Derivation of sediment Hg Quality Standards based on ecological assessment in river basins

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ABSTRACT

The Environmental Quality Standards (EQS) directive was an important improvement of long-term water quality monitoring at the European level, leading to the use of sediments and biota as relevant matrices for assessing priority substances under the European Water Framework directive. Currently, commonly accepted sediment EQS for Hg are missing in Europe. In this study we present a new, tiered approach to deriving sediment quality standards for Hg: the derivation of Predicted No-Effect Concentration (PNEC) from data in the literature, followed by adjusting values at regional scale, using ecological field data (macroinvertebrate community assessment) and field sediment ecotoxicity bioassays. The limited set of effect data available for Hg spiked-sediment ecotoxicity tests has resulted in unreliable PNEC values for sediment quality assessment. Field reference sites (n = 40) where the macroinvertebrate community status was assessed as High or Good were used to define the ecological background and threshold levels in sediments in northern Spain. Sediment QS developed in other areas were not suitable for specific basins in our study area, since they were within the range of our Hg background levels. Temporary sediment Quality Standards (QS) for Hg were developed for the Nalón River basin (where several mining districts occur), using field effect-based approaches such as sediment ecotoxicity data from *Tubifex tubifex* chronic bioassays and ecological assessment of macroinvertebrate communities. A proposal for Hg quality assessment in freshwater sediments of northern Spain is made based on ecologically relevant QS values, providing benchmark values for No-Effect and Effect Hg sediment concentrations.

Keywords: Environmental Quality Standards; Predicted No-Effect Concentration; Threshold concentration; Species Sensitivity Distribution; Macroinvertebrate community assessment; Reference Condition Approach.

***Capsule**: Sediment quality standards for Hg are developed using background and threshold levels in sites with Good or High ecological status, and a classification is proposed based on Hg sediment levels associated with increasing risk of ecotoxicity and macroinvertebrate community alteration.*

Highlights

- Derived sediment Hg PNECs are not reliable in due to limited data
- Ecological background and threshold Hg levels are defined on field biological data
- Ecological background Hg sediment levels in North Spain are mostly < 0.5 mg kg^{-1} dw
- Hg ecological threshold sediment levels in North Spain range from 0.49–1.21 mg kg^{-1} dw
- HC_{50} estimated from the SSD model with sensitive taxa abundances is 0.90 mg kg^{-1} dw

1. INTRODUCTION

The Environmental Quality Standards (EQS) directive 2008/105/EC (EC, 2008) for priority substances was an important improvement for long-term water quality monitoring at the European level under the Water Framework Directive (WFD: EC, 2000). However, until recently, the only mandatory requirement by the European Directives (2000, 2008) for sediment and biota quality was that contamination levels should not increase significantly in the long-term (i.e., stand-still criterion). In Directive 2013/39/EU (henceforth, EQS directive: EC, 2013), by amending Directives 2000/60/EC and 2008/105/EC regarding priority substances in the field of water policy, it was established that EQS for priority substances in sediment and biota are required in water quality monitoring programs. Priority substances represent a significant direct toxicity risk to benthic biota (sediment-dwelling organisms) or tend to bioaccumulate, so they pose a significant risk of secondary poisoning to predators along the food chain. Several metals are included in the priority substances list (Appendix A: EQS directive), such as Cd, Hg, Ni and Pb (and their compounds). According to EQS directive, for the first time, EQS for priority substances should be taken into account in river basin management plans covering the period 2015 to 2021, and good surface water chemical status for existing priority substances should be met by the end of 2021. However, for the sediment compartment, only a few State Members have developed EQS for a limited number of chemical substances (Bakke et al., 2010; Crommentuijn et al., 2000; de Deckere et al., 2011).

The European Technical Guidance document (TGD) for Deriving Environmental Quality Standards No. 27 (EC, 2011) compiles inputs and improvements for the field of EQS derivation that can serve the State Members to identify priority substances. The recommended approaches for EQS derivation rely mostly on laboratory data with spiked chemicals, with further consideration of field and mesocosm data (Bakke et al., 2010; Buchwalter et al., 2017; Crane et al., 2007; Kwok et al., 2008, 2014; Leung et al., 2005). Laboratory toxicity data for EQS derivation should consider a range of species, including all available data for any taxonomic group or species relevant for the sediment compartment, according to the REACH legislation (ECHA, 2011). Therefore, relevant and reliable ecotoxicity data on sensitive species and endpoints should be used as the basis for deriving and extrapolating the quality standards via deterministic (i.e., the Assessment Factor or Equilibrium Partitioning methods) or probabilistic (i.e., the Species Sensitivity Distribution method or Dose-Response Regression models) approaches. The method selection depends on the quality and quantity of available data. Although some examples may be found in which the probabilistic method has been used in the sediment compartment, e.g., Cu Risk Assessment Report (EU-V RAR, 2008), the Assessment Factor method is used more frequently, given the scarcity of sediment ecotoxicity data, which limits the application of probabilistic approaches for many substances (ECHA, 2014, 2017).

TGD No. 27 foresees the use of field data to corroborate the choice of Assessment Factor, as another line of evidence to support a proposed quality standard. Under specific local geological circumstances, the TGD No. 27 states that policy makers should consider, on a case-by-case basis, the suitability of generic guidelines and incorporate relevant aspects of environmental chemistry and species sensitivity to known pollutants. Field data may consist of a range of effect levels matching chemical concentration and biological data. However, the levels of naturally occurring substances ("background" concentrations) should also be considered as another strong supporting line of evidence to determine the EQS (EC, 2013). Reimann and Garret (2005) reviewed the common definitions of background levels used in the literature and showed that the definitions varied depending on the objective of the study. In the present study, we define the "ecological background" concentration as the natural variation of metal concentration in river sediments of a geographical area, not due to local-point anthropogenic sources, from sites assessed as High or Good ecological status (although unidentified diffuse pollution or atmospheric deposition cannot be discarded). Based on this concept, we define the "ecological threshold" value as the upper limit of the background fluctuation below which alteration of the ecological status is unlikely.

The study region in North Spain includes mining districts linked to As and Hg-rich minerals in the Nalón River basin (Asturias) (Loredo et al., 2003; Ordoñez et al., 2013). A recent study of rivers and streams in this area (Méndez-Fernández et al., 2015) revealed that metal levels in reference sediments of the Nalón basin might be naturally enriched (especially for As and Hg) and in the most toxic sites, the sediment Hg concentrations can reach 300 mg kg^{-1} dw. However, the absence of SQGs or EQS for the sediment compartment in Spain has limited the development of sound environmental risk assessments and water quality protection goals in the area. Therefore, our underlying hypothesis is that sediment Hg EQS can be developed to assess sediment quality in rivers based on a sounded evaluation of ecological background and threshold levels, as defined in this paper. The new Hg EQS should be able to assess both increasing risk of toxicity and macroinvertebrate community alteration. When addressing Hg sediment pollution and ecotoxicity assessment, the following questions arised: 1) Which approach should be best to determine Hg background and threshold levels? 2) What is the relationship between the ecological background and the toxic metal concentrations in the field? 4) Are the ecological threshold levels comparable to different SQGs proposed elsewhere?

To answer these questions, we followed a stepwise approach that included three tiers: 1) we estimated the Predicted No-Effect Concentrations (PNECs) for Hg using spiked-sediment toxicity test data from the literature; 2) we determined the Hg background and No-Effect threshold levels from a set of sediments from several river catchments in North Spain with High or Good ecological status, by using a Reference Condition Approach (RCA, Reynoldson et al. 2002); and 3) we estimated the Hg Effect concentration based on a range of risk probabilities, measured as biological responses (ecotoxicity and alteration of field macroinvertebrate community) to Hg sediment concentrations. The Nalón River basin was selected as a case-study area of concern for assessing the Hg levels in sediment because it has a historical record of Hg mining activity that might require locally relevant environmental assessments. Finally, we provide a sediment Hg quality classification proposal for North Spain based in Hg Quality Standards derived from different data sources. This contribution aims to be regarded under the Minamata convention agreement (UNEP, 2017), which aims to protect human health and the environment from anthropogenic emissions and releases of Hg and its compounds.

2. MATERIAL AND METHODS

The derivation of Hg quality standards for freshwater sediments in North Spain relies on the procedure described in TGD No. 27 (EC, 2011), based on the effect-based approach to biota integrity used in the REACH directive (EC 1907/2006) for PNEC derivation with consideration of field data. The procedure allows for deriving temporary quality standards (QS) for chemicals in sediments, considering several lines of evidence (LOE). In the present study, the LOEs were the ecological background and ecological threshold concentrations in sediments, that is Hg levels at reference sites (which provide the benchmarks related to High and Good Ecological Status); the exposure-response models of the relationships of total Hg concentration in the sediment with field ecotoxicity data from sediment bioassays using benthic organisms; and the exposure-response models related to field metrics on the biological status for macroinvertebrate communities (Fig. 1).

