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Baseline tissue levels of trace metals and metalloids to approach ecological threshold concentrations in aquatic macroinvertebrates

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ABSTRACT

Within the framework of the European Environmental Quality Standards Directive, the biota was recognized as a suitable matrix for monitoring water quality. In the Nalón River basin (North Spain), a catchment subject to historical mining activities, ten macroinvertebrate taxa (4 mayflies, 1 perlid stonefly, 2 caddisflies, 2 oligochaete worms, and 1 blackfly) were collected from unpolluted reference sites in the study area, and the sites were assessed as having High or Good ecological status based on their macroinvertebrate communities to establish a metal bioaccumulation reference condition. For each taxon, tissue concentrations of seven metals (Cd, Cr, Cu, Hg, Ni, Hg, and Zn) and two metalloids (As and Se) were measured and interpreted as natural baseline tissue concentrations that reflected the natural variability of the region. The taxa selected as biomonitors represented 5 different feeding styles in the benthic community: deposit-feeders, scrapers, filterers, generalists and predators, and bioaccumulation was analyzed both by taxon and feeding style as well as general habits (endo- *vs* epi-benthic) and river type. For each taxon, ecological threshold tissue concentrations (ETTC) were calculated as the 90th percentile (P90) of the baseline data distribution. In most instances, the deposit-feeders (aquatic lumbricid and microdrile oligochaetes) showed the highest ETTCs, except for Cu and Zn, which were mainly bioaccumulated by generalist Ephemerellidae, followed by scraper Heptageniidae in the case of Cu. The P90 values were derived from organisms in unaltered reference conditions as estimates of the no-observed-effect concentrations (NOEC), and should provide an approach to ETTCs for the field macroinvertebrate taxa of the region below which the alteration of the benthic community is unlikely. For each metal and metalloid, the P90s for the 10 taxa were entered in a species-sensitivity-distribution model, and the median hazard concentration (HC_{50}) for the macroinvertebrate community was calculated. The ecological threshold concentrations in the biota calculated in this study are proposed for use as a screening tool in the environmental risk assessment of the Nalón River basin and the Cantabrian region, allowing metal exceedance in the selected biomonitors to further research using other lines of evidence under the European Water Framework directive.

1. Introduction

Under the European Water Framework directive (WFD, EC, 2000), sediment and biota have recently been recognized as suitable matrices for monitoring long-term changes in the quality of European water bodies (Carère et al., 2012; EC, 2008, 2010), but in practice, Environmental Quality Standards (EQSs) for chemical substances in these compartments have only been developed by some member states. In Spain, the directive 2013/39/EU (EC, 2013, transposed and published in the Spanish RD 817/2015) expressed that when priority substances (e.g., Cd, Pb and Hg and their compounds) with significant bioaccumulation potential are present, the surface water as well as the sediment and biota matrices remain important for informing water quality policies. In this context, national water authorities should provide sufficient field data for reliable, long-term trend analysis of such substances, and it is compulsory that sediment and biota EQSs be established for them. It is also remarkable that, for the first time, this directive recognized that this information should be incorporated into river management plans for the period from 2015 to 2021.

In accordance with the WFD (EC, 2000), the core of the ecological status classification systems for water bodies is a reference condition approach (RCA), in which the assessment of the effects of human

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activity is based on the degree of deviation of any biological variable measured in a test site from its reference condition value (Bailey et al., 2004; Reynoldson and Wright, 2000). In Europe, this concept mostly relies on the use of minimally disturbed streams and rivers (Stoddard et al., 2006) for each type or ecoregion (characterized by type-specific chemical and hydromorphological conditions and inhabited by specific biotic communities), as well as agreement with a given set of anthropogenic pressure threshold criteria that would ensure the absence of significant alterations at those sites (Pardo et al., 2012). In addition, when assessing naturally occurring substances, such as metals, background levels in the studied matrices should also be considered (EC, 2010, 2013) to correctly evaluate metal levels against the relevant EQSs.

In this context, several terms, such as “background”, “baseline” and “threshold” levels, have been used interchangeably with different regulatory purposes by scientists and environmental authorities. Here, we described tissue baseline concentrations as the range of variation of the metal and metalloid (jointly referred to as “metals” henceforth) concentrations measured in a number of selected potential biomonitors (macroinvertebrate taxa) from a set of reference sites in a particular river catchment or ecoregion. Baseline concentrations are the natural background levels that, according to Reimann & Garrett (2005), reflect natural processes uninfluenced by human activities, although diffuse contamination cannot be excluded. The upper percentile value of the data distribution of the baseline metal concentrations in taxa from unaltered reference sites should provide an estimate of the ecological threshold tissue concentration (ETTC) below which alterations in the macroinvertebrate communities of a particular region are unlikely. The term “hazard” is used as the potential of a substance bioaccumulated to cause damage in the benthic community (tissue residue-effects approach: Meador et al., 2011). Metal tissue hazardous concentrations for the macroinvertebrate community are approached using the ETTCs of several taxa, and hypothesized as a benchmark for tissue metal residues below which there is a low probability of adverse effects in the field communities.

