndez-Fernández et al., 2014). Moreover, the applicability of the SEM/AVS model to benthic organisms may be also compromised by the fact that this model is based on the chemistry of anoxic sediment (Casado-Martinez et al., 2010), a condition that rarely occurs in river (lotic) habitats. Additionally, because bioavailability of metals also depends on organism behavior, feeding selectivity and physiology (Luoma and Rainbow, 2005; De Jonge et al., 2010), the assessment of bioaccumulation using a single species cannot provide the necessary information to evaluate risk at the community level. Moreover, differential bioaccumulation among metals and metalloids (hereinafter, referred to as metals) depends on metabolic essentiality, which in turn can explain differences among taxa in uptake rates, assimilation efficiencies, and detoxification mechanisms (Adams et al., 2011; Wang and Rainbow, 2008). Taking into consideration the aforementioned limitations, we approached the risk assessment of metal bioaccumulation in a different way, using several taxa and based on their departure from the baseline tissue residues previously measured in the mining region (Rodriguez et al., 2018).

In Europe, the core of the ecological status classification systems for water bodies under the Water Framework Directive (WFD: EC, 2000) is based on the selection of a spatial network of reference sites, and on the degree of deviation of biological variables from the reference values. The Nalón River basin has a long history of metal extraction, and its sediments can harbor high levels of certain metals due to the legacy of abandoned Hg and Cu mining activities. These metals have been shown to cause ecotoxicity and alterations to the macroinvertebrate community (Méndez-Fernández et al., 2015; Costas et al., 2018). In the absence of tissue quality standards for metals in the European regulations (except for Hg: EC, 2013), bioaccumulation risk assessment in the study region is addressed here using the ratio of the metal tissue residues in field organisms to the baseline value of the corresponding taxon (ETTC, Rodriguez et al., 2018). This ratio will provide information on the degree of departure of the metal bioaccumulation from background levels, and on the metal bioavailability gradients within the river basin. It was hypothesized that these gradients could also be related to the alterations measured in macroinvertebrate communities.

The present study measured the metal tissue concentration in several sites potentially affected by metal pollution, with the aim of performing a bioaccumulation risk assessment in the Nalón River basin through the integrative assessment of the most relevant metals in selected biomonitors. Therefore, the specific objectives of this study are: (1) to provide criteria to select a suitable set of relevant metals and macroinvertebrate taxa as biomonitors for the bioaccumulation risk assessment; (2) to provide a methodological framework to assess metal bioaccumulation through integrative scores for mixed-metal pollution situations; and (3) to provide tools based on stream macroinvertebrate bioaccumulation that can assist in the decision making process in monitoring and restoration programs of water bodies in mining districts.

2. Material and methods

2.1. Study area

This study was conducted in the Nalón River basin (Asturias, northern Spain), the largest basin within the Cantabrian water district (total area of 4907 km^2). A total of 29 sites were sampled for macroinvertebrate tissue residue analysis during summer, in 2014 and 2015. The sampling design included 14 reference sites and 15 test sites, whose environmental characteristics have been partially reported in previous publications (Méndez-Fernández et al., 2017; Costas et al., 2018; Rodriguez et al., 2018). These sites correspond to four Spanish river types (Types R-T21, R-T25, R-T28 and R-T31: BOE, 2015) (Table S-1). Reference sites (REF group) were selected according to the European WFD criteria by the absence of significant anthropogenic pressures and validated following the procedure by Pardo et al. (2012). The test sites were selected as being potentially affected by different mining pressures and were identified based on previous data of the authors: 3 sites downstream from abandoned Cu mining activities (CU group); 8 sites downstream from abandoned Hg mining areas (HG group); and 4 sites close to an active gold mine (AU group: 1 site upstream (P1) and 3 sites downstream from the mine (Table S-1). The ecological status at each river site was assessed by the EQR (Ecological Quality Ratio) score of the NORTI predictive model (NORThern Spain Indicators, Pardo et al., 2014), which measures the similarity of the faunal composition of a test sample to that of the reference community of the corresponding river-type predicted from a multivariate model.

2.2. Sampling strategy and metal analysis in sediment and tissue

At each site, 0.5 L of a composite sediment sample was collected with a stainless-steel spade from the upper 5–10 cm layer of fine sediment settled in submerged depositional areas, along an approximately 25-m river reach segment. Samples were taken to the laboratory on ice and stored at 4 °°C, in the dark. Dissolved oxygen in the field was always > 8.5 mg L^{-1}, pH = 5.8–8.7 (mean = 7.8), conductivity = 31–1263 µS cm^{-1} (mean = 452), TOC% in sediment =0.4–6.3 (mean = 2.0), silt-clay % in sediment= 0.4–28.1 (mean = 5.7). Other physical and chemical water characteristics were published in Costas et al. (2018).

Macroinvertebrates were kick-sampled in a variety of lotic habitats and river margins, using a hand-net (maximum depth in sediment about 10 cm). Selected biomonitors belong to 10 taxa (8 insect families plus 2 oligochaete groups): Baetidae, Ephemerellidae, Ephemeridae, Heptageniidae, Hydropsychidae, Lumbricidae, Microdrile oligochaetes (mostly *Lumbriculidae*), Perlidae, Rhyacophilidae, and Simuliidae. These taxa can be regarded as representative of different metal exposure routes, based on their feeding styles (scrapers, filterers, deposit-feeders, generalist and predators), in addition to their general habits (epibenthic vs endobenthic); all are widespread within the study area.

Macroinvertebrate samples for bioaccumulation analyses were obtained at each site following a multi-habitat sampling scheme. For each taxon, 3 replicates consisting of 1–20 individuals were collected per site, depending on the individual size (see details in Rodriguez et al., 2018); when number or size of the sample was limited, individuals were pooled to obtain enough biomass for the metal analysis (see Table S-2). In some occasions, the exceptionally large size of some taxa (e.g. Perlidae) required the analysis of only part of the body or the selection of medium-sized individuals. Organisms were sorted on site, held for 5–10 h in river water on ice and identified before being frozen. By then, they would have totally or largely emptied their guts (4–6 h clearing period in Cain et al., 1992). In aquatic oligochaetes, the gut content after the purging period represents a low fraction of the body weight. For example, after a 9-h purging period under 4 °°C, the remaining sediment in the gut of *Tubifex tubifex* represents only 11% of the total fecal production in 24 h24 hours, and 5% of the mean worm's body (tissue) weight (unpublished data from authors).

All sampled taxa were analyzed (*nn* = = 117). Procedures related to the sediment and tissue acid digestion and measurement methods were described by Méndez-Fernández et al. (2017) and Rodriguez et al. (2018), and are briefly summarized here. Nine trace metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn) were analyzed in tissue and in the < 63 µm fraction of sediments. Macroinvertebrate samples were digested in nitric acid (70% Baker® Instra-Analyzed) and hydrogen peroxide (30% RP Suprapur Merck®). Sediment samples were digested using a microwave extraction method, in concentrated nitric acid and concentrated hydrochloric acid, following US EPA 3051 protocol (US EPA, 2007). All analytical samples included Mussel Tissue Standard Reference Material (NIST 2976), or Buffalo River sediment (RM8704, USA) and Sewage Sludge-3 (CRM 031–040, UK) as reference materials for quality control. All concentrations in present study are given on dry-weight (dw) basis.

To assess sediment pollution, we used the SedPoll score (Costas et al., 2018), an integrative sediment pollution score based on 6 metals (As, Cd, Cu, Hg, Pb and Se), selected as relevant for the macroinvertebrate community composition in the study area. This score is calculated for each site as the average of the ratios of each of the actual metal sediment concentrations to the corresponding 90th percentile of the REF group sediment concentration.

