

# Evaluating water quality and ecotoxicology assessment techniques using data from a lead and zinc effected upland limestone catchment

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## Abstract

Point and diffuse sources associated with historical metal ore mining are major causes of metal pollution. The understanding of metal behaviour and fate has been improved by the integration of water chemistry, metal availability and toxicity. Efforts have been devoted to the development of efficient methods of assessing and managing the risk posed by metals to aquatic life and meeting national water quality standards. This study focuses on the evaluation of current water quality and ecotoxicology techniques for the metal assessment of an upland limestone catchment located within a historical metal (lead ore) mining area in northern England. Within this catchment, metal toxicity occurs at circumneutral pH (6.2-7.5). Environmental Quality Standards (EQSs) based on a simple single concentration approach like hardness based EQS (EQS-H) are more overprotective, and from sixteen sites monitored in this study more than twelve sites (> 75%) failed the EQSs for Zn and Pb. By increasing the complexity of assessment tools (e.g. bioavailability-based (EQS-B) and WHAM- $F_{TOX}$ ), less conservative limits were provided, decreasing the number of sites with predicted ecological

risk to seven (44%). Thus, this research supports the use of bioavailability-based approaches and their applicability for future metal risk assessments.

**Keywords:** *water quality standards, bioavailability, ecotoxicology, metal assessment, river water, limestone catchment*

## 1. Introduction

Humans have impacted upland catchments for centuries through the process of mining. Exposure of metal-bearing minerals to oxygen and water, both subsurface and through dumping of mine wastes above the surface, can result in increased dissolved concentrations of metals in water bodies. Once metals have entered aquatic ecosystems, they interact with a broad spectrum of biotic and abiotic components via dynamic interrelated processes, resulting in a large variety of compounds (Luoma and Rainbow, 2008).

In natural waters, the behaviour of metals depends on their speciation. Such species include free ions, inorganic complexes, organic complexes, and metal sorbed to or incorporated within colloids or particulate matter. The formation of metal complexes depends on the concentration of all dissolved components, pH, and ionic strength (Namieśnik and Rabajczyk, 2010). Free metal ions can bind to dissolved organic matter (DOM; particularly humic acids), forming complexes that regulate the concentrations of metals available for interaction with organisms (Tipping, 2002). The complexity of these interactions in aquatic environments makes it difficult to measure the distribution of chemical forms. Knowledge of chemical speciation is important because the relative distribution of different forms controls metal interactions with organisms, including toxic effects. For instance, dissolved metals, particularly free metal ions, have been related to freshwater ecotoxicity (Campbell, 1995; De Schamphelaere and Janssen, 2002). More recently, the concentration predicted to be bound to humic acid has been found to be a good proxy for both organism body burdens (Stockdale et al., 2010; He and Van Gestel, 2015) and accumulation by aquatic bryophytes (Tipping et al., 2008).

In the last few decades, a variety of in situ analytical approaches have been developed to understand the dynamics of metals in natural waters (Buffle and Horvai, 2000). One such technique is Diffusive Gradients in Thin-films (DGT) for assessing a range of active species, depending upon their mobility (diffusion coefficients) and kinetics (dissociation rates) across a thin film of polyacrylamide gel (Zhang and Davison, 2015). This technique has been used as a monitoring tool for providing kinetic information on labile metal species in rivers, soil or sediment impacted by mines and coastal waters (Unsworth et al., 2006; Warnken et al., 2009). In addition, DGT has been applied to investigate potential metal availability in freshwater ecosystems, for instance the accumulation of cadmium in communities of algae (periphyton) has been evaluated by Bradac et al. (2009).