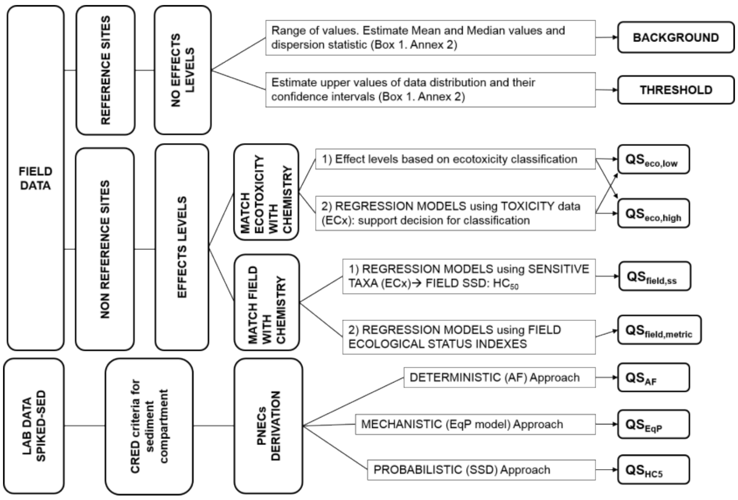

Fig. 1. Flowchart showing the different approaches followed to derive temporary Quality Standards (QS). See the text in section 2.1 and 2.3.3 for definitions of each QS.

In present study, we measured total Hg concentrations in river sediments, but not other mercury compounds, e.g. methyl mercury. In freshwater sediments, it has been observed that the percentage of methyl mercury accounts for a small fraction of the total mercury (usually <1%, as reported by Eisler, 2007; Gascon-Diez et al., 2016), and its presence is associated to either water acidic conditions (Vieira et al., 2018), or anoxic sediment with high organic content where abundant sulphate-reducing bacterial activity takes place (Paranjape and Halla, 2017). This conditions where not observed in the study area (see section 2.2.1).

2.1. PNEC DERIVATION BASED ON SPIKED-SEDIMENT ECOTOXICITY DATA

The calculation of PNECs is the most common procedure used for the derivation of EQS in Europe, and they are mainly derived from literature data on spiked-sediment toxicity tests (EC, 2011). Within our study, we conducted a literature search for Hg ecotoxicity data in freshwater benthic invertebrates, including those from the Euro Chlor Hg risk assessment for the marine environment (Euro Chlor, 1999), the EQS substance data sheet (EC, 2005) and the recent review from Conder et al. (2015). The retrieved studies (Table A-1) were assessed for reliability and relevance using an adapted version of the CRED (Criteria for Reporting and Evaluating Ecotoxicity Data) methodology for aquatic studies (Moermond et al., 2016), with specific criteria for assessing spiked-sediment toxicity test data (Appendix A; Casado-Martinez et al., 2017). Some references were not publicly available but have been used in previous risk assessments (e.g., Euro Chlor, 1999; EC, 2005), and were used assuming reliability and relevance in what we considered master references. Once sediment toxicity data are validated, either deterministic or probabilistic approaches can be used to obtain a proposal for Hg-QS (Appendix A).

2.2. ESTIMATION OF BACKGROUND AND THRESHOLD CONCENTRATIONS IN NORTH SPAIN USING A REFERENCE CONDITION APPROACH

2.2.1. Data gathering

The first step to estimate regional background levels was the appropriate selection of reference sites for the derivation process. Several sources of information were considered. First, the selection of sites was based on the reference monitoring networks developed by the Water Authorities in Spain (Cantabrian Hydrographical Confederation and Ebro Hydrographical Confederation) (35 sites), according to the WFD criteria (Pardo et al., 2012, 2014; Rodriguez et al., 2018). Second, we selected additional sites that belong to the operative surveillance monitoring networks (but not to the reference networks) with a historical record of High or Good Ecological Status (5 sites) fulfilling the following criteria: a) macroinvertebrate communities were evaluated as having High or Good Status; b) riparian vegetation and hydromorphology were mostly unaffected; and c) site anthropogenic pressures, as evaluated by the Water Authorities, were absent or had a low to medium impairment. When data were available, sites were additionally validated by their absence of ecotoxicity using *Tubifex tubifex* sediment chronic bioassays (data from years 2004 to 2011). The ecotoxicity criteria for sediment classification were reported in previous publications (Méndez-Fernández et al., 2017; Rodriguez et al., 2011).

The application of these criteria resulted in 40 sites being used as representative of the reference (unpolluted) conditions for the Hg in sediments from North Spain (Table B-1). These sites belong to different types of water bodies as established by the Spanish Government, (MAGRAMA, 2015) (Table B-2). The sediment Hg concentrations at these sites were used to calculate the ecological background and threshold concentrations associated with no biological field effects on macroinvertebrate community and sediment ecotoxicity. For further statistical treatment see 2.3.3. The full details of water chemistry and sediment characterization at each site have been previously published (see Costas et al., 2018; Méndez-Fernández et al., 2015, 2017; Rodriguez et al., 2011, 2018). However, a brief summary is given in section 2.4, and Tables B-1 and B-2. Finally, methyl mercury was expected to represent a very low percentage of the total Hg measured in the sediments given that the studied rivers mean water temperature was below 17ºC, high oxygenation levels where measured with basic or neutral pH, and that low organic content in the sediment were recorded (see Tables B-1 and B-2, in Appendix B).

2.2.2 Statistical analyses

The differences in the sediment Hg concentration due to the different MAGRAMA types and river basins were assessed through univariate non-parametric tests: the Kruskal-Wallis followed by the pairwise multiple comparisons Dunn´s test (Zar, 1996) in IBM® SPSS 24 (2016). Based on the range of Hg background sediment concentrations, several methods were used to derive metal sediment threshold values: 1) the mean + 2 times the standard deviation (SD); 2) the median + 2 times the median absolute deviation (MAD); 3) the Tukey inner fence (TIF); and 4) the 90 and 95 bootstrapping percentiles (P90, P95). These statistics are among the most widely used to establish the geochemical background variation in soils from United States (Smith et al., 2014), Europe (Ander et al., 2013; Cave et al., 2012) and Australia (Reimann and de Caritat, 2017). The assumption of normal distribution for sediment metal concentrations was evaluated following the criteria described in Table B-3. As the untransformed Hg data showed a positive skew, they were log transformed and the calculated statistics were returned to natural numbers using anti-log. The TIF was calculated as [TIF= P75 + 1.5* IQR], where P75 stands for the 3rd quartile or percentile 75 and IQR is the inter quartile range (75th-25th percentile). Descriptive statistics were calculated in IBM® SPSS 24 (2016), except for MAD, which was calculated in the R software. Bootstrap percentiles and confidence intervals at 95% were calculated (1000 re-samples), using IBM® SPSS 22 (2013).

2.3. MERCURY EFFECT LEVELS IN THE NALÓN RIVER BENTHIC COMMUNITIES

For calculating the Hg Effect levels, two types of data were used to match the observed effects in the biota with sediment Hg concentrations: 1) ecotoxicity data from *T. tubifex* sediment bioassays (see 2.3.1 and Table B-1) conducted in 25 sites of the Nalón River basin (Méndez-Fernández et al., 2015, 2017) and 2) abundance data for 9 macroinvertebrate taxa that were identified as sensitive to a metal pollution gradient (Costas et al., 2018) and field macroinvertebrate community alteration scores in the Nalón basin, calculated by the METI multimetric index (Pardo et al., 2010) that averages several invertebrate community structure metrics, and by the NORTI predictive model (Northern Spain Indicators system; Pardo et al., 2014). Both systems are based in the Ecological Quality Ratio (EQR) assessment, and vary from 0 to >1, with sites with an EQR > 0.93 being in high ecological status (corresponding to a reference condition defined for each typology on a spatial base of reference sites). Lethal and Effective Concentrations (LCx and ECx) were estimated for ecotoxicity endpoints, abundance of sensitive taxa, and field community assessment indices (METI and NORTI) using dose-response models. Finally, the effective concentrations estimated for sensitive macroinvertebrate taxa were entered in the Species-Sensitivity-Distribution (SSD) model and Hg Hazard Concentrations (HCx) with their 95% confidence intervals were calculated (see 2.3.3, for statistical analysis).

2.3.1. Sediment bioassays: Toxicity assessment

Sediment bioassays were run in the laboratory of Animal Ecotoxicity and Biodiversity at the University of the Basque Country (UPV/EHU, Spain). The 28-d sediment chronic bioassay with *T. tubifex* was based on Reynoldson et al. (1991) and ASTM (2005) and the detailed methods were described in Méndez-Fernández et al. (2015, 2017). At the end of the 28-d bioassay, we measured survival (%), reproduction (number of Total Cocoons, TCC; number of Empty Cocoons, ECC; and number of Total Young, TYG), and growth endpoints (Total Growth Rate, TGR; see Maestre et al. 2007). Sediment toxicity data were reported by Méndez-Fernández et al. (2015) for the Nalón basin, and toxicity assessment of the sites was performed site by site in the multivariate space of the reference sites using probability ellipses of 80% and 95%, following the procedure described by Rodriguez et al. (2011). Test sites were assessed as Non-Toxic (NT) when they were ordered 80% probability ellipse of the reference sites in the n-MDS space and were thus evaluated as “equal to reference”. Sites outside the 95% probability ellipse were assessed as Toxic (T).