2.3. *Integrated bioaccumulation scores*

The Ecological Threshold Tissue Concentration (ETTC) for each metal and taxon (Rodriguez et al., 2018) was used as benchmark value to express the bioaccumulation in each site as a metal hazard quotient. Next, for each taxon, these quotients were averaged by the number of metals and expressed as Tissue-residue Ratio to Threshold (TRT) score TRT= $\left(\sum_i (TR_i / B_i)\right)/n$, where i is the metal, TR_i the tissue concentration of each metal in the taxon, B_i the baseline or low threshold for each metal in that taxon, and n the number of metals. This index is based on the Cumulative Criterion Unit (Clements et al., 2000) and assumes metal mixture interactions as additive. Finally, for every site, an INtegrated TISSue score (INTISS) was calculated as the mean of the TRT scores of the selected taxa, and used as the base for the site classification.

Site bioaccumulation assessment used both the taxon TRT and the site INTISS scores, following a classification in four general categories: (1) Similar to reference, when bioaccumulation scores are $\leq$ 1.0 (boundary of 0 in log-scale); (2) Low bioaccumulation when scores are 1.1–2.0 (boundary of 0.3 in log-scale); (3) Medium bioaccumulation when scores are 2.1–10.0 (boundary of 1.0 in log-scale); (4) High bioaccumulation when scores are $>$ 10.

2.4. *Statistical analyses*

Regression analyses and univariate statistics were performed with IBM SPSS® software (IBM Corp, 2017). Metal concentration in the biota expressed in the form of integrative scores (taxon TRT and site INTISS) were related to the sediment pollution scores (SedPoll), and to the ecological status of benthic macroinvertebrate communities (EQR scores) using linear regression models. Metal tissue concentrations were not usually normally distributed (Shapiro-Wilk test), hence data were normalized using log-transformation. The standardized residuals were always within ± 3 standard deviations from zero.

Additionally, several multivariate analyses were performed for individual taxa by means of PRIMER 6 software (Clarke and Gorley, 2006). Using the Euclidean distance of the log-transformed and normalized tissue residue data, the one-way ANOSIM procedure (999 permutations) (Clarke, 1993) was applied to the tissue residue dissimilarity matrices to test the possible influence of the following site-grouping factors: (1) ecological status based on Good/Not Good classification of sites using a NORTI-EQR score boundary of 0.700; and (2) the anthropogenic pressures related to mining activities (REF, CU, HG and AU groups). Null hypotheses in ANOSIM were rejected when $p < 5\%$. The contribution of tissue metal residues in different taxa to the site group dissimilarities was analyzed through SIMilarity PERcentage analysis (SIMPER procedure) (Clarke and Gorley, 2006).

Table 1

Presence (✓)/absence (x) of taxa selected as biomonitors in the study sites (identified by the potential mining influence on each site). Percentage of appearance at reference (REF) sites ($n = 14$, in Rodriguez et al., 2018) and test sites ($n = 15$) is indicated. **Abbreviations**: SC Scraper, CF Collector-Filterer, G Generalist, P Predator, DF Deposit Feeder, [Ep] epibenthic, [En] endobenthic.

Biomonitor Taxa	Functional Group	CU group			HG group								AU group				Percentage of Appearance	
		N4	N6	NAL013	N9	N10	N11	N13	N14	N15	NAL056a	NAL056b	P1	P2	P3	P4	TEST-Sites	REF-Sites
Ephemeroptera																		
Baetidae	SC [Ep]	✓	✓	✓	✓	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	93	100
Ephemerellidae	G [Ep]	✓	✓	✓	x	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	87	86
Ephemeridae	CF [En]	✓	✓	x	x	✓	✓	✓	✓	x	✓	✓	✓	x	✓	x	67	86
Heptageniidae	SC [Ep]	✓	✓	✓	x	x	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	80	100
Plecoptera																		
Perlidae	P [Ep]	x	✓	x	x	x	x	✓	✓	x	✓	x	✓	✓	✓	x	47	71
Trichoptera																		
Hydropsychidae	CF [Ep]	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	✓	✓	x	✓	✓	87	93
Rhyacophilidae	P [Ep]	✓	✓	✓	x	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	87	93
Diptera																		
Simuliidae	CF [Ep]	✓	✓	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	x	x	x	73	79
Annelida Clitellata																		
Lumbricidae	DF [En]	✓	✓	✓	x	✓	x	✓	✓	✓	x	x	✓	✓	✓	✓	73	100
Microdrile oligochaetes	DF [En]	✓	✓	✓	✓	x	✓	✓	x	✓	✓	✓	✓	✓	✓	✓	87	86
Total taxa per site		9	10	8	3	7	8	9	9	4	9	8	10	7	9	7	––	––

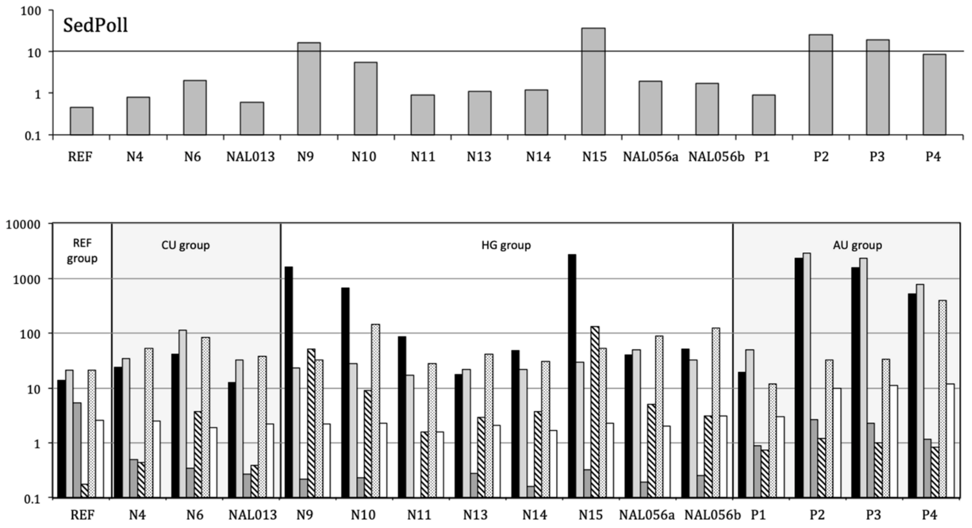

Fig. 1. Sediment pollution score (SedPoll) (above) and sediment concentration of 6 metals (below) in the study sites. Metal codes (by order in the graph): As (black), Cu (light grey), Cd (dark grey), Hg (striped), Pb (dotted) and Se (white). For the reference sites (*n* = = 14), the median value is represented.

3. Results

3.1. *Biomonitors: first-level selection process*

The selection of suitable biomonitor taxa in a reference condition approach depends primarily on their presence in both reference and polluted sites; thus, the selection of biomonitors should consider their tolerance to metals and other anthropogenic stressors. In this study, all the biomonitor taxa selected were present in more than 70% of reference sites and in most test sites (in $\geq$ 70% sites, except for Ephemeridae and Perlidae in 67 and 47% of sites, respectively) (Table 1). The low presence of perlids suggests that this predator taxon might not be an appropriate biomonitor in the Nalón River basin, unless it appears to be suitable for trophic transfer studies. Hence, in the selection of biomonitors, it seemed necessary to choose several taxa relatively tolerant to anthropogenic pressures to prevent lack of information from sites with high mortality of most sensitive organisms, either due to pollution or to lack of suitable habitat. The next requirement is that the abundance and body size of potential biomonitors should be sufficient for tissue residue analysis (Table S-2). Some of the taxa are very small, e.g. Baetidae and Simuliidae, and the collecting time required to get a minimum biomass was higher than for other taxa; however, these were usually very abundant. Finally, in an effort to represent the range of tissue concentrations at the sites, we also followed a complementary criterion: the selection of several biomonitor taxa with different functional traits (feeding styles and habits) as a strategy to represent the main metal uptake routes.