In view of the importance of DOM in complexing metals, comprehensive speciation models have been developed for predicting the distribution of chemical species accounting for complexation with inorganic and organic ligands as well as competition for organic binding sites between different metals, and between metals and protons. The Non-Ideal Competitive Adsorption (NICA) coupled with a Donnan electrostatic sub-model uses a continuous distribution approach to describe metal and proton bindings to organic matter (Benedetti et al., 1995; Kalis et al., 2006). A discrete site approach is used in the Humic Ion Binding Model VII (Tipping et al., 2011). This is coupled with an inorganic thermodynamic code, the Windermere Humic Aqueous Model (WHAM) (Tipping, 1994). The WHAM code has been successfully applied in a variety of research and regulatory areas related to water quality criteria for zinc and copper in the United States and Europe (Hamilton-Taylor et al., 2011; Balistrieri and Mebane, 2014; Balistrieri and Blank, 2008; Cheng et al., 2005). In addition, it has been used across Europe as part of the Critical Loads approach related to atmospheric deposition and soil pollution by cadmium, lead, mercury and copper (de Vries et al., 2007; Hall et al., 2006; Tipping et al., 2003). Over recent decades, WHAM/Model V was incorporated into the Biotic Ligand Model approach to improve prediction of metal toxicity to aquatic organisms and support the implementation of Environmental Quality Standards (EQS).

Metal bioavailability and toxicity have long been recognized to be a function of water chemistry (Paquin et al., 2002). Until recently environmental regulations considered only hardness-based conditions to derive EQS (Beane et al., 2016). However, latest research has increased understanding of the influence of physicochemical variables in metal speciation, and ecotoxicological studies have extended our knowledge of metal effects on biota. Consequently, bioavailability-based approaches such as the Biotic Ligand Models (BLMs) have been implemented within EQS for chemical and ecological assessment, to evaluate the overall quality of a given waterbody (Niyogi and Wood, 2004). In the UK, the BLMs have been simplified to create a user-friendly method known as the Metal Bioavailability Assessment Tool (M-BAT), which use pH, DOC and calcium as input data to account for toxicity of single metals in freshwaters within a regulatory context (e.g. Zn-BLM, Cu-BLM, Mn-BLM and Ni-BLM) (WFD-UKTAG, 2014; De Schamphelaere and Janssen, 2002; Steenbergen et al., 2005; Lock et al., 2007). The M-BAT tools parameterised to date include those for Zn, Cu, Mn, Ni and Pb. Some of these tools have been incorporated into a tiered-approach monitoring scheme to implement the bioavailability-based water quality guidelines. However, they have limitations for assessing metal mixture effects since they are based on BLMs for specific metal-organism interactions, and they do not include all dissolved chemical species. To overcome this issue, an alternative bioavailability-based model (WHAM- $F_{TOX}$ ) developed by Stockdale et al. (2010) offers a plausible option for quantifying mixture toxicity and its potential effects on aquatic organisms. WHAM- $F_{TOX}$ , in common with the BLM, uses organisms as reactants. However, WHAM- $F_{TOX}$  assumes that toxicity is related to non-specific binding to organism surfaces rather than specific biotic ligands (Stockdale et al., 2010). Several studies have applied WHAM- $F_{TOX}$  for predicting metal toxicity to aquatic biota in laboratory experiments and linking the effects of chemical speciation of metals and protons to field species richness of freshwater macroinvertebrates (e.g. Ephemeroptera, Plecoptera and Trichoptera (SR<sub>EPT</sub>)) and zooplankton species diversity (Tipping and Lofts, 2013; 2015; Stockdale et al., 2010; 2014; Qiu et al., 2015).

Despite the scientific and regulatory improvements for the establishment of EQSs, achieving good ecological and chemical status of water bodies according to the Water Framework Directive (WFD) (European Commission, 2000) is still a significant challenge (Environment Agency, 2008a). In the UK, the major impediment to meet this aim is related to diffuse pollution, including metal contamination generated by abandoned mines and mined wastes (Jarvis and Younger, 2000). At a national scale, comprehensive data exist for certain areas but there are substantial gaps for other regions, therefore the assessment of the extent and severity of metal pollution from mining activities is partial (Mayes et al., 2009). As a consequence, the prioritisation of threatened sites to address remediation of metal pollution in a logical and cost-effective manner is a difficult task. River Basin Management Plans (RBMP) provide a good framework for the implementation of mitigation measures. But the application of bioavailability-based standards (EQS-B) offers a significant alternative for the selection of sites at real ecological risk, reducing the burden of remediation targets for efficiently achieving a better quality of water bodies (Environment Agency, 2008b; Harmsen and Naidu, 2013).