2.3.2. Field community data

In a recent publication on the mining impacts in the Nalón River basin (Costas et al, 2018), a total of 9 macroinvertebrate families were identified as sensitive (Baetidae, Elmidae, Heptageniidae, Hydropsychidae, Leuctridae, Leptophlebidae, Nemouridae, Scirtidae and Sericostomatidae) to the sediment metal pollution gradient, using TITAN (Threshold Indicator Taxa Analysis in R software) (Baker and King, 2010). This method can detect both the location of taxon-specific change points and the response direction along an environmental gradient. The identified sensitive taxa belong to 4 different insect orders (Coleoptera, Ephemeroptera, Plecoptera and Trichoptera) and cover 4 different feeding styles (collector-gatherers, collector-filterers, scrapers and shredders). The macroinvertebrate ecological community status of the Nalón basin was evaluated with the METI and NORTI classification methods following WFD. The Ecological Quality Ratios (EQRs) derived from both systems range from 0 to >1, as the NORTI and METI observed values are divided by the expected median reference value of each river type. Collection and processing of macroinvertebrate samples were done according to the Spanish official protocol ML-Rv-I-2013 (for more details see Pardo et al., 2014 and Costas et al., 2018).

2.3.3. Statistical analyses

We followed the terminology proposed in TGD No. 27 referring to the temporary Quality Standards (QS), as follows:

$QS_{eco,low}$= quality standard based on ecotoxicity data for low adverse effects

$QS_{eco,high}$= quality standard based on ecotoxicity data for high adverse effects

$QS_{field,ss}$= quality standard based on field sensitive taxa using a Species Sensitivity Distribution (SSD) model

$QS_{field,metric}$= quality standard based on EC_{50} values using field metrics (METI and NORTI) from dose-response models

To derive the ecotoxicity effect levels, a modified version of the de Deckere et al. (2011) approach was followed after classifying the sites into different toxicity categories (Non Toxic, NT, and Toxic, T) using probability ellipses (see 2.3.1). $QS_{eco,low}$ was calculated as "$\sqrt{}$ (P_{10} T * P_{50} NT)", and $QS_{eco,high}$ as "$\sqrt{}$ (P_{50} T * P_{90} NT)", where P_{10}, P_{50} and P_{90} are the percentiles for the sediment Hg concentration within each toxicity category.

Dose-response regression models with the R software package *drc* (Ritz and Streibeig, 2005) were used to estimate lethal and effective concentrations (LC_x and/or EC_x) for *T. tubifex* endpoints, sensitive species abundances and EQR assessment methods. Model selection was carried out using Akaike´s information criterion (AIC), and model validation was based on graphical assessment. Goodness-of-fit was assessed by the Neill´s lack-of-fit test ($p > 0.05$) for no-replicates included in the *drc* package (see Méndez-Fernández et al., 2015 for more details).

The EC_{50} values derived for the METI and NORTI indices defined $QS_{field,metric}$ as the concentration of Hg in sediment related to a 50% reduction in the whole macroinvertebrate community richness, diversity and functioning. Additionally, the EC_{50} values calculated from the dose-response models for the abundance of 9 sensitive macroinvertebrate families (see 2.3.2) where entered in a species sensitivity log-logistic distribution model of field sensitive taxa related to the sediment Hg concentration, and fitted to estimate the hazardous concentrations for 5% (HC_5) and 50% (HC_{50}) of the species, with their 95% confidence intervals, using the ETX v.2.1

program (Van Vlaardingen et al. 2004). Data distribution was checked for normality according to the program specifications, using the Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises Normality tests. The HC_{50} for the SSD of sensitive taxa was taken as the $QS_{field,ss}$, representing the highest Hg concentrations at which sensitive taxa occur at the study sites.

2.4. METAL ANALYSIS FROM FIELD SEDIMENTS

Sediment sampling was conducted under a low flow regime, from July to September, when most of the fine-grained suspended sediments become deposited on the riverbed (Mudroch and Azcue, 1995), and when the worst conditions for biota toxicity are expected to occur (AQEM 2002). At each site, sediment characterization was conducted for particle size distribution, total organic content (TOC%) and metal analysis (silt-clay fraction: < 63 µm), as published in previous papers (Méndez-Fernández et al., 2017). All the Hg concentrations were analyzed on the fine-sediment fraction (< 63 µm) and samples were microwave acid digested (following EPA3051 or EPA3052 procedures). For better clarification, all procedures related to the methodology for acid digestion and analytical measurements used in different studies are compiled in Table B-4. Pearson´s correlation coefficients were calculated for Hg concentration and sediment TOC% or the silt-clay fraction (in %) at the reference sites. The metal sediment concentrations were always reported on a dry weight basis (mg kg^{-1} dw), and values are referred to total inorganic Hg.

3. RESULTS

3.1. SEDIMENT Hg PNEC DERIVATION

The literature review resulted in little reliable and relevant ecotoxicity data derived from chronic single-species toxicity tests (see Appendix A-1 and A-2). Only data for two different sediment-relevant species were assigned to category Q1 and could be used for PNEC derivation, via the AF method: the freshwater amphipod *Hyalella curvispina* (4 NOEC values) and the insect *Chironomus riparius* (2 NOEC values). The NOEC value for *H. curvispina* was included despite being a non-native species, due to similar Hg sensitivity compared to other representative organisms (Peluso et al. 2013). *C. riparius* toxicity tests were done using spiked OECD artificial sediment that had been aged for 7 days (see Appendix A-2 for more details of the retrieved studies). The TOC% of the sediments from the literature database varied between 2.0 and 7.0%. Effect concentrations were normalized to a standard sediment with TOC of 5% and 1%, according to the decrease in Hg bioavailability to benthic organisms at increasing TOC concentrations (Boeing 2000). The lowest reliable value was a 21-d NOEC of 4.3 mg kg^{-1} (at 5% TOC) for *H. curvispina* growth (Peluso et al., 2013), which is close to the 28-d NOEC of 4.8 mg kg^{-1} (at 5% TOC) for *C. riparius* emergence (Chibunda, 2009). The effect concentration for *C. riparius* exposed to spiked natural sediments was much higher (801.7 mg kg^{-1}, reported by Thompson et al. (1998), cited in EuroChlor, 1999) and previously used for QS derivation (EC, 2005).

Differences in the physico-chemical properties of the sediment used in the ecotoxicity tests, the spiking protocol and the different sediment matrix used for quantification of chemical concentration may all contribute to the variability of effect concentrations, rather than differences in test species or population sensitivity among both studies. Consequently, the lowest reliable effect datum reported as the long-term ecotoxicity endpoint was 4.3 mg kg^{-1} (for 5% TOC) corresponding to 0.86 mg kg^{-1} (at 1% TOC) (see Table A-1). These values can be used as critical data for Hg sediment concentration according to TGD No. 27 (EC, 2011).

Hence, given the limited number of data, the AF method foresees the application of an AF= 50 to the lowest credible datum when the only available data are two long-term tests with different species representing different living and feeding styles (TGD No. 27: EC, 2011). The rigorous application of an AF= 50 resulted in a generic Quality Standard (QS_{AF}) of 0.09 mg kg^{-1} (normalized at 5% TOC) or 0.02 mg kg^{-1} (normalized at 1% TOC). In accordance with the TGD, it may be necessary to adapt the applied AF if the estimated QS_{AF} is below background concentrations (see section 3.2).

3.2. SEDIMENT Hg ECOLOGICAL BACKGROUND AND ECOLOGICAL TRESHOLD ESTIMATION USING A REFERENCE CONDITION APPROACH

Sediments from reference sites in North Spain (n=40) had a low organic content (median of 2.1% TOC; range= 0.4–11.1%), their median silt-clay content was 5.1% (range= 0.4–62.1%), and their Hg concentration ranged from 0.02 to 2.92 mg kg^{-1} (Table B-1). The highest Hg concentration was measured at the Nalón River Basin (2.92 mg kg^{-1}). Otherwise, values were usually < 0.5 mg kg^{-1}. No significant correlations were found between the Hg concentration and sediment TOC% or silt-clay composition % (Pearson r, $p > 0.05$). There were no significant differences in sediment Hg concentration attributed to MAGRAMA river types or river basins (Fig. B-1) (Kruskal-Wallis test, $p > 0.05$). Types 25 and 27 did not have enough data (n = 1), and sites L196, MIE002 and OIA044 could not be grouped with other river basins and were thus not included in the multiple comparison analyses. Given these results, all data were grouped to estimate ecological background and threshold concentrations.