3.2. *Metal tissue residues in macroinvertebrate taxa and their relationship to sediment metal concentrations*

Sediment metal concentrations per site are shown in the supplementary Table S-3. The highest values for As, Cu and Se were recorded in the AU group, with levels above the Probable Effect Concentration (PEC, MacDonald et al., 2000). Based on the Hg quality standards for sediments proposed by Méndez-Fernández et al. (2019), all reference sites (except N12) were classified as Good chemical status ($<$ 0.49 µg Hg g^{-1} dw), while test sites in the HG group had Moderate to Bad chemical status, with particularly high levels at N9 and N15 ($>$ 50 µg g^{-1} dw).

Overall, the sediment quality assessed by the SedPoll score (Fig. 1) indicated that the REF group of sites was not polluted (scores $\leq$ 1). In the HG group, site N10 was assessed as moderately polluted (score 2.1 – 10), while sediments in N9 and N15 were highly polluted (scores 10.1 – 50). In the AU group, SedPoll scores indicated that sites P2 and P3 were highly polluted, and that there was a reduction to moderate pollution level at the downstream site, P4. The remaining test sites, including the CU group, were assessed as similar to the reference or with low metal pollution (scores $\leq$ 2).

For each biomonitor taxon, metal availability from sediments was approached through linear regression models between the log-transformed metal concentration in sediment and tissue residues, including both reference and tests sites (Table 2). Linear regression analysis for As suggested that it is both highly bioavailable to all macroinvertebrate taxa in the Nalón River ($r^2_{adj} > 0.700$) and linearly dependent on sediment concentration (*bb* = = 0.7–1.2). Filterer taxa showed $b > 1.0$, suggesting that the increase in tissue residues can be the result of the ingestion of small, metal-rich sediment particles or a slow elimination pattern. In assessing As bioaccumulation, several taxa identified 4 sites in the HG group (N9, N10, N11, N15) as having Medium to High bioaccumulation and 3 sites (P2, P3 and P4) in the AU group (TR $>$ 10 times the baseline ETTC, see Table S-4). No single taxon discriminated the 7 sites, as each was absent from some of the sites. Nevertheless, Baetidae, Hydropsychidae and Microdrile oligochaetes could identify 5 or 6 out of the 7 aforementioned sites.

The fit of the linear models for Cu was better for the infaunal taxa that burrow into the sediment, i.e. Ephemeridae and oligochaetes, as well as for their potential predators (Rhyacophilidae) ($r^2_{adj} > 0.700$, Table 2). According to the slope values (*bb* = = 0.3–0.7), these models suggest a variable degree of regulation of the body concentration by these taxa when exposed to increasing Cu levels in the sediment. Regarding site assessment for Cu, the 3 sites in the AU group (P2 to P4) with the highest sediment loadings were evaluated as having Medium to High bioaccumulation by all the taxa, except for Ephemerellidae (see Table S-4).

The fit of the linear regression models for Hg was low to moderate ($r^2_{adj} = 0.224$–0.579), but higher for the Lumbricidae (Table 2). According to the slope values (*bb* = = 0.2–0.6), these models suggest that Hg body concentration was kept at lower levels than expected when exposed to increasing Hg levels in the sediment, either due to body barriers or detoxification processes. However, the models are not easy to interpret due to the absence of many taxa from sites with higher Hg levels (e.g., N9 and N15), which presumably decreased both the slope and fit of the models. Medium to High Hg bioaccumulation was assessed for the 2 sites with the highest sediment values (N9 and N15) by Baetidae, Hydropsychidae, Simuliidae, Lumbricidae and Microdrile oligochaetes. Bioaccumulation assessments for N10, N11 and N13 were Low

Table 2

Linear regression of the log-transformed metal tissue residues by taxon on the log metal concentration in the sediment. Only significant models are included (F test, $p < 0.05$).

Taxon	Metal	r^2_{adj}	b (SE)	a
Baetidae	As	0.801	0.801 (0.076)	−0.645
($n = 28$)	Cd	0.441	0.877 (0.186)	0.709
	Cu	0.430	0.327 (0.071)	0.992
	Hg	0.434	0.340 (0.073)	−0.639
	Zn	0.275	0.814 (0.243)	0.739
Ephemeridae	As	0.867	1.164 (0.099)	−1.140
($n = 22$)	Cd	0.333	1.223 (0.360)	0.436
	Cr	0.392	0.712 (0.187)	−0.697
	Cu	0.830	0.716 (0.070)	0.225
	Hg	0.338	0.407 (0.119)	−0.588
Ephemerellidae	As	0.789	0.710(0.072)	−0.340
($n = 27$)	Cd	0.222	0.596 (0.206)	0.785
	Cu	0.454	0.300 (0.063)	1.334
Heptageniidae	As	0.748	0.747 (0.086)	−0.530
($n = 26$)	Cd	0.508	0.833 (0.161)	0.095
	Cr	0.124	0.533 (0.250)	−0.575
	Cu	0.516	0.454 (0.086)	1.135
	Hg	0.363	0.326 (0.084)	−0.721
	Zn	0.465	0.793 (0.166)	0.749
Hydropsychidae	As	0.855	1.039 (0.085)	−1.252
($n = 26$)	Cd	0.145	0.485 (0.212)	−0.583
	Cu	0.567	0.319 (0.055)	0.743
	Hg	0.463	0.426 (0.090)	−0.548
	Se	0.143	0.440 (0.194)	0.129
Lumbricidae	As	0.718	0.797 (0.103)	−0.105
($n = 24$)	Cr	0.206	0.419 (0.159)	−0.253
	Cu	0.789	0.469 (0.050)	0.349
	Hg	0.579	0.577 (0.101)	−0.310
	Se	0.220	0.419 (0.153)	0.785
Microdrile oligochaetes ($n = 25$)	As	0.845	0.800 (0.070)	−0.037
	Cu	0.749	0.601 (0.071)	0.290
	Hg	0.498	0.363 (0.073)	−0.190
	Se	0.223	0.493 (0.176)	0.451
Perlidae	As	0.891	0.877 (0.079)	−1.421
($n = 16$)	Cu	0.692	0.315 (0.053)	0.997
	Hg	0.433	0.317 (0.090)	−0.738
Rhyacophilidae	As	0.764	0.691 (0.076)	−1.140
($n = 26$)	Cd	0.383	0.876 (0.216)	−0.062
	Cr	0.182	0.811 (0.317)	−1.011
	Cu	0.787	0.276 (0.029)	0.840
	Hg	0.224	0.205 (0.072)	−0.604
	Ni	0.143	0.900 (0.396)	−1.681
	Se	0.178	0.440 (0.173)	0.254
	Zn	0.177	0.352 (0.139)	1.648
Simuliidae	As	0.917	1.177 (0.075)	−1.117
($n = 23$)	Cr	0.397	0.755 (0.196)	−0.552
	Hg	0.493	0.427 (0.090)	−0.288

to Medium, depending on the taxa, and predator taxa showed Low or No bioaccumulation of Hg (≤ ETTC) in all the test sites (Table S-4).

A moderate fit of Cd TR as a function of the sediment concentration was obtained in linear regression models for Heptageniidae ($r^2_{adj} = 0.508$) and Baetidae ($r^2_{adj} = 0.441$), and one of their potential predators, Rhyacophilidae ($r^2_{adj} = 0.383$) (Table 2). For Cr, Ni, Se and Zn, only a few taxa showed a significant (but mostly low) fit to the linear model. No relationship was found for Pb. These results can be partly due to the low concentration of these metals in sediments, which can also explain to some extent the low TR measured in many taxa. None of these metals reached the high bioaccumulation class (TR > 10 times the ETTC) in any of the biomonitor taxa (Table S-4).