This study aims to evaluate different approaches used to assess water quality. Focusing on an upland limestone catchment affected by historical mining, we compare hardness based EQS with approaches that consider more detailed water chemistry such as BLM based EQS and WHAM- $F_{TOX}$ . Data from a single biological survey were used to give context to the results from the chemical approaches. Additionally, we evaluate the ability of the DGT technique to yield dynamic dissolved concentrations that could be applied to EQS as the WFD allows for water quality criteria to be set based upon dynamic methods incorporating chemical speciation. This contribution complements a companion paper (Valencia-Avellan et al., 2017), which provided an assessment of the geochemical and hydrological processes controlling the main sources of metal pollution.

## 2. Methods

### 2.1. Site description

The study area is located within the Yorkshire Dales National Park, northern England (Figure 1). Hebden Beck is a headwater catchment (12 km long) containing extensive historical lead and zinc mining operations, which flows through limestone bedrock surrounded by sheep pasture and peat-rich moorland (Jones et al., 2013). Evidence of mining and smelting exists along the length of the river system, from mine tailings and slag to mine water drainage adits and abandoned buildings. A detailed description of the study area is provided in (Valencia-Avellan et al., 2017).

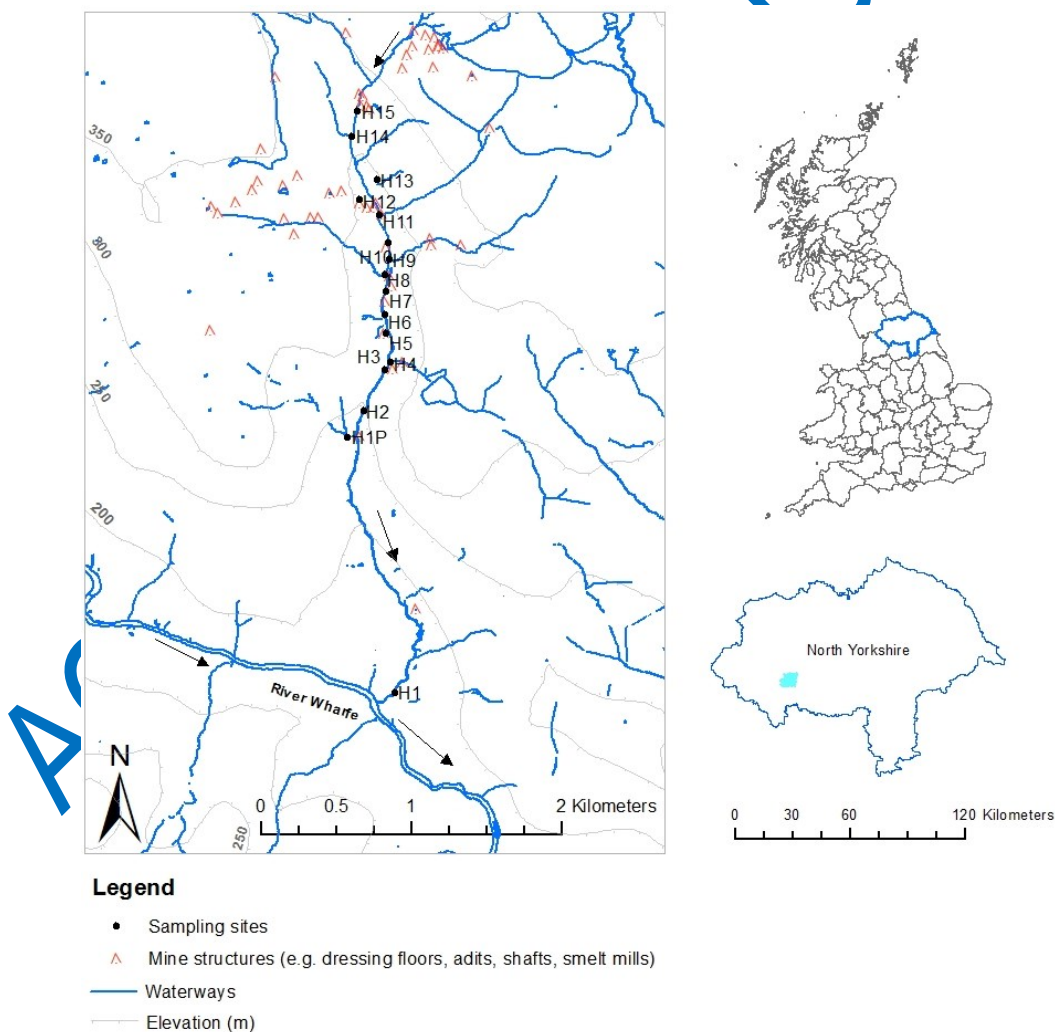


Figure 1. Hebden Beck discharging into the River Wharfe. Black dots represent sampling stations, they are ordered from upper to lower catchment, therefore from H15 to H1. Black arrows indicate the direction of flow.