Reference sediments have a mean Hg concentration (± SD) of 0.28 ± 0.47 mg kg^{-1} and a median value (± MAD) of 0.18 ± 0.16 mg kg^{-1} (Table 1), and these concentrations were interpreted as an approach to the ecological background Hg concentration in river sediments from North Spain. The lowest threshold value was calculated as the median plus two times the median absolute deviation (0.49 mg kg^{-1}), whereas the most extreme threshold value was approached as the mean plus two times the standard deviation (1.21 mg kg^{-1}). Both P90 and Tukey´s Inner Fence provided the same threshold of 0.60 mg kg^{-1}, and the upper confidence limit at 95% of the P90 (0.91 mg kg^{-1}) was similar to the P95 value (0.92 mg kg^{-1}). These results show that the Hg ecological threshold value, i.e. the threshold below which there are low probabilities of alteration on the ecological status of the macroinvertebrate communities can range between 0.49 and 1.21 mg Hg kg^{-1} for sediments in North Spain.

Table 1. Mercury sediment ecological background and threshold concentrations derived using a Reference Condition Approach, from river sites with High or Good ecological status in North Spain. Abbreviations: SD, standard deviation; MAD, Median Absolute Deviation; TIF, Tukey Inner Fence; P_{90} and P_{95}, 90% and 95% percentiles with their confidence intervals (CI) at 95%.

Ecological Background levels (mg kg^{-1} dw)	
Range	0.02–2.92
Mean (SD)	0.28 (0.47)
Median (MAD)	0.18 (0.16)
n	40
Ecological Thresholds levels (mg kg^{-1} dw)	
Mean + 2SD	1.21
Median + 2 MAD	0.49
TIF	0.60
P_{90} (CI95%)	0.60 (0.32–0.92)
P_{95} (CI95%)	0.91 (0.34–2.92)

3.3. MATCHING ECOTOXICITY, FIELD COMMUNITY ECOLOGICAL STATUS AND Hg SEDIMENT CONCENTRATION: A CASE STUDY IN THE NALÓN RIVER BASIN

Using the modified method of de Deckere et al. (2011) on a data set from sediment chronic ecotoxicity data with *Tubifex tubifex*, a Hg $QS_{eco,low}$ value of 1.0 mg kg^{-1} was obtained, and the $QS_{eco,high}$ value was 11.2 mg kg^{-1}. Using regression models, we explored the values of several ecotoxicity endpoints (Table 2) and found that the EC_{50} was higher for survival (8.58 mg kg^{-1}) than for total growth rate (TGR = 3.47 mg kg^{-1}), and that the reproduction EC_{50} values (endpoints: TCC, ECC and TYG) fell in the range of 6.39 to 7.94 mg kg^{-1}. However, survival and growth had similar EC_{10} and EC_{20} values, with EC_{10} being ≤ 0.5 mg kg^{-1} and EC_{20} being approximately 1 mg kg^{-1} (Table 2). The $QS_{eco,low}$ was close to the *T. tubifex* EC_{20}s estimated and was interpreted as a threshold for Hg concentration below which toxicity is unlikely to occur. On the other hand, the value obtained for $QS_{eco,high}$ was higher than the EC_{50} calculated for any of the *T. tubifex* toxicity endpoints, suggesting that it can be interpreted as a high concentration, above which it is likely to cause more than 50% adverse effects on the survival, growth and reproduction of *T. tubifex*.

Table 2. Chronic effective concentrations for Hg (EC_x) (mg kg^{-1} dw) estimated through non-linear regression models for several toxicity endpoints from chronic sediment bioassays with *T. tubifex* (data from Méndez-Fernandez et al., 2015). Abbreviations: SE: Standard error of the mean; LN.2= 2 parameter log-normal model; EXD.3= 3 parameter exponential decay model; W1.4, W2.3 and W2.4: type 1 and 2 Weibull models with 3 and 4 parameters.

Endpoints	Toxicity parameter	EC_x	SE	Best model
Survival	EC_{10}	0.38	0.05	LN.2
	EC_{20}	1.10	0.12	
	EC_{50}	8.58	0.85	
Total Growth Rate (TGR)	EC_{10}	0.53	0.29	EXD.3
	EC_{20}	1.12	0.62	
	EC_{50}	3.47	1.93	
Total cocoons per adult (TCC)	EC_{50}	6.39	2.08	W2.4
Hatched cocoons per adult (ECC)	EC_{50}	7.94	5.10	W1.4
Total Young per adult (TYG)	EC_{50}	7.21	4.95	W2.3

From the 9 macroinvertebrate sensitive taxa investigated in unpolluted and polluted sites in the Nalón basin, dose-response regression models could be fitted for all but the collector-filterer Hydropsychidae (Table 3). The abundances of some taxa were variable, not necessarily related to contamination but in some cases to habitat preferences, which can explain the large standard error of the Hg-EC_{50}. EC_{50} values were ordered as follows: Elmidae < Scirtidae ≈ Heptageniidae < Sericostomatidae < Leuctridae ≈ Nemouridae ≈ Leptophlebiidae < Baetidae. Thus, the families of the Order Coleoptera were among the most sensitive taxa to Hg sediment concentration, and the family Baetidae was the most resistant among the sensitive families evaluated. Mercury EC_{50} values estimated from regression models using the biological assessment approaches in use in the Cantabrian region of North Spain (according to the WFD criteria) were 4.87 ± 0.40 and 5.79 ± 2.87 mg kg^{-1} for METI and NORTI EQRs, respectively (Table 3).

Table 3. Sediment Hg EC_{50} and standard error (SE) (mg kg^{-1} dw) estimated for abundance of sensitive taxa (see section 2.3.2) and site ecological status assessment by the predictive model NORTI and the multimetric METI (see section 3.2).

Order	Family	Feeding style	Hg-EC_{50} (abundance)	SE	Best fitted model
Coleoptera	Scirtidae	Shredder	0.08	0.06	W1.4
	Elmidae	Scraper	0.02	0.05	W2.3
Plecoptera	Leuctridae	Shredder	3.69	3.87	W1.3
	Nemouridae	Shredder	3.72	1.41	G.3
Ephemeroptera	Baetidae	Collector-gatherer	30.63	63.22	W1.3
	Leptophlebiidae	Collector-gatherer	4.84	9.67	G.3
	Heptageniidae	Scraper	0.08	1.14	W2.3
Trichoptera	Hydropsychidae	Collector-filterer	NOT APPLICABLE		
	Sericostomatidae	Shredder	1.43	2.22	W2.3
Ecological status (assessment using macroinvertebrate communities)		**Approach**	**Hg-EC_{50}** (ecological status)	**SE**	**Best fitted model**
		NORTI	5.79	2.87	LN.4
		METI	4.87	0.40	LL.4

The Species Sensitivity Distribution (SSD) model was constructed with the Hg-EC_{50} values of the sensitive taxa abundance variation (Fig. 2) to the Hg sediment concentration. The assumption of normality was accepted, according to the Anderson-Darling, Cramer von Mises and the Kolmogorov-Smirnov normality tests ($p > 0.01$, ETX v.2.1). The Hazardous Concentration at 5% (HC_5) was 0.01 mg kg^{-1} (CI 95%: 0.00–0.08), and at 50% (HC_{50}) was 0.90 mg kg^{-1} (CI 95%: 0.16–4.90). The HC_5 values were in the lowest range of Hg sediment concentrations at the reference sites in the Nalón River basin (see Table B-1) and were below their mean and median values. On the other hand, the HC_{50} was within the range of the ecological threshold levels calculated for the reference sites (0.49-1.21, see Table 1). It is also noteworthy that the upper confidence limit of the estimated HC_{50} for sensitive species abundance matched with the EC_{50} calculated using the METI and NORTI EQRs assessment methods (see Table 3). From here, we estimate the $QS_{field,ss}$ as equal to 0.90 mg kg^{-1}, and the $QS_{field,metric}$ within a range of 4.87–5.79 mg kg^{-1}.