The fact that not all measured metals are equally available for bioaccumulation, or are likely to affect the macroinvertebrate community ecological status, led us to propose the use of integrative metal scores on a selection of relevant metals. For each biomonitor, the TRT scores were calculated using different number of metals (3 to 9 metals, TRT_3 to TRT_9). The metals selected for the combined index TRT_3 were As, Hg and Cu, based on the relationships of the tissue residues with sediment concentrations described above. The addition of metals to the TRT had an effect on the index similar to a dilution of the relevant metals (see Table S-4 for comparison of TRT_3 and TRT_9). Log-transformed values of SedPoll and TRT scores for the different metal combinations were fitted to linear regression models for all taxa; in all instances, models were significant, showed positive slopes and moderate to good fit depending on the taxa and metal combinations ($r^2_{adj} = 0.486–0.849$, Table 3). In half of the cases, the highest determination coefficients were obtained for a three-metal combination (As, Cu, Hg = TRT_3). Only 3 cases were better adjusted for TRT_6 and 2 cases for TRT_9, but differences in model fit were not large. These models suggest, first, that the combination of metals considered in each case was highly bioavailable for the taxa. Additionally, those taxa with regression coefficient (b) closer to 1 allowed for a more straightforward prediction of bioaccumulation related to increased sediment pollution levels than those with low b values (e.g. Ephemerellidae and Heptageniidae), where either their feeding style, the regulation of the uptake or the active elimination processes of metals may make interpretation difficult.

3.3. Relationship between TRT and field community scores (EQR)

Linear regression analyses between the log-transformed TRT scores and EQR values were performed to evaluate the potential of the bioaccumulation scores to predict the ecological status of the macroinvertebrate communities. As expected, a reduction in the macroinvertebrate community EQR scores was associated with increasing metal bioaccumulation in most taxa (Fig. 2). Linear regression models were significant for 8 of the 10 taxa assessed (none were significant for Ephemeridae and Heptageniidae) (Table 3). Models based on TRT_3 (As, Cu and Hg) provided the best fit, and the regression equations indicated that a doubling of the TRT_3 score can reduce the EQR by less than 0.1 units, but a 10-fold increase can reduce it by 0.1–0.2 units, depending on the taxon.

In Fig. 2, test sites with no field community alteration (EQR ≥ 0.700) and no bioaccumulation fell in the upper left side of the graph, above the EQR line and to the left of $TRT_3 = 1$; sites with field alteration and high bioaccumulation fell in the right lower side, below the EQR line and to the right of $TRT_3 = 10$. In this way, only sites N9, N10, N15 (in HG group), and P2, P3 and P4 (in AU group) showed alterations attributable to moderate or high metal bioaccumulation for most taxa present at those sites. Finally, several sites (NAL056a and N4 and N11) appeared in a variable position, and often in the limit of the boundary lines, thus indicating a need for specific surveillance programs.

3.4. Integrated risk assessment of bioaccumulation using several biomonitor taxa in a mixed-metal pollution situation

Multivariate analyses using metal tissue residues (TR) for each taxon, both at reference and test sites, gave a good representation of the actual site dissimilarities (Table 4). One-way ANOSIM on metal TR, using two EQR classes (Good/Not Good) as a factor, resulted in significant differences between EQR classes for most taxa examined separately (Global $RR = = 0.317–0.632$, $pp \le \le 1\%$), except for Ephemeridae and Perlidae (Table 4). These analyses identified Lumbricidae, Microdrile oligochaetes, Hydropsychidae and Heptageniidae as the taxa with highest differences (ANOSIM Global $R > 0.500$), that is, tissue residues in these taxa were responsible for a greater dissimilarity between sites with Good and Not Good EQR values. One-way ANOSIM analysis using the anthropogenic pressures (HG, CU, AU mining and REF group) as factors resulted in Global R values of 0.308–0.605 ($p < 3\%$) for all taxa, except for Perlidae. In examining pairwise dissimilarities in metal TR, higher differences were observed between REF vs AU groups ($RR = = 0.646–0.972$, $pp \le \le 2.2\%$) for all examined taxa, compared with the lower differences between the REF vs HG groups ($RR = = 0.246–0.470$, $p < 5\%$). This may be due, in part, to absence of several taxa in sites of the HG group. No significant dissimilarity was observed between CU vs REF groups, except for Microdrile oligochaetes ($RR = = 0.319$, $pp = = 4.4\%$).

Table 3

Linear regression of the log *SedPoll* (X) vs log *TRT* (Y) scores and log *TRT* (X) vs *NORTI-EQR* scores (Y), calculated for 3, 4, 6 and 9 metals (see text) over ten biomonitor taxa in the Nalón River basin. Highest determination coefficients (r^2_{adj}) for each taxon marked in bold. **Abbreviations**: SC scraper, F filterer, O opportunistic, DF deposit-feeder, P predator, ns non significant.

	Metals	log *SedPoll* (X) vslog *TRT* (Y)			log *TRT* (X) vs*NORTI-EQR* (Y)		
		r^2_{adj}	b (SE)	a	$r^2 a_{dj}$	b (SE)	a
Baetidae	As Cu Hg	**0.544**	0.712(0.175)	0.104	**0.712**	−0.234 (0.041)	0.758
(SC)	As Cd Cu Hg	0.539	0.688 (0.171)	0.036	0.685	−0.238 (0.044)	0.741
	As Cd Cu Hg Pb Se	0.508	0.620 (0.163)	−0.027	0.638	−0.249 (0.051)	0.722
	All 9 metals	0.446	0.536 (0.158)	−0.063	0.577	−0.260 (0.060)	0.706
Ephemeridae	As Cu Hg	0.733	1.063 (0.210)	−0.010		ns	
(F)	As Cd Cu Hg	0.722	1.029 (0.208)	−0.102		ns	
	As Cd Cu Hg Pb Se	0.754	0.949 (0.178)	−0.164		ns	
	All 9 metals	**0.761**	0.844 (0.155)	−0.199		ns	
Ephemerellidae	As Cu Hg	**0.486**	0.487 (0.139)	−0.011	**0.539**	−0.191 (0.049)	0.725
(O)	As Cd Cu Hg	0.468	0.422 (0.124)	−0.023	0.435	−0.198 (0.062)	0.719
	As Cd Cu Hg Pb Se	0.471	0.384 (0.112)	−0.119	0.425	−0.216 (0.069)	0.698
	All 9 metals	0.337	0.287 (0.018)	−0.131	0.248	−0.204 (0.092)	0.688
Heptageniidae	As Cu Hg	0.501	0.393 (0.113)	0.129		ns	
(SC)	As Cd Cu Hg	0.512	0.411 (0.116)	0.084		ns	
	As Cd Cu Hg Pb Se	**0.540**	0.399 (0.107)	−0.014		ns	
	All 9 metals	0.486	0.349 (0.103)	−0.037		ns	
Hydropsychidae	As Cu Hg	**0.650**	1.014 (0.210)	0.019	**0.524**	−0.086 (0.023)	0.733
(F)	As Cd Cu Hg	0.631	0.949 (0.205)	−0.020	0.488	−0.088 (0.025)	0.728
	As Cd Cu Hg Pb Se	0.626	0.874 (0.190)	−0.076	0.465	−0.094 (0.028)	0.722
	All 9 metals	0.598	0.793 (0.183)	−0.131	0.403	−0.095 (0.032)	0.714
Lumbricidae	As Cu Hg	**0.494**	0.816 (0.249)	0.094	**0.653**	−0.115 (0.026)	0.749
(DF)	As Cd Cu Hg	0.479	0.786 (0.246)	0.013	0.649	−0.118 (0.027)	0.739
	As Cd Cu Hg Pb Se	0.481	0.738 (0.230)	−0.058	0.628	−0.124 (0.029)	0.730
	All 9 metals	0.412	0.628 (0.222)	−0.073	0.549	−0.129 (0.036)	0.722
Microdrile oligochaetes	As Cu Hg	0.734	0.740 (0.127)	0.102	**0.470**	−0.192 (0.056)	0.749
(DF)	As Cd Cu Hg	0.737	0.681 (0.116)	0.063	0.452	−0.206 (0.062)	0.741
	As Cd Cu Hg Pb Se	**0.749**	0.644 (0.106)	−0.032	0.436	−0.216 (0.067)	0.721
	All 9 metals	0.748	0.562 (0.093)	−0.081	0.421	−0.244 (0.078)	0.707
Perlidae	As Cu Hg	**0.849**	0.945 (0.160)	−0.081	**0.603**	−0.156 (0.049)	0.757
(P)	As Cd Cu Hg	0.832	0.881 (0.159)	−0.118	0.559	−0.162 (0.055)	0.748
	As Cd Cu Hg Pb Se	0.815	0.826 (0158)	−0.211	0.554	−0.170 (0.059)	0.731
	All 9 metals	0.734	0.702 (0.0168)	−0.205	0.490	−0.184 (0.071)	0.723
Rhyacophilidae	As Cu Hg	0.505	0.546 (0.150)	−0.012	**0.568**	−0.177 (0.043)	0.726
(P)	As Cd Cu Hg	0.537	0.539 (0.1040)	−0.093	0.546	−0.181 (0.046)	0.712
	As Cd Cu Hg Pb Se	0.579	0.510 (0.122)	−0.179	0.515	−0.192 (0.052)	0.695
	All 9 metals	**0.620**	0.450 (0.099)	−0.212	0.488	−0.220 (0.062)	0.683
Simuliidae	As Cu Hg	0.711	1.187 (0.235)	−0.015	**0.619**	−0.157 (0.038)	0.719
(F)	As Cd Cu Hg	0.702	1.124 (0.227)	−0.061	0.598	−0.163 (0.041)	0.710
	As Cd Cu Hg Pb Se	**0.782**	1.023 (0.169)	−0.059	0.618	−0.190 (0.046)	0.713
	All 9 metals	0.748	0.934 (0.169)	−0.134	0.589	−0.201 (0.051)	0.695