## 2.2. Water sampling

Monthly field surveys were conducted from November 2013 to December 2014 at sixteen sampling sites. Sampling locations (Figure 1) include a source pool, tributaries and the main river channel, covering the most mining impacted area (5 km<sup>2</sup>). The detailed sampling strategy and analytical procedures are described in Valencia-Avellan et al. (2017). Parameters recorded *in situ* such as temperature, pH, dissolved oxygen, conductivity and flow, together with measured concentrations of dissolved metals (defined as filterable with a 0.45 µm filter) (Zn<sup>2+</sup>, Pb<sup>2+</sup>, Cd<sup>2+</sup>, and Cu<sup>2+</sup>), major anions (SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>) and dissolved inorganic and organic carbon (DIC, DOC) were considered as model input parameters for metal speciation assessment. Concentrations of dissolved metals were also used to evaluate their association with the presence of labile-inorganic species in the river water. Detection limits for metals in water samples were Zn:  $5.1 \times 10^{-7}$  mol/l and Pb:  $2.4 \times 10^{-13}$  mol/l. For statistical analysis, annual average concentrations were calculated for each site.

## 2.3. Determination of $C_{DGT}$ in river water

Diffusive Gradients in Thin-films (DGT) devices (DGT Research Ltd, Lancaster, UK) incorporated a Chelex metal binding layer, a 0.8 mm thick diffusive hydrogel (polyacrylamide gel crossed by agarose cross-linker) and a polyethersulphone filter membrane. DGT devices were subject to minimal handling and transported to and from the site in acid cleaned zip-lock bags. A total of 121 DGT measurements were performed bimonthly from December 2013 to November 2014 across the sampling sites as part of the water sampling surveys. Deployment time averaged approximately one month but varied from 18 days to 43 days. Duplicate devices were deployed at two of the sixteen sites and a field blank was processed in the field once per visit. All DGTs were processed in the laboratory within 24 hours: this involved separating the layers of the probe using acid-washed Teflon tweezers. Each resin gel (Chelex) layer was placed in a 2 ml polypropylene tube with 0.7 ml of 1 M HNO<sub>3</sub> (ARISTAR) then stored at 4°C.

Immediately before ICPMS analysis, the eluent was diluted 10x with Milli-Q water (18.2 M $\Omega$ ·cm).

Labile metal concentrations were determined following the method of Zhang and Davison (1995). First, the mass of metal ( $M$ ) in the Chelex resin is calculated (Eq. 1), where  $C_e$  is the concentration of metals in the 1 M HNO<sub>3</sub> elution solution (in  $\mu\text{g/l}$ ) provided by the ICPMS analysis,  $V_{\text{gel}}$  is the volume of the resin gel,  $V_{\text{HNO}_3}$  is the volume of HNO<sub>3</sub> added to the resin gel, and  $f_e$  is the elution factor for each metal, typically 0.8. Once mass is determined, the concentration of labile metals ( $C_{\text{DGT}}$ ) can be calculated as per Zhang and Davison (1995) (Eq. 2), where,  $M$  is the mass of metal in the Chelex resin,  $\Delta g$  is the thickness of the diffusive gel (0.8 mm) plus the thickness of the filter membrane (0.13 mm),  $D$  is the diffusion coefficient of metal in the gel reported at <http://www.dgtresearch.com/>,  $t$  is deployment time and  $A$  is the exposure area (3.14 cm<sup>2</sup>).