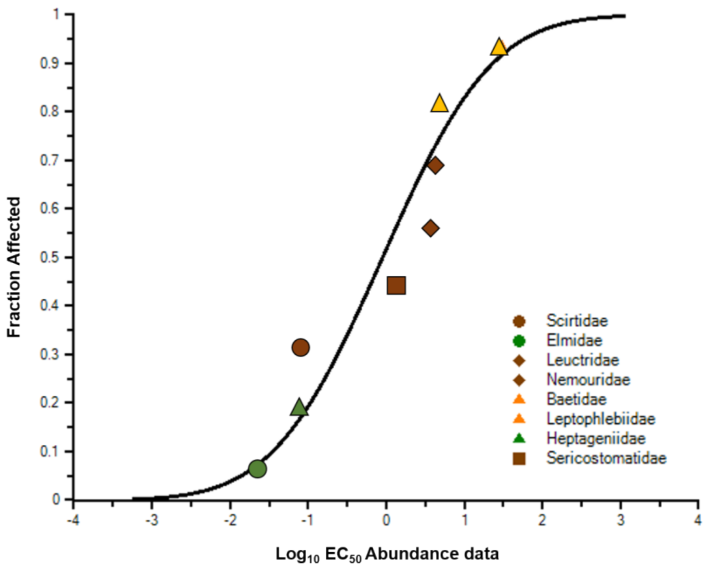

Fig. 2. Species Sensitivity Distribution model for the abundance of sensitive taxa, based on sediment Hg EC_{50} values (see Table 3). Symbols indicate the same insect Order (circle= Coleoptera; diamond= Plecoptera; triangle= Ephemeroptera; square= Trichoptera). Symbol filling indicates the taxa feeding style (green= scraper; brown= shredder; orange= collector-gatherer).

4. DISCUSSION

4.1. PNEC DERIVATION BASED ON SPIKED-SEDIMENT DATA

For most chemicals, information regarding sediment ecotoxicity in different trophic groups across different benthic taxa is very limited and requires the use of assessment factors that, in our study area, result in unreliable PNEC values. The estimated QS_{AF} of 0.09 mg kg^{-1} (at 5% TOC) or 0.02 mg kg^{-1} (at 1% TOC), as derived from the lowest reliable effect datum related to the long-term endpoints, is lower than the sediment median and mean ecological background levels in North Spain (0.18 to 0.28 mg kg^{-1}). Only the beetles Elmidae and Scirtidae, which are among the most sensitive taxa, can show adverse effects in their abundance at this Hg concentration (EC_{50} of 0.02 and 0.08 mg kg^{-1}, respectively), as can the mayfly Heptageniidae (EC_{50}= 0.08 mg kg^{-1}). Thus, the application of an AF of 50 to the lowest effect datum for the derivation of the QS_{AF} might be too conservative and, thus, unreliable because more than 70% of the reference sites in North Spain would be misclassified as affected by Hg pollution when they actually are not.

On the other hand, the use of a critical value at 1% TOC, without the application of an assessment factor, gives a QS = 0.86 mg kg^{-1}, which was in accordance with the range for the ecological threshold concentrations (0.60-1.21 mg kg^{-1}). Hence, the background, threshold and effect values from field studies can provide in the future trustworthy evidences to adjust the AF value and to derive a reliable QS_{AF} with an ecological meaning.

4.2. ECOLOGICAL BACKGROUND, ECOLOGICAL THRESHOLD AND EFFECT VALUES BASED ON MACROINVERTEBRATE COMMUNITY DATA

To our knowledge, this is the first study attempting to derive the background values of freshwater sediments using a Reference Condition Approach, i.e. supported by the site assessment as High or Good ecological status, which includes benthic macroinvertebrate community with good conservation status. The Hg ecological background values in sediments from North Spain (0.18 to 0.28 mg kg^{-1}), were in agreement with those determined in the same region using a geochemical approach in streams from unmineralized areas (< 1 mg kg^{-1}: Ordóñez et al., 2013), in estuaries from pre-mining levels (0.33 mg kg$^{-1:}$ García-Ordiales et al., 2017), or from unpolluted estuarine sediments (0.27 mg kg^{-1}: Rodriguez et al., 2006).

The most extreme value at the reference sites was found at the Nalón River basin (2.92 mg kg^{-1}) and represented one of the highest (and exceptional) natural levels in North Spain, probably related to the catchment lithology, and its location in a mining district which was the world's third leading Hg producer in the 1960–70s (Loredo et al., 2003). In other Hg mining areas in Spain, such the Almadén mines (the largest Hg mining district in the world) the Hg levels were very similar (2.2 ± 0.6 mg kg^{-1}) in control sites upstream the mining activity area (García-Ordiales et al., 2016). In the Idrija mines (Slovenia) the values in pristine reference river sediments upstream from the mines reached 5 mg kg^{-1} (Žižek et al., 2007). These data suggest that the influence of geology on sediment background levels in local or regional river basins can be very important, and the sediment QS derivation should be validated by biological assessment of the field communities or by sediment ecotoxicity bioassays (Reimann and Garret, 2005).

When comparing ecological threshold Hg levels in our study area with data in the literature concerning Hg-SQG Low (lack of toxicity) reviewed by Conder et al. (2015), we found no significant differences ($p \geq 0.05$) (0.18 and 0.17 mg kg^{-1}, respectively). However, there were significant differences ($p < 0.05$) between the North Spain reference median values and Conder et al.'s median High (probable, moderate or severe toxicity) Hg-SQGs (0.87 mg kg^{-1}). However, the latter fell within the range of the ecological threshold concentration determined in the present study (0.49–1.21 mg kg^{-1}), below which No-Effect on the macroinvertebrate communities is expected to occur. These data indicated that SQGs in use in other geographical areas cannot be directly applied in North Spain, because they can fall within the range of the natural background and ecological threshold levels of No-Effect.

The SSD model built on the EC_{50} for abundances of the sensitive taxa gave an HC_{50} value of 0.90 mg kg^{-1},that is similar to with the upper limit of natural background sediment concentration fluctuation in North Spain (Table 1), and slightly below both $QS_{eco,low}$ value and 20% chronic effects in *T. tubifex* (EC_{20} for survival and growth, Table 2). These values are also below the NOEC values reported for Hg from sediment ecotoxicity tests with other freshwater benthic organisms exposed to spiked-sediments (see Table A-1). The different approaches used in present study lead towards a low effect value between 0.90–1.20 mg kg^{-1} as the boundary between Good and Moderate status in North Spain, which supports the $QS_{eco,low}$ value of 1 mg kg^{-1}. This threshold value is around 3–7 times the Hg ecological background levels in the study area, an effect concentration that can be considered relevant since it is more than two-fold above the background level, after MacDonald et al., 1996).

In Fig. 3 we compared the current boundary value of the METI and NORTI EQRs for classifying macroinvertebrate communities into Good to Moderate classes (green line, Fig. 3) set at 0.7, MAGRAMA (2015) with the METI and NORTI EC_{50} values calculated in present study (in black, Fig. 3). We see that the actual Good/Moderate EQR boundary to assess the ecological status of macroinvertebrate communities would allow for a reduction in the macroinvertebrate community EQRs due to the Hg sediment concentration of almost 50%. This is far from desirable and suggests the need for revising the EQR values in basins affected by Hg pollution, as in the Nalón River basin.

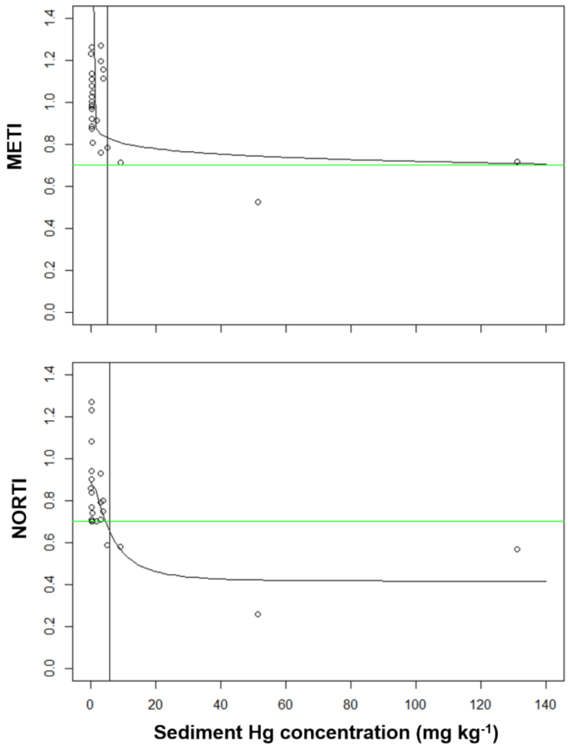

Fig. 3. METI and NORTI EQRs for macroinvertebrate ecological status in relation to sediment Hg concentration in the Nalón River basin. Vertical black line represents the EC_{50} value of 4.87 and 5.79 mg kg^{-1} for METI and NORTI EQRs, respectively. The horizontal green line represents the EQR boundary between Good to Moderate (MAGRAMA, 2015).