Finally, the SIMPER procedure was applied for metal TR in each taxon across the anthropogenic pressure classes. The analysis showed that As was the main contributor to dissimilarities between REF vs HG groups, followed by Hg (37–66% contribution for As alone or As and Hg, depending on the taxon), except for Perlidae. The main metals contributing to dissimilarities between REF vs AU group were Cu, Ni and As (from higher to lower) (Table 4). Analyses of individual taxa using SIMPER dissimilarities between the two EQR classes showed that As, Cu, Hg and Cd were the main contributors (44–75%, depending on the taxon and metal combination) (Table 4).

These results, and others from previous sections, showed that a selection of 3 biomonitor taxa with demonstrated bioaccumulation potential can minimize sampling and analysis efforts in a bioaccumulation risk assessment, without loss of relevant information. In this regard, we explored various taxa combinations through INTISS scores (see Section 2.3) calculated with TRT_3 (As, Cu and Hg). INTISS was calculated over the TRT_3 scores of all biomonitor taxa present at a site ($INTISS_{all}$) and also over a selection of 3 taxa representative of different feeding styles ($INTISS_3$: the scraper Baetidae, the deposit-feeder Microdrile oligochaetes and the filterer Hydropsychidae).

Linear regression models of the log $INTISS_3$ on the log SedPoll score showed an increase in INTISS in response to sediment pollution (Fig. 3). Additionally, the regression model of the EQR scores on the log $INTISS_3$ showed a reduction in the ecological status with increasing INTISS values (Fig. 3). The fit of the models built with the 3 selected taxa was similar to those with all the biomonitor taxa present at the site (log $INTISS_3$ and log $INTISS_{all}$ vs log SedPoll: r^2_{adj}= 0.720 and 0.732, respectively; NORTI-EQR vs log $INTISS_3$ and log $INTISS_{all}$: $r^2_{adj} = 0.556$ and 0.583, respectively).

The graphical classification of sites based on the regression model followed the same rationale as Fig. 2. Thus, two sites in CU group were classified with No bioaccumulation and Good ecological status; several sites among the HG and AU groups showed Medium to High bioaccumulation, consistent with their Moderate to Poor ecological status; and sites N4 and N11 were at the boundary of Good/Not Good ecological status, though the latter fell within the Medium bioaccumulation class, requiring additional monitoring. The remaining sites were classified as Good ecological status with No or Low bioaccumulation risk, except for NAL056a, which showed alterations in the field community above what was expected from its low TRT_3 score, probably due to reasons other than metal bioaccumulation.

The risk assessment of test sites due to metal bioaccumulation, using INTISS with 3 vs all taxa gave similar results for most sites (Table 5). In most instances, INTISS site assessment for bioaccumulation is consistent with that provided by the EQR scores on the ecological status.