Approximately 25 years ago, Kiffney and Clements (1993) concluded that monitoring the metal concentrations in a set of aquatic macroinvertebrates belonging to different functional groups was a good indicator of the metal bioavailability in lotic benthic communities. Due to several physicochemical processes that occur in water and sediment matrices, metals can be present in different chemical forms that might differ in their bioavailability (Simpson and Batley, 2007) and toxicity to organisms. However, once metals are bioavailable to aquatic organisms, they can be bioaccumulated, and the tissue residues in field taxa can be related to any measurable effect on the aquatic community (Luoma et al., 2010). Benthic community metrics have been used as benchmarks for screening and monitoring sediment quality as well as for deriving EQSs (Meador et al., 2014), and in recent years, there is growing evidence that the tissue metal concentration in target macroinvertebrates is linked to metal-induced ecological effects measured in macroinvertebrate communities, such as decreased abundance and/or richness of sensitive taxa (Bervoets et al., 2016; De Jonge et al., 2013; Luoma et al., 2010; Rainbow et al., 2012; Schmidt et al., 2011). The advantage of this approach is that tissue residues are likely to be less variable among species and exposure conditions than toxic responses expressed as a function of environmental concentrations (i.e., the tissue residue-effects approach: Meador et al., 2011).

The European directive 2013/39/EU (EC, 2013) does not designate freshwater macroinvertebrates for the derivation of EQS, although a few European member states (e.g., CIPAIS: the Joint Commission for the Protection of Italian-Swiss Waters against Pollution) have established quality standards based on whole-body macroinvertebrates as well as fish species (EC, 2014). However, fish are absent in many river reaches, either polluted or unpolluted, which limits the risk assessment of water and sediments based on bioaccumulation in only these organisms. Benthic macroinvertebrate taxa have several advantages as biomonitors of bioaccumulation, as noted by several authors and reviewed by Goodyear and McNeil (1999): 1) they are representative of a variety of habitats and trophic levels and are relatively sedentary, thus representing local conditions; 2) the variability due to sex and maturity level are minimal since most insect taxa live in aquatic habitats in their larval stage; oligochaetes can reach maturity but are hermaphrodites; 3) they represent a food source for fish and are therefore suitable for trophic transfer and biomagnification studies (Besser et al. 2001; Quinn et al. 2003); and 4) they can be crucial for the assessment of sites where fish populations are not present or where the species that are present are not adequate for bioaccumulation monitoring, e.g., protected species.

The present work is part of a larger study on ecological risk assessment in the Nalón River basin (North Spain), where historical Hg and Cu mining activities have introduced a flux of toxic metals into aquatic ecosystems by erosion or leaching (Loredo et al., 2006, 2010; Méndez-Fernández et al. 2015). Data from the same study area were reported by Méndez-Fernández et al. (2017), who compared bioaccumulation in oligochaetes in the field and in laboratory bioassays in a broader geographic context, i.e., the Cantabrian region. Here, we report the metal concentrations in a set of aquatic macroinvertebrates representative of various feeding styles, including the aquatic oligochaetes. We hypothesized that tissue metal levels in biomonitor macroinvertebrate taxa in reference conditions should provide the benchmark against which any test site belonging to the same ecoregion could be assessed. For that purpose, the reference condition was independently established based on macroinvertebrate community metrics. The specific objectives of present study were 1) to analyze and compare tissue metal concentrations in ten macroinvertebrate taxa as determined by their feeding style and habits, and by river type; 2) to provide baseline or reference values and estimate ecological threshold metal and metalloid concentrations (ETTC) in macroinvertebrate taxa of the Nalón River basin; and 3) to evaluate the potential of the studied taxa as biomonitors according to their different bioaccumulation patterns and feeding styles. Overall, we aim to contribute to the future establishment of biota EQSs under the European WFD and to stress the potential of the voluminous database on the ecological state of macroinvertebrate communities to correctly identify and harmonize metal threshold data across different European regions.

2. Materials and methods

2.1. Study area

A total of 14 reference sites were studied during the summers of 2014 and 2015 in the Nalón River basin (Asturias, North Spain), and there were 2–5 reference sites per river type (Table 1). Eight study sites had previously been included in the reference site network of the Cantabrian Hydrographical Confederation (CHC) (Pardo et al., 2010), and the remaining sites were validated as references according to the criteria proposed by WFD (EC, 2000), by the absence of significant anthropogenic pressures in their basins (Pardo et al., 2012), by using geographical information system databases, and by *in situ* expert judgment (I. Pardo). The ecological status of all 14 sites was validated as High or Good during the survey performed in 2015 to calculate the ecological quality ratios (EQRs) of the sites based on the macroinvertebrate community composition and structure (Table 1). The macroinvertebrate community assessment was done using a predictive model built to assess the ecological status of rivers in Northern Spain (NORTI, the NORThern Spain Indicators System: Pardo et al., 2014). The NORTI uses the Bray–Curtis similarity in taxa composition to the reference biological community of the river type to assess how similar a test site is to the reference. Four macroinvertebrate community–based river types of the Cantabrian region were sampled (Types 1, 2, 4 and 5) according to the classification provided by Pardo et al. (2014). The NORTI value is classified from High to Bad following Water Framework