4.3. PRELIMINARY CLASSIFICATION OF SEDIMENT QUALITY STANDARDS FOR Hg AND IMPLICATIONS FOR WFD OBJECTIVES

Considering the different Hg QS values determined through different approaches in the present study, four quality classes are proposed for freshwater sediments (Table 4), similar to those proposed for marine (Bakke et al., 2010) and estuarine sediments (Rodriguez et al., 2006), in compliance with WFD class boundaries. Thus, Class I for High chemical status is defined by ecological mean background levels at reference conditions in North Spain, ≤ 0.28 mg kg^{-1}. In a further step, we defined three more classes based in different risk levels, from low to high adverse effects based on sediment ecotoxicity and macroinvertebrate community alteration using specific data derived for the Nalón River. Therefore, Class II indicates Good chemical status, where sediment Hg concentration is ≤ 0.49 mg kg^{-1}, as defined by ecological threshold levels, the quality standard based on field sensitive taxa, $QS_{field,ss}$ (obtained from the SSD-HC_{50}), and *T. tubifex* bioassays highest EC_{20} value. Class III indicates Moderate chemical status at a sediment Hg concentration ≤ 1.22 mg kg^{-1} and is defined by the concentration causing > 20–50% effects in *T. tubifex* bioassays and > SSD-HC_{50} decrease of the sensitive taxa abundances. Class IV indicates Bad chemical status and it is set a sediment Hg concentration ≥ 3.47 mg kg^{-1} and defined by high adverse effects in the biota, i.e., > 50% probability of alteration in the whole macroinvertebrate community structure (> METI or NORTI-EC_{50}), and > 50% probability of effects on survival, growth and reproduction in *T. tubifex* bioassays (> chronic EC_{50}). This classification may still require expert judgment for defining the boundaries and classification of sediments with Hg concentration, including the uncertainty levels of the proposed boundaries

Table 4. Summary of environmental Hg quality standards (QS, in mg kg^{-1}) and preliminary classification based on reference condition, field toxicity data (ecotoxicity sediment 28-d bioassays with *Tubifex tubifex*) and ecological status assessment based on benthic community conservation status. Abbreviations: $QS_{eco,low}$= quality standard based on ecotoxicity data for low adverse effects; $QS_{eco,high}$= quality standard based on ecotoxicity data for high adverse effects; $QS_{field,ss}$= quality standard based on field sensitive taxa using a Species Sensitivity distribution model; $QS_{field,metric}$= quality standard based on EC_{50} values using field metrics (METI and NORTI) EQRs from dose-response models.

LEVEL OF EFFECTS	Definition	Descriptors	Description	Hg QS mg kg^{-1}	Class boundary	CLASS
NO-EFFECTS	ECOLOGICAL BACKGROUND	Reference sites: High or Good Ecological status	Mean & Median North Spain	0.18-0.28	0.28	I
	ECOLOGICAL THRESHOLD		Thresholds North Spain	0.49-1.21		II
ADVERSE EFFECTS	LOW	Macroinvertebrate community	$QS_{field,ss}$	0.90		
		Toxicity	20% effect *T. tubifex*	1.12		
			$QS_{eco,low}$	1.00	1.21	
	MODERATE	Macroinvertebrate community	> 50% hazard of affection on sensitive taxa	1.22-3.46	3.46	III
		Toxicity	> $QS_{eco,low}$ 20-50% effects *T. tubifex* bioassay			
	HIGH	Macroinvertebrate community	$QS_{field,metric}$	4.87-5.79		IV
		Toxicity	> 50% effects *T. tubifex* bioassay	3.47-8.58		
			$QS_{eco,high}$	11.2	≥ 3.47	

In a previous publication related to macroinvertebrate community assessment of the Nalón basin, the authors showed the relevance of metals as biotic community stressors (Costas et al., 2018) and indicate the need for incorporating metal pollution as a significant pressure in the impact analyses that may put at risk the achievement of the Directive's environmental objectives (EC, 2003). The methodology for the development of the ecological threshold and QS values used here for mercury includes different approaches based on the risk assessment of field biological communities and ecotoxicity that help in the decision process for developing adequate sediment EQS criteria for priority substances that assist to the achievement of the Good Ecological Status for European water bodies. These criteria will also allow to identify and adequately set recovery objectives for each water body, as required under the WFD.

5. CONCLUSIONS

Different lines of inquiry improved the understanding of the ecological implications of the contaminant concentrations in the field, which cannot be fully addressed using only data based on traditional spiked-sediment ecotoxicity bioassays. The proposed Hg-QSs and criteria used for the sediment classification using background, threshold and effect-based biological criteria require a future validation in different basins of North Spain or other European areas to evaluate their ecological reliability and relevance when applied to basins with different lithology. By the other side, it is likely that the Hg-QSs proposed here are applicable to sediments characterized by low organic content, non-acidic and highly oxygenated waters, where methylation is not expected. The Hg transfer from the sediment to the aquatic biota will be evaluated in a separate contribution, to assess Hg bioaccumulation and biomagnification potential that may pose a risk to the aquatic organisms.

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APPENDIX A

In this annex we give a detailed overview of the ecotoxicity data reviewed and their assessment performed using the CRED (Criteria for Reporting and Evaluating Ecotoxicity Data) methodology (Moermond et al., 2016) adapted for evaluating data from spiked sediment toxicity test studies (Casado-Martinez et al., 2017).

A-1 Effect data (from spiked-sediment toxicity tests) evaluation

According to recommendations in the TGD, PNECs derived within published Risk Assessment Reports should be normally adopted as QS because the assessments and associated data have undergone through peer review, promoting consistency between chemical assessment and control regimes. The following documents are available:

- Common Implementation Strategy for the Water Framework Directive, Environmental Quality Standards (EQS), Substance Data Sheet Priority Substance No. 21, Mercury and its compounds, CAS-No. 7439-97-6, Final version 15 January 2005
- Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region – North Sea. Mercury- August 1999

A literature search was completed for new ecotoxicity data on December 2016 to update the Hg QS report completed in 2005. The reviewed documents were assessed for relevance and reliability using the CRED methodology (Moermond et al. 2016) adapted for evaluating data from spiked-sediment toxicity test studies (Casado-Martinez et al. 2017). They were classified in the following categories:

- Q1: the results can be used for the calculation of QSs without restriction.
- Q2: the results of the study can be used for the calculation of QSs with restriction: they will be used as supportive information.
- Q3: the results cannot be used for the calculation of QSs due to relevance and/or reliability issues.
- Q4: the study is not assignable due to insufficient details on relevance and/or reliability.

For metals, the TGD recommends a 3-step approach for developing QSs, starting with a $QS_{generic}$ adopting a reasonable worst-case approach, i.e. based on conditions of high bioavailability and on total risk approach (without accounting for background concentrations). According to the limited available data, total organic carbon (TOC%) seems to mitigate Hg toxicity in sediments. Thus the compiled database of effect concentrations was normalized to 5% TOC content (standard sediment in the EU TGD) and 1%. No acid volatile sulphide (AVS) normalization could be performed.

A-2 Overview of the Q1 rated studies based on reliability and relevance

1. **Peluso et al., 2013**

- Species: *Hyalella curvispina*
- Origin: laboratory culture
- Experimental sediment: Artificial OECD Guideline 219 for testing of chemicals (2004) sediment with 3.5% organic matter and 75% quartz sand, 20% kaolinite and 5% sphagnum moss peat and calcium carbonate. One additional test sediment consisting of artificial OECD with virtually no organic matter was used but effect data was not included in the derivation

according to its low relevance. A stream sediment with 12% organic matter was also used. Background Hg concentrations are <0.05 mg kg^{-1}. No AVS measurements, for OECD (2004) AVS can be assumed to be approx. 0.05 mmol kg^{-1}.

- Spiking and equilibration time: dried sediments rehydrated to 30% water, then mercury additions done from a stock solution of 1 g L^{-1} prepared from $HgCl_2$ (Anedra®) in distilled water. The corresponding volume was added, then stirred manually. Seven days of equilibration at same light and temperature as toxicity testing.
- Overlying water: dechlorinated tap water. Dissolved oxygen, pH, conductivity, ammonia, hardness and alkalinity at the beginning and end of test.
- Metal and other analyses during the test: Hg analyses after acid digestion by cold vapour atomic absorption spectrometry with hydride generator. One replicate for chemical analyses at start and end of test for each exposure concentrations and control. Controls <0.05 mg kg^{-1}; artificial sediment six concentrations ranging from 1.6 and 1.3 mg kg^{-1} to 11.8 and 9.5 mg kg^{-1} at start and end of the test, respectively. Natural sediment with four concentrations ranging from 4.5 and 4.0 mg kg^{-1} to 10.2 and 8.5 mg kg^{-1} at start and end of test, respectively. Concentrations in overlying water increasing with increasing sediment concentrations from start to end of test. Higher concentrations in overlying waters in the artificial sediment than in the natural sediment for a same sediment concentration.
- Bioassays: 7-14d old juveniles. Seven replicates per concentration, 10 organisms per replicate. 21d of exposure at 21°C on 16:8 light:dark. Organisms fed fish food and boiled lettuce ad libitum one every five days, before renewal of water.
- Test endpoints: survival and growth (length).
- Statistics: normality and homoscedasticity tested using Shapiro-Wilk's and Barlett's tests. One way ANOVA followed by Dunnet's test. Data arcsine-transformed for % survival and log-transformed for length data. $p < 0.05$
- NOEC: > 10.7 mg kg^{-1} and 2.05 mg kg^{-1} for survival and growth in artificial sediment; >9.4 mg kg^{-1} and 4.2 mg kg^{-1} in natural sediment. This corresponds to a concentration in overlying water of 0.008 and 0.003 mg L^{-1} and 0.008 and 0.003 mg L^{-1} at the end of exposure for the artificial and natural sediment, respectively.