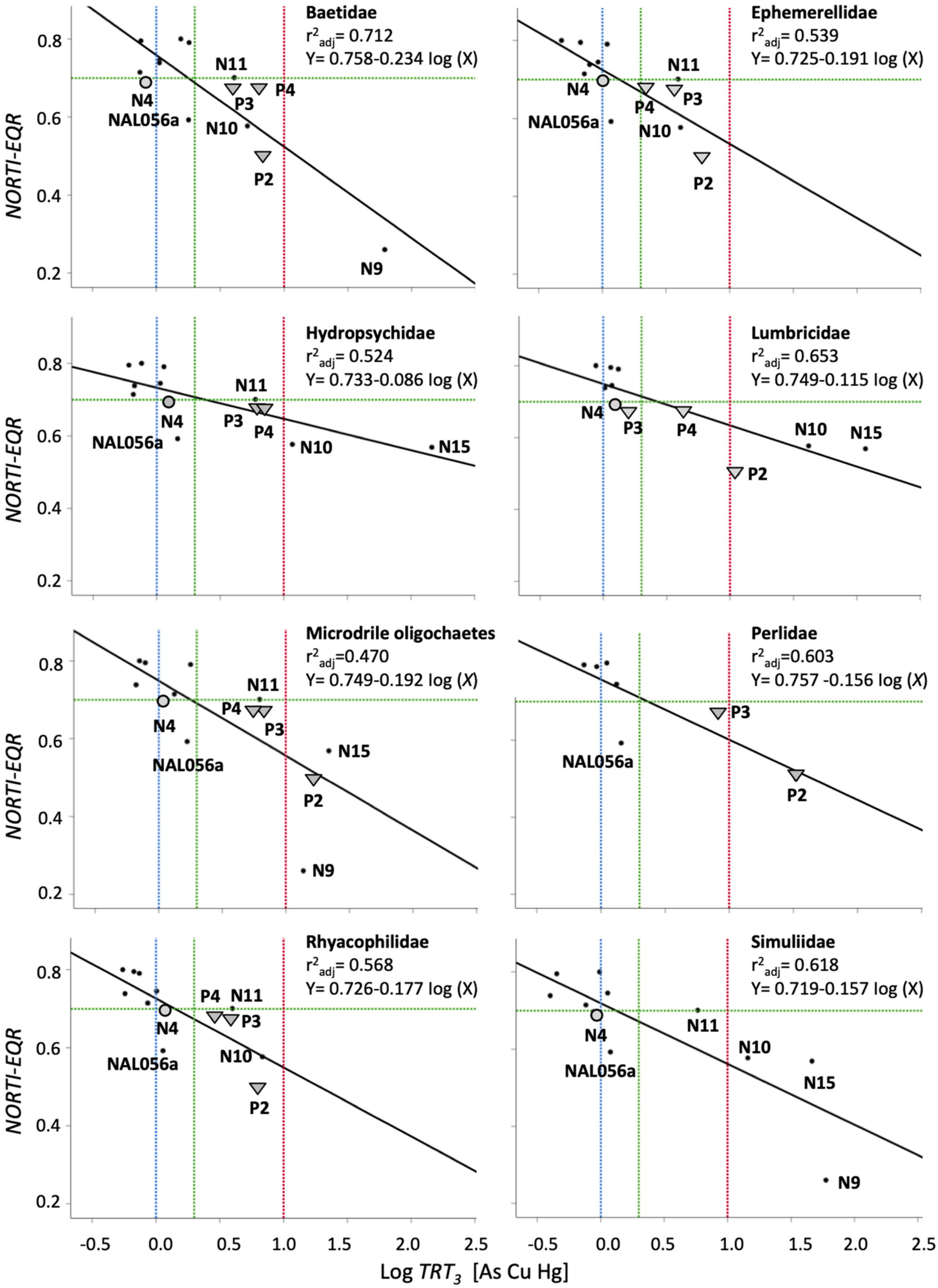

Fig. 2. Linear regression models of the NORTI-EQR score on the log-transformed TRT score calculated for As, Cu and Hg tissue concentration in taxa from the Nalón River basin (regression non-significant for Ephemeridae and Heptageniidae). Horizontal green line: EQR boundary (=0.700) for Good/Moderate ecological status. Vertical overlaid lines: bioaccumulation boundaries of TRT scores: blue, No/Low bioaccumulation; green, Low/Medium; red, Medium/High. Mining area codes: black circle, mercury mines; gray circle, Cu mines; triangle, Au mines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Multivariate analyses of the metal tissue residues, separately for each taxon. One-way ANOSIM Global R values, using as factors two NORTI-EQR classes (Good and Not Good ecological status), and the main potential anthropogenic pressures (CU, HG and AU mining groups and REF, Reference sites). For each taxon, SIMPER procedure identified the metals (ordered by their % contribution) mainly contributing to dissimilarity between EQR site classes (Good and Not Good) and between anthropogenic pressure classes. ns: no significant, $p \geq 5\%$.

TAXA	ANOSIM (factor: EQR) Global R (p %)	SIMPER (factor: EQR) (cumulative %)	ANOSIM (factor: pressures) Global R (p%)	Pairwise R (p%) REF vs CU groups	Pairwise R (p%) REF vs HG groups	Pairwise R (p %) REF vs AU groups	SIMPER (factor: pressures) (cumulative %)
Baetidae	0.397	As Cu Hg (48%)	0.422	ns	R= 0.269	R= 0.972	REF *vs* HG: Hg As Cr (51%)
	0.6		-0.1		2	0.1	REF *vs* AU: Cu Ni Cd (59%)
Ephemeridae	0.215	Cu As Zn (53%)	0.269	ns	R= 0.246	R= 0.646	REF *vs* HG: As (52%)
	ns		-2.6		-3.3	-2.2	REF *vs* AU: As Cu (75%)
Ephemerellidae	0.317	As Cu Cd (48%)	0.455	ns	R= 0.305	R= 0.712	REF *vs* HG: As Pb (57%)
	-0.6		-0.2		-1.2	-0.1	REF *vs* AU: Pb Cu As (69%)
Heptageniidae	0.503	Cu As Cd (46%)	0.605	ns	R= 0.470	R= 0.996	REF *vs* HG: As Cu (63%)
	-0.4		-0.1		-0.4	-0.1	REF *vs* AU: As Pb (62%)
Hydropsychidae	0.542	Cu As Hg (51%)	0.382	ns	R= 0.315	R= 0.913	REF *vs* HG: As (65%)
	-0.1		-0.1		-0.4	-0.2	REF *vs* AU: As Pb (71%)
Lumbricidae	0.632	As Hg Cu (47%)	0.352	ns	R= 0.351	R= 0.692	REF *vs* HG: Hg As (48%)
	-0.1		-0.9		-3.9	-0.1	REF *vs* AU: Cu Ni Se (56%)
Microdriles	0.63	Cu As Hg (43%)	0.489	R= 0.319	R= 0.441	R= 0.821	REF *vs* HG: Hg As Zn (55%)
	-0.1		-0.1	-4.4	-0.3	-0.2	REF *vs* AU: Cu Se Ni (53%)
Perlidae	0.328	As Cu Ni (58%)	ns	–	–	–	REF *vs* HG: Pb Cr Ni (57%)
	ns						REF *vs* AU: As Pb (51%)
Rhyacophilidae	0.498	Cu As Cd (44%)	0.308	ns	ns	R= 0.797	REF *vs* HG: Hg As Se (52%)
	-0.5		-1.1			-0.4	REF *vs* AU: Cu Cd Ni (43%)
Simuliidae	0.462	As Hg (75%)	0.317	ns	R= 0.289	ns	REF *vs* HG: As (66%)
	1		1.4		1.3		REF *vs* AU: Ni Cu (65%)

4. Discussion

Metal accumulation in tissues is not necessarily related to toxicity or trophic transfer (Chapman, 2008). Thus, environmental risk assessment derived from bioaccumulation has to be associated with the onset of adverse effects on the organisms. These are conceptually related to the metabolically available accumulated metal fraction, but at this time we cannot measure that concentration in a tissue (Rainbow, 2018). Moreover, exposure conditions as well as the multiple ecological, physiological and genetic factors that differentiate species are some of the obstacles to extrapolating tissue residue data from laboratory bioassays to field communities, and from one region to another. Therefore, it is not surprising that at the beginning of this century there was still a low emphasis on bioaccumulation in environmental regulations, and a limited knowledge of dose-effect relationships at the level of field populations and communities. More recently, there has been greater interest in investigating the range of tissue residues associated with adverse effects on field invertebrate communities (Adams et al., 2011). In his valuable review, McCarty et al. (2011) addressed the need for future efforts to enlarge the tissue residue-effect databases, to develop a new risk-based framework linking toxicology and ecology, and to improve regulatory guidance incorporating tissue residue-based approaches. We implemented this approach in this study by linking sediment levels of contaminants in the Nalón River basin to tissue concentrations in biomonitors, covering a variety of feeding styles. This procedure allowed us to model alterations in the community ecological status by using integrative bioaccumulation scores in mixed-metal site scenarios.

In rivers impacted by historical or active metal mining, differences in metal concentration among macroinvertebrate taxa from the same sites can be related to differences in exposure (infaunal vs epifaunal) and to the various uptake routes, reflecting differences in bioavailability (Luoma, 1989; Luoma and Rainbow, 2005). Thus, we were able to approach the bioaccumulation risk assessment using integrative scores applied to three taxa (Baetidae, Hydropsychidae and Microdrile oligochaetes) with different functional traits. The three invertebrate taxa selected for the Integrative scores (INTISS) have been also used as biomonitors by other authors (e.g. Luoma et al., 2010; Méndez-Fernandez et al., 2017, respectively). However, the selection in this study was not made *a priori*, but was the result of comparison with other possible biomonitors in the community, as described in Section 3 (see also Fig. 4). These taxa met the following criteria: they are high bioaccumulators of the most relevant metals and are well represented in both reference and test sites, allowing for the calculation of tissue residue ratios between both conditions. Taxa with higher tissue residues are potentially better biomonitors, as the higher bioavailability implies multiple uptake routes (Rainbow, 2018). These taxa were not only high bioaccumulators of the relevant metals, but their selection was supported by the good fit of linear regression models of the metal tissue concentration with sediment concentration and community ecological status.