$$M = (C_e (V_{\text{gel}} + V_{\text{HNO}_3}) / f_e) / 1000 \quad (1)$$

$$C_{\text{DGT}} = (M\Delta g / (DtA)) / 1000 \quad (2)$$

#### 2.4. Speciation modelling using Windermere Humic Aqueous Model (WHAM/Model VII)

WHAM/Model VII calculates the chemical speciation of metals using humic (HA) and fulvic acids (FA) as active DOM components. In this study, default model parameters were used together with the measurements for the following inputs; temperature, pH, dissolved anions (Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup>), dissolved metals (Zn<sup>2+</sup>, Pb<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, Fe<sup>3+</sup>, and Al<sup>3+</sup>), dissolved inorganic carbon (DIC; input as carbonate) and DOC. For the modelling DOM was assumed to be composed of 50% carbon and the active fraction considered to be 65% fulvic acid, and hence was calculated from DOC values (FA = DOC  $\times$  1.3) (Tipping et al., 2008). The activity of both Al and Fe was calculated from the measured total filtered concentration and from the equations derived for Al by Tipping (2005) and for Fe by Lofts and Tipping (2011), with the lower of the two values being adopted. This avoids over-estimation of Al or Fe activity in



filtrates containing colloidal forms of the metals. Two different scenarios were considered for modelling. Firstly, Al and Fe oxide precipitates were not considered to be active with respect to surface chemistry. In a second scenario it was considered that the oxides contain active binding sites and that one mole of oxide has 90 g/mol for Fe (Dzombak and Morel, 1990), and 61 g/mol for Al (Lofts, 2012) of active phase. Concentrations of metals bound to oxides were calculated from the output data. These scenarios were applied to allow comparison with the DGT results.

### 2.5. Estimating metal mixture toxicity using WHAM- $F_{TOX}$

WHAM- $F_{TOX}$ , a parameterised version of WHAM established by Stockdale et al. (2010) was applied to evaluate the combined impact of protons ( $H^+$ ) and metals ( $Al^{3+}$ ,  $Zn^{2+}$ ,  $Pb^{2+}$  and  $Cu^{2+}$ ). In WHAM- $F_{TOX}$ , the metal toxicity function ( $F_{TOX}$ ) is considered a product of toxic cation-bound concentrations ( $v_i$ , mmol/g) and the toxicity coefficient of each analyte ( $\alpha_i$ ) and  $i$  refers to each toxic cation (Eq. 3).

$$F_{TOX} = \sum \alpha_i v_i \quad (3)$$

Concentrations of  $v_i$  were obtained from the WHAM/Model VII modelling as described above without active oxides and with the addition of  $10^{-6}$  g of colloidal humic acid. Toxicity coefficients for aluminium ( $\alpha_{Al}$ : 2.24), zinc ( $\alpha_{Zn}$ : 2.69), lead ( $\alpha_{Pb}$ : 2.51) and hydrogen ( $\alpha_H$ : 1) were adopted from Stockdale et al. (2010). The Pb toxicity coefficient (with a published p-value of 0.91) was used in absence of an alternative published value. We suggest the application of  $\alpha_{Pb}$  with caution as it may be conservative compared with other Pb toxicity coefficients reported for trout species (cutthroat trout, 6.7; rainbow trout, 4.6) (Tipping and Lofts, 2015).

For these calculations, the following conditions were applied: i) if  $F_{TOX}$  is lower than 2.33 ( $F_{TOX-LT}$ ) the maximum  $SR_{EPT}$  will be 23 and no toxicity occurs (Eq. 4), ii) a graded linear toxic response occurs as  $F_{TOX}$  increases (Eq. 5), and iii) if  $F_{TOX}$  is higher than 5.20 ( $F_{TOX-UT}$ ) no species are predicted to be present (Eq. 6). Obtained values were converted to a percentage

of the maximum  $SR_{EPT}$  (90% percentile). A detailed description of these calculations is provided by Stockdale et al. (2010).