→ Data are accepted as Q1, although the concentrations in overlying water increased from the start to the end of the exposure. Unbounded NOECs are assigned the label Q2.

2. Chibunda, 2009

- Species: *Chironomus riparius*
- Origin: laboratory culture originated from Ghent University, at 20°C and 12:12 dark: light.
- Experimental sediment: Artificial OECD (2004) sediment with 2.5% TOC and 75% sand, 20% kaoline clay and 5% sphagnum moss peat and calcium carbonate pH 7.0. AVS < 0.06 mmol kg^{-1}.
- Spiking and equilibration time: dried sediments rehydrated to 30-50% water, then mercury additions from a stock solution of $HgCl_2$ reagent grade (Merck-Germany) to reach the concentrations then mixed thoroughly. Addition of distilled and double deionized water 1:3 sediment: water ratio and left for 7 days at 4°C in the dark. Then overlying water discarded.
- Overlying water: EPA-medium, moderately hard water with a hardness of 85 mg L^{-1} as $CaCO_3$. Temperature, dissolved oxygen, pH, ammonia, hardness measured 3 times per week, before renewal of water.
- Metal and other analyses during the test: Hg analyses after acid digestion by atomic absorption spectrometry with cold vapour generator technique. One replicate for chemical analyses at end of test for each exposure concentration and control and measurements at start of test. Controls <0.02 mg kg^{-1}; artificial sediment six concentrations ranging from 0.59 and 12.68 mg kg^{-1}, not

reported if time-weighted. Concentrations in pore water increasing with increasing sediment concentrations from <0.00002 to 0.8 mg L^{-1}. The Maximum Permissible Addition ($MPA_{water,SSD}$, EU 2005) is 0.047 µg L^{-1} therefore toxic effects caused solely by exposure through porewater in the lowest effect concentration are excluded.

- Bioassays: 48h old juveniles. Eleven replicates per concentration, 5 for 14d survival and growth, 5 for survival, growth and emergence at 28d and one for chemical analysis. 10 organisms per replicate. 28d of exposure at 20°C on 12:12 light: dark. Organisms fed fish food daily 0.5 mg first 10 days, 1 mg afterwards. Water renewal three times a week.
- Test endpoints: survival, growth (dry weight), emergence success.
- Statistics: data arcsine square root transformed before analysis. Normality and homogeneity using Shapiro-Wilk tests. One way ANOVA followed by Fisher LSD test. $p < 0.05$
- NOEC: 2.42 mg kg^{-1} for 28d emergence success, 2.42 mg kg^{-1} for 14d survival and 0.93 mg kg^{-1} for 14d growth. This corresponds to a concentration in pore water of 0.142 mg L^{-1} and 0.085 mg L^{-1}, respectively.

→ Data at 28d are accepted as Q1 even if the concentrations in overlying water were not measured, but were quantified in pore waters.

3. Thompson et al. (1998) cited in: Euro Chlor, 1999

- Species: *Chironomus riparius*
- Origin: laboratory culture
- Experimental sediment: natural sediment with 5.8% TOC. No gran size or AVS measurements.
- Spiking and equilibration time: $HgCl_2$. No information on spiking and equilibration.
- Overlying water: no information on water quality parameters, only Hg concentrations.
- Metal and other analyses during the test: five concentrations and control measured at start, middle and end of test. Concentrations ranging from 81-94% of nominal. Highest Hg concentrations in overlying water measured at test start of 0.0062 mg L^{-1} at the highest sediment concentration, with no clear relationship with sediment concentrations. At 14 and 28d concentrations < 0.001 mg L^{-1}.
- Bioassays: < 24h post-hatching juveniles. Triplicates, no information on number of organisms per replicate. 28d of exposure at 20°C, partial renewal at day 14. No further information.
- Test endpoints: emergence.
- Statistics: no information.
- NOEC: 930 mg kg^{-1} measured.

→ Data reported in this publication are accepted as Q1, based in their acceptance in two master references (Euro Chlor 1999; EC 2005).

Table A-1. Overview of sediment Mercury chloride (mg kg^{-1} dw) chronic toxicity for freshwater species, NOEC values, and assigned categories after evaluating the studies for relevance and reliability. In all instances, the total Hg concentration in sediments was measured, and exposure was under static/renewal conditions. Abbreviations: NOEC: NO-Effect Concentration; TOC: Total Organic Carbon; Q1, Q2: reliability and relevance categories (see Appendix A); n.a.: not available.

Species	**Higher Taxa**	**Test duration (days)**	**Effect parameter**	**NOEC**	**NOEC (5% TOC)**	**Dose – response reported**	**Test sediment TOC (%)**	**Sediment conditions**	**Equilibration time (days)**	**Reference**	**Category**
Hyalella curvispina	Amphipoda	21	Survival	**> 10.7**	**-**	Yes	2.0	Artificial OECD substrate: AVS 0.05 µmol/g *	7	Peluso et al. 2013	Q2
Hyalella curvispina	Amphipoda	21	Survival	**> 9.4**	**-**	Yes	7.0	Natural sediment	7	Peluso et al. 2013	Q2
Hyalella curvispina	Amphipoda	21	Growth	**2.0**	**4.9**	Yes	2.0	Artificial OECD substrate: AVS 0.05 mmol/kg*	7	Peluso et al. 2013	Q1
Hyalella curvispina	Amphipoda	21	Growth	**6.0**	**4.3**	Yes	7.0	Natural sediment	7	Peluso et al. 2013	Q1
Chironomus riparius	Diptera Chironomidae	28	Emergence	**2.4**	**4.8**	Yes	2.5	Artificial OECD substrate: AVS < 0.06 µmol/g	7	Chibunda 2009	Q1
Chironomus riparius	Diptera Chironomidae	28	Emergence	**930.0**	**801.7**	Yes	5.8	Natural sediment	n.a.	Thompson et al. 1998, in Eurochlor 1999	Q1

*Not reported, quantified in other studies for same composition of artificial sediment.

APPENDIX B

Table B-1. Sediment Hg concentration (mg kg^{-1} dw) at 40 reference sites from North Spain. For each site the following information is indicated: the MAGRAMA (2015) macroinvertebrate river type (**Type**), the geographic coordinates in UTM (Universal Transverse Mercator), the sampling year, their reference water quality monitoring network (**Ref net**), the toxicity assessment performed by *T. tubifex* chronic bioassay (**Tox Assess**), the silt-clay fraction (**SC%,** < 63 µm), and the Total Organic Carbon (Total Organic Carbon, **TOC%)**. **n.a.=** not available data.

Site	Type	River	Basin	Municipality	UTMX	UTMY	Sampling year	Ref net	Tox Assess	SC %	TOC %	Hg
ZBA088	11	Barrundia	Ebro	Barria	546046	4751571	2010	Yes	Yes	10.6	2.3	0.16
RCVA178	11	Najerilla	Ebro	Villarvelayo	501228	4664283	2006	Yes	Yes	14.4	3.0	0.25
URS40	11	Nela	Ebro	Nela	501639	4659606	2006	Yes	Yes	5.1	3.9	0.62
URS65	11	Mayor	Ebro	Villaeslada de Cameros	524326	4661172	2006	Yes	Yes	7.8	1.4	0.23
URS66	11	Urbión	Ebro	Viriegra de Abajo	510910	4662733	2006	Yes	Yes	22.3	2.3	0.34
URS67	11	Tirón	Ebro	Fresneda de la Sierra Tirón	490020	4682917	2006	Yes	Yes	7.4	2.2	0.42
41	12	Ega	Ebro	Murieta	569332	4722831	2006	No	Yes	20.4	3.5	0.11
D166	12	Jerea	Ebro	Palazuelos	470415	4737357	2006	Yes	Yes	17.7	2.0	0.05
RCVA169	12	Oca	Ebro	Villalmondar	472535	4700115	2006	Yes	Yes	21.5	4.2	0.68
URS61	12	Rudrón	Ebro	Tablada de Rudrón	431779	4729853	2006	Yes	Yes	20.7	2.1	0.07
NAL011	21	Turón	Nalón	Mieres	283040	4788140	2014	Yes	No	1.2	2.1	0.28
NAL047	21	Villabre	Nalón	Yermes y Tameza	246779	4794815	2014	Yes	No	4.3	1.6	0.06
R2	21	Lindes	Nalón	Quirós	262789	4777652	2015	Yes	No	8.7	1.3	0.20
NAL042	21	Onón	Nalón	Cangas del Narcea	219588	4790741	2015	Yes	No	1.1	1.2	0.28
NAL043	21	Genestaza	Nalón	Tineo	227060	4795591	2014	Yes	No	1.1	0.6	0.07
NAL029	21	Pumar	Nalón	Cangas del Narcea	203262	4786717	2015	Yes	No	0.4	0.4	0.25
NAL038	21	Coto	Nalón	Cangas del Narcea	198031	4778564	2015	Yes	No	0.6	1.0	0.19
LA001	22	Lamason	Nansa	Lamason	379667	4792545	2008	Yes	Yes	4.5	0.8	0.34
SB002	22	Saja	Saja-Besaya	Los Tojos	395193	4777339	2011	Yes	Yes	3.7	0.5	0.10