The methodological framework followed in the selection of biomonitors and relevant metals, and the calculation of Integrative scores (TRT and INTISS) for a bioaccumulation risk assessment in mining areas

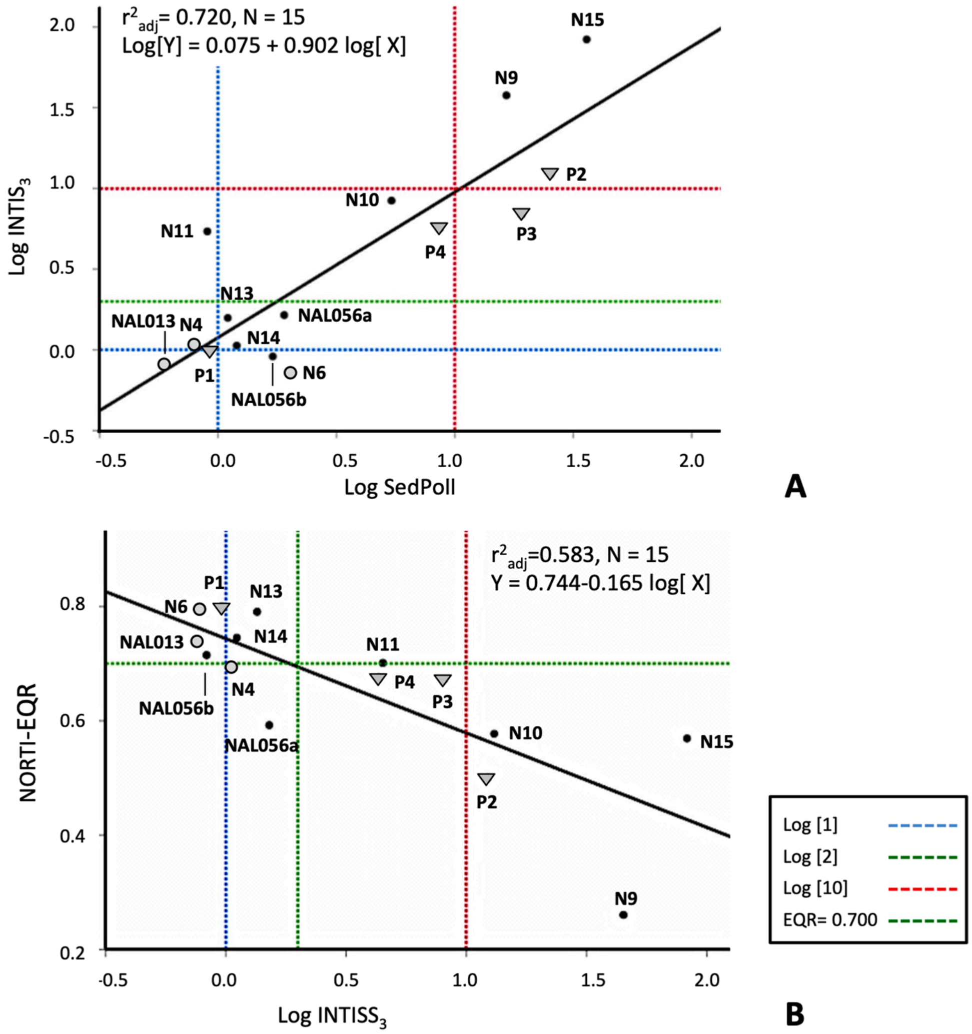

Fig. 3. Linear regression models between (A) the log SedPoll score and the log $INTISS_3$ [As, Hg, Cu for 3 taxa; and (B) the log $INTISS_3$ and NORTI-EQR scores. Mining area codes: black circle, mercury mines; gray circle, Cu mines; triangle, Au mines.

is summarized in Fig. 4. This approach has also emphasized the objective to achieving good ecological status of macroinvertebrate assemblages by reducing the bioaccumulation of metals to baseline levels.

A key issue to be considered when assessing metal bioaccumulation is the difference in metabolic essentiality of some metals. Essential metals (e.g. Cu or Zn) are necessary for the normal growth, development and reproduction of organisms (Chapman and Wang, 2000), and in our results, some of these metals attained very high tissue concentrations, unlike the most toxic, non-essential metals (e.g., Cd, Hg) that are usually present at low levels. Some non-essential metals also do not show adverse effects at moderate tissue residues in field organisms, probably due to efficient detoxification processes (complexation, sequestration and/or storage as inactive substance) (McCarty et al., 2011), but also to genetic adaptation (Klerks and Bartholomew, 1991). Therefore, there is not an absolute definition of what is high or low tissue concentration of a particular metal across invertebrates (Rainbow and Luoma, 2011). Consequently, risk assessment applied in other river basins worldwide need to be adjusted at regional scale with a set of calibrated biomonitors, each with known baseline metal concentrations, for a reliable comparison.

Bioaccumulation risk assessment in the present study followed a reference condition approach based on the ratios of actual tissue residues in field organisms to the baseline thresholds (ETTC) set for each taxon from reference, unaltered or minimally disturbed sites of the same region (Fig. 4). This procedure can help to interpret the causes of aquatic community alteration in other areas affected by historic or current mining activities. The ETTC can also provide reliable objectives for metal TR reduction in restoration programs of different Water Authorities. The levels of metals above the defined thresholds in the selected biomonitors can be used as a surrogate measure of ecotoxicologically significant effects on the community (Adams et al., 2011). The approach presented here can further refine these thresholds based on additional monitoring data.

Previous studies have demonstrated the relationships between metal bioaccumulation in particular biomonitors (Perlidae, Ephemerellidae, Hydropsychidae or Simuliidae) and some adverse effects measured in field macroinvertebrate communities, e.g. loss of taxa richness, loss of abundance of specific bioindicator groups (such as Heptageniidae) or reduction in community monitoring scores (Luoma et al., 2010; Schmidt et al., 2011; de Jonge et al., 2013; Bervoets et al., 2016). Here, we focused our analyses on integrative tissue residue scores (TRT and INTISS) after a critical selection of both relevant metals and biomonitors. These scores respond to increases in sediment pollution and to the reduction of the ecological status of the macroinvertebrate community, and these relationships were modelized. The site classification using boundaries of 2 and 10 times a baseline value will indicate the priorities for management actions in particular locations to reduce bioaccumulation, or to monitor the effectiveness of the measures applied in reducing the deviation from the Good class. The site INTISS scores in the Nalón River basin demonstrated that the atypically high tissue

Table 5

Bioaccumulation risk assessment in 15 test sites of the Nalón River basin, in 3 mining areas (CU, HG and AU groups). For each taxon, the TRT scores were calculated for 3 relevant metals (As, Cu, Hg). The integrative bioaccumulation score (INTISS) for each site was calculated as the mean of the TRT_3 scores in a selection of 3 taxa (BAET, MICRO, HYDRO) and on all taxa present at the site. **Abbreviations**: BAET Baetidae, EPHE Ephemeridae, EPHELL Ephemerellidae, HEPTA Heptageniidae, HYDRO Hydropsychidae, LUMBR Lumbricidae, MICRO Microdrile oligochaetes, PERLI Perlidae, RHYA Rhyacophilidae, SIMU Simuliidae. *TRT* and *INTISS* color codes: No bioaccumulation < 1.0 (white); low 1.12.0 (green); moderate 2.1–10.0 (yellow); high > 10.0 (red). (1) from Costas et al., 2018).