$$\text{If } F_{TOX} \leq F_{TOX-LT}, \text{ then } SR_{EPT} = SR_{EPT-max} \quad (4)$$

$$\text{If } F_{TOX} > F_{TOX-LT}, \text{ then } SR_{EPT} = SR_{EPT-max} - \left( SR_{EPT-max} \times \frac{F_{TOX} - F_{TOX-LT}}{F_{TOX-UT} - F_{TOX-LT}} \right) \quad (5)$$

$$\text{If } F_{TOX} > F_{TOX-UT}, \text{ then } SR_{EPT} = 0 \quad (6)$$

## 2.6. Water quality assessment tools

Tools with different level of complexity were applied to a consistent set of water chemistry data to assess their contrasting levels of compliance with their respective standards. Firstly, water hardness was calculated at each sampling point for applying established metal limits under hardness-based standards (Environment Agency, 2011). For the hardness based values a risk characterization ratio (RCR) was estimated dividing the dissolved metal concentrations or Predicted Effect Concentration (PEC) by the calculated hardness-based limits or No Predicted Effect Concentrations (PNEC). Water quality standards were exceeded (i.e., over accepted limits) if  $RCR \geq 1$  ( $RCR = PEC/PNEC$ ).

Secondly, assessment tools based on the BLM were applied. One tool, known as the Metal Bioavailability Assessment Tool (M-BAT) was used for Zn (and is also parameterised for Cu, Mn and Ni) and the “Final Pb Screening Tool” was used for Pb, these are both available from the UK Water Framework Directive Technical Advisory Group (WFD-UKTAG, 2014). An additional Pb tool is also available from the International Lead Association that combines bioavailability models with detailed species sensitivity distribution (SSD) analyses (International Lead Association, 2017). These two Pb tools are referred to as Pb-FST and Pb-SSD for the UK-TAG and ILA versions, respectively. For these BLM based tools, an alternate risk characterisation ratio (RCR) was calculated, where PEC values are the bioavailable metals based on dissolved concentrations, and PNEC are the site-specific values obtained from the tools, if  $RCR \geq 1$  water quality standards were exceeded (Merrington and Peters, 2013; WFD-UKTAG, 2014). For these calculations, Zn-BAT considered dissolved

concentrations and the water chemical parameters  $\text{Ca}^{2+}$ , pH and DOC as input data, Pb-FST only required concentrations for Pb and DOC. In the case of the Pb-SSD, it required additional parameters such as  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{CO}_3^{2-}$ . Thirdly, the model WHAM- $F_{\text{TOX}}$  derived from WHAM/Model VII was applied to predict the effect of water chemistry on the maximum species richness ( $\text{SR}_{\text{EPT-max}}$ ) as described above.

## 2.7. Biological survey

A single macroinvertebrate survey was carried out at all sites in August 2014. Samples were collected using a standard kick sampling technique and modified from the Water Framework Directive compliant methodologies (EU-STAR project, 2004). Given the small size of watercourses in the survey, one-minute kick samples were taken at thirteen sites, 30 second kick samples were taken at 2 narrow sites and one sweep sample was taken in a pond at the top of the catchment. Kick samples were followed by a stone search to collect organisms attached to the substrate. Specimens were analysed live at each site. Several were preserved and taken back to the lab for identification confirmation. Invertebrates were analysed to family level. All relevant families within the sample were scored on a presence or absence basis and frequency scale, using the Biological Monitoring Working Party (BMWP) index which rates invertebrates based on their tolerance to organic pollution. BMWPs greater than 100 are associated with unpolluted streams, while the scores of heavily polluted streams are less than 10. In addition, the average sensitivity of the macroinvertebrate families known as the Average Score Per Taxon (ASPT) was determined by dividing the BMWP scores by the number of taxa present. Scores for ASPT are considered from 0 to 10, where 10 indicates a unpolluted site containing large numbers of high scoring taxa (Armitage et al., 1983) (Table 1). Impacts of metal pollution might not be reflected by the BMWP and associated average score per taxon (ASPT) scoring systems as they were originally developed as indicators of organic pollution (Tipping et al., 2009). Despite this caveat, BMWP scores, number of taxa and ASPT values were compared with metal concentrations and outcomes from water quality assessment tools.

Table 1. Scale of water quality categories based on BMWP and ASPT scores.