L196	22	Lea	Lea	Oleta	540110	4799215	2004	No	Yes	3.4	2.2	0.22
BID2	23	Bidasoa	Bidasoa	Oronoz-Mugaire	612923	4777241	2005	No	Yes	4.4	0.7	0.32
ON1	23	Onin	Bidasoa	Lesaka	604036	4789210	2005	No	Yes	62.1	11.1	0.13
OIA044	23	Oiartzun	Oiartzun	Aritxulegi	595819	4792944	2015	Yes	No	n.a.	n.a.	0.20
NAL009	25	Huerna	Nalón	Lena	262769	4767937	2014	Yes	No	5.5	2.0	0.07
NAN001	26	Nansa	Nansa	Poblaciones	382612	4771787	2008	Yes	Yes	10.0	1.6	0.04
NAN002	26	Nansa	Nansa	Poblaciones	384970	4773968	2008	Yes	Yes	2.4	1.1	0.04
OMTU136	26	Tumecillo	Ebro	Fresneda	494540	4747042	2005	No	Yes	17.9	1.4	0.12
URS41	26	Erro	Ebro	Sorogain	629520	4760509	2006	Yes	Yes	1.8	0.4	0.25
URS6	26	Esca	Ebro	Burgui	663198	4731389	2006	yes	Yes	50.9	2.2	0.02
RCVA53	27	Subordan	Ebro	Hecho	684599	4734594	2006	Yes	Yes	30	2.7	0.92
NAL050	31	Teverga	Nalón	Proaza	743042	4794471	2014	Yes	No	1.2	1.4	0.09
NAL055	31	Lena	Nalón	Lena	270698	4777131	2014	Yes	No	3.3	2.2	0.16
NAL031	31	Arganza	Nalón	Tineo	216040	4795670	2015	Yes	No	0.7	0.7	0.15
MIE002	32	Miera	Miera	Miera	443271	4793458	2008	Yes	Yes	1.8	0.5	0.02
SB003	32	Argonza	Saja-Besaya	Los Tojos	406066	4774490	2008	Yes	Yes	13.8	8.1	0.29
SB017	32	Barranco	Saja-Besaya	San Felices de Besaya	416579	4790017	2008	Yes	Yes	2.4	1.0	0.11
SB022	32	Saja	Saja-Besaya	Los Tojos	395300	4780409	2008	Yes	Yes	3.2	1.0	0.05
R4	0	Mosa	Nalón	Proaza	257059	4793047	2014	Yes	No	5.6	2.3	0.04
NO2259	0	Reguera de Brañanueva	Nalón	Riosa	264220	4789220	2014	Yes	No	6.6	1.4	0.20
N12	0	Rubial	Nalón	Lena	268088	4785479	2015	Yes	No	3.9	2.1	2.92

Table B-2. Mean water physical and chemical parameters in the study rivers, according to each of the River Types (MAGRAMA, 2015). Abbreviations: DO: dissolved oxygen. Note (1): 3 sites were not assigned to any known MAGRAMA type since their catchment area was <10 km^2.

River type	Description	DO %	DO mg l^{-1}	°C	pH	µS cm^{-1}	No. Sites
11	Siliceous Mediteranean Mountain rivers	93	9.0	11.3	8	308	**6**
12	Calcareous Mediterranean Mountain rivers	90	8.3	14.2	7.8	516	**4**
21	Siliceous Cantabrian-Atlantic rivers	100	9.6	16.3	6.9	208	**7**
22	Calcareous Cantabrian-Athlantic rivers	97	9.7	14.1	8.0	252	**3**
23	Basque-Pyrenean rivers	90	9.2	10.4	7.6	202	**3**
25	Siliceous Humid Mountain rivers	100	9.8	11.2	8.2	660	**1**
26	Calcareus Humid Mountain rivers	95	9.2	12.4	7.8	334	**5**
27	High mountain rivers	98	9.7	8.9	7.8	208	**1**
31	Small Cantabrian-Atlantic Siliceous river axes	92	8.9	15.8	7.8	390	**3**
32	Small Cantabrian-Atlantic Calcareous river axes	92	8.9	15.8	7.8	390	**4**
0	Small rivers without defined type (1)	92	9.1	16.3	8.2	361	**3**

Table B-3. Preliminary data inspections for the selection of background values in geochemical studies (based in Ander et al., 2013; Reimann et al., 2005)

1. Display Empirical Cumulative Distribution Functions on linear and normal probability scales: Graphical assessment of normality.

2. Compute the mean and standard deviation (SD) of the data (sub)set, and then the coefficient of variation (CV%).

3. If the CV > 100% plots on a logarithmic scale should be prepared. If the CV is between 70% and 100%, the inspection of logarithmically scaled plots will likely be informative.

4. Calculate the octile skewness coefficient (OC). OC= $((P_{87,5}-P_{50})-(P_{50}-P_{12,5}))/(P_{87,5}-P_{12,5})$. Data require transformation if OC is outside the range [-0.2, 0.2].

5. Calculate [mean+2 SD], [median+2 MAD], Tukey inner fence (TIF), and Percentile approaches (P90, P95, P98).

6. If Step 3 and 4 indicates that logarithmic displays would be informative, log-transform the data, repeat the calculations, and anti-log the results to return them to natural numbers.

Table B-4. Summary of analytical procedures conducted to measure sediment metal concentration. All analyses were performed in the < 63 µm sediment fraction.

Sampling Year, Month	Acid Digestion procedure and Metal analysis	Metal Recoveries
2004-2006, September	EPA3051, ICP-AES and ICP-MS Hg: CV AAS or FI HG AAS	Not reported
2008-2011, September	EPA3052, ICP-AES and ICP-MS Hg: EPA method 6020A	RM8704, USA 82.5-104.4%
2014-2015, September-July	EPA3051, ICP-MS	RM8704 and CRM 031-040 80-118 %

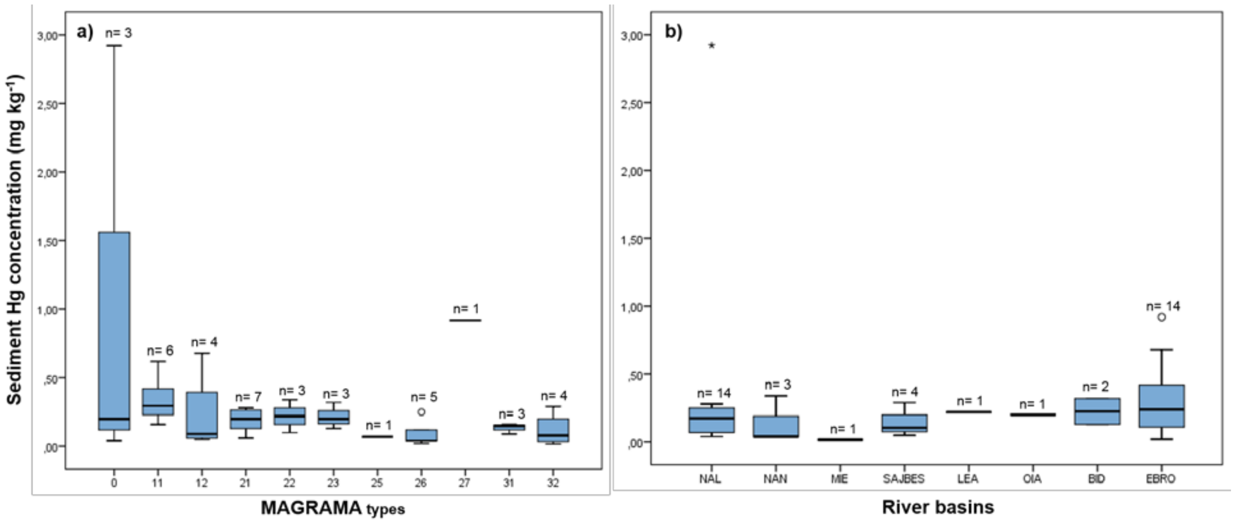

Fig. B-1. Sediment Hg concentration at reference sites in North Spain, ordered by (a) MAGRAMA river types (see Table B-2 for class type description), and (b) by the main river basins. Abbreviations: NAL= Nalón; NAN= Nansa; MIE= Miera; SAJABES= Saja-Besaya; LEA= Lea; OIA= Oiartzun; BID= Bidasoa; EBRO= Ebro. See text for grouping details.