		[As Cu Hg] *TRT₃*										*INTISS₃*	*INTISS_all*	*NORTI-*
	Sites	**BAET**	**EPHE**	**EPHELL**	**HEPTA**	**HYDRO**	**LUMBR**	**MICRO**	**PERLI**	**RHYA**	**SIMU**	**3 taxa**	**all taxa**	**EQR(1)**
CU group	**N4**	0.8	0.7	1.0	1.0	1.3	1.3	1.2	–	1.2	0.9	1.1	1.2	Moderate
	N6	0.8	1.0	0.7	1.0	0.6	1.1	0.8	0.7	0.7	0.5	0.7	0.7	Good
	NAL013	1.1		0.8	0.9	0.7	1.0	0.7	–	0.6	0.4	0.8	0.7	Good
HG group	**N9**	61.6		–	–	–	–	13.8	–	–	59.6	37.7	13.8	Poor
	N10	5.2	7.5	4.1	–	11.7	41.6	–	–	6.8	14.4	8.4	7.5	Moderate
	N11	4.1	3.2	3.9	2.8	6.0	–	6.2	–	4.0	5.8	5.4	5.0	Good
	N13	1.8	1.2	1.1	2.2	1.1	1.3	1.8	0.9	0.7	–	1.6	1.2	Good
	N14	1.1	1.3	0.9	1.0	1.1	1.2	–	1.3	1.0	1.1	1.1	1.0	Good
	N15			–	–	145.7	117.0	21.9	–	–	46.2	83.8	83.8	Moderate
	NAL056a	1.8	1.3	1.2	2.5	1.5	–	1.7	1.4	1.1	1.2	1.6	1.4	Moderate
	NAL056b	0.7	0.8	0.7	0.7	0.7	–	1.3	–	0.9	0.8	0.9	0.9	Good
AU group	**P1**	1.6	0.8	0.5	1.8	0.8	0.9	0.7	1.1	0.5	1.0	1.0	0.6	Good
	P2	6.8	–	6.0	4.7	–	11.4	17.7	33.2	6.0	–	12.2	9.9	Moderate
	P3	6.5	29.9	3.7	4.6	7.1	1.6	7.0	8.2	3.8	–	6.9	5.4	Moderate
	P4	4.2	–	2.2	3.8	6.6	4.5	6.2	–	2.8	–	5.6	4.5	Moderate

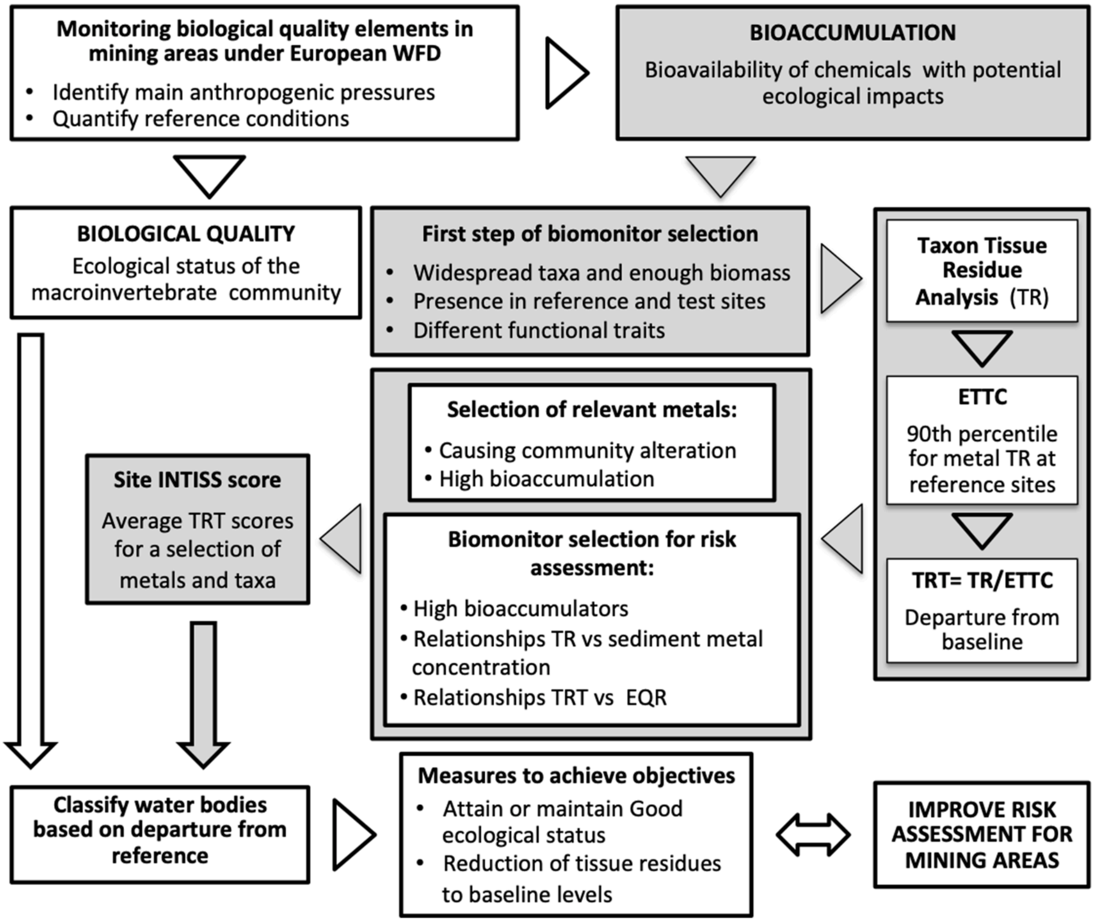

Fig. 4. Schema of the study design proposed for bioaccumulation risk assessment using integrative scores for the protection of the aquatic community ecological status.

concentrations in sites of historic mercury mines and active gold mining could predict high risk of community alteration.

It is also important to acknowledge some of the limitations of the present approach. First, the lack of information about metal speciation could explain differences in metal tissue residues measured in the selected taxa, but it would require further research, focused on each metal and taxon. Second, although the calculation of TRT and INTISS scores is based on the assumption of additive effects of the most relevant chemicals in the basin, we are aware that in metal mixtures there can be also synergetic or antagonistic effects (Norwood et al., 2007). More complex mixing models for the presence of several metals could also improve the bioaccumulation risk assessment on the community ecological status in the future. Recently, some methods have been applied to assess field effects derived from the mixture of metals (the Chronic Criterion Accumulation Ratio: Schmidt et al., 2010; or the 90th quantile mixture regression model: De Jonge et al., 2013). Lastly, further improvement of the regional database is required to expand the range of tissue residue data for other metals, such as Cd, Ni, Pb or Zn, which are important in other mining districts in northern Spain.

The European strategy for water quality risk assessment using reference conditions as a baseline is a suitable starting point for the application of the proposed methodology. A comparison of site bioaccumulation and ecological status assessments, based on their departures from reference levels, showed that water quality monitoring programs can be optimized by combining the two approaches.

5. Conclusions

Our results show that bioaccumulation levels in field organisms from mining areas of the Nalón River can be expressed as gradients in metal bioavailability for As, Cu and Hg.

An integrative score (INTISS) based on a combination of macroinvertebrate biomonitors, representing different feeding styles and levels of metal bioaccumulation, should facilitate the identification of pollution stressors causing loss of ecological integrity in freshwater ecosystems. Specifically, tissue residues in Baetidae, Hydropsychidae and Microdrile oligochaetes have the potential to be used as early warning signals for metals that are likely to result in adverse effects for the macroinvertebrate community in the Nalón River.

This procedure for assessing metal bioaccumulation can also assist in the decision-making process of ongoing or specifically designed biomonitoring programs for rivers affected by mining activities. The methodology proposed includes criteria for sampling decisions, selection of biomonitor taxa and the calculation of integrative scores that can assist in environmental risk assessment in other mining areas, applying a reference condition approach.

CRediT authorship contribution statement

Pilar Rodriguez: Conceptualization, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition. **Iñigo Moreno-Ocio:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Maite Martínez-Madrid:** Investigation, Writing – review & editing, Funding acquisition. **Noemi Costas:** Investigation, Formal analysis, Data curation. **Isabel Pardo:** Investigation, Writing – review & editing, Funding acquisition. **Leire Méndez-Fernández:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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