BMWP score	ASPT score	Water Category	Interpretation
0-10		Very poor	Heavily polluted
11-40	< 4	Poor	Polluted or impacted
41-70	> 4	Moderate	Moderately impacted
71-100	> 5	Good	Clean but slightly impacted
> 100	> 6	Very good	Unpolluted, un-impacted

### 3. Results

General water chemistry for Hebden Beck has been reported in detail by Valencia-Avellan et al. (2017). It is dominated by the underlying geology of the catchment (i.e. buffered with high Ca and circumneutral pH values), whilst metal levels are associated with the weathering and erosion of Pb and Zn minerals exposed by past mining. Mine-impacted water is characterised by circumneutral pH (6.3-7.5) and elevated metal concentrations. Maximum concentrations of Pb, Cd, Zn, and Cu were measured in site H12 corresponding to an ephemeral tributary flowing through spoil mine wastes ( $1.4 \times 10^{-6}$ ,  $4.18 \times 10^{-7}$ ,  $6.5 \times 10^{-5}$ ,  $3.12 \times 10^{-7}$  mol/l, respectively) (Table S1). Full chemistry data are included in Tables S1-S3. Considering the significantly higher concentrations of Zn and Pb, we focus primarily on these metals. Associations between DIC/DOC, sulfate and metals were identified. DIC was strongly correlated with Ca and Mg ( $R^2 = 0.9$ ), whilst DOC was more closely correlated with levels of Fe, Al, Pb and Cu ( $0.1 \geq r \leq 0.7$ ). Sulfate correlated strongly with Zn and Cd ( $R^2 = 0.6, 0.7$ ;  $p < 0.001$ ), possibly associated with the dissolution of sulfide minerals (Table S4).

#### 3.1. Dynamic metal ( $C_{DGT}$ ) concentrations

From DGT measurements at each site, annual average Zn- $C_{DGT}$  concentrations ranged from  $2.8 \times 10^{-5}$  to  $4.8 \times 10^{-9}$  mol/l and for Pb- $C_{DGT}$  from  $1.1 \times 10^{-7}$  to  $1.1 \times 10^{-10}$  mol/l. Highest Zn- $C_{DGT}$  identified in perennial tributaries were H7:  $2.8 \times 10^{-5}$  Zn mol/l and highest Pb- $C_{DGT}$  in

ephemeral tributaries H13 and H12:  $1.1 \times 10^{-7}$  Pb mol/l, while in main channel maximum concentrations were observed in H6:  $7.5 \times 10^{-6}$  Zn mol/l and H10:  $2.3 \times 10^{-8}$  Pb mol/l (Table 2).

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Table 2. Annual averages and standard deviation ( $\pm$ SD) of dissolved metals ( $M_D$ ) in water samples (mol/l), metal concentrations from DGT devices- $C_{DGT}$  (mol/l) and exceedances of Environmental Quality Standards (EQS) by comparing different tools EQS-H<sup>a</sup>, EQS-B (Bioavailability assessment tool-BAT<sup>b,c</sup>, Final screening tool-FST<sup>d</sup> and Species sensitivity distribution-SSD<sup>e</sup>) and WHAM- $F_{TOX}$  ( $SR_{EPT} = 23$  no toxic effects,  $SR_{EPT} < 23$  toxic effects, and  $SR_{EPT}$  indicated as %) for assessing effects of Zn and Pb pollution. RCR: Risk Characterisation Ratio, PEC: Predicted Effect Concentration/ PNEC: Predicted No Effect Concentration. Description of sites are indicated as reservoir (RS), main channel (MC), ephemeral tributaries (ET) and perennial tributaries (PT).

Sites	Description	Zn <sub>D</sub> - measured (mol/l)	Zn- $C_{DGT}$ (mol/l)	Zn RCR		Pb <sub>D</sub> - measured (mol/l)	Pb- $C_{DGT}$ (mol/l)	Pb RCR			WHAM- $F_{TOX}$	
				EQS-H	EQS-B			EQS-H	EQS-B(FST)	EQS-B(SSD)	$SR_{EPT}$	% $SR_{EPT}$
H15	RS	$3.1 \times 10^{-5}$ ( $\pm 7.2 \times 10^{-6}$ )	$1.2 \times 10^{-5}$ ( $\pm 6.9 \times 10^{-6}$ )	253.5	54.0	$1.3 \times 10^{-6}$ ( $\pm 4.0 \times 10^{-7}$ )	$5.7 \times 10^{-8}$ ( $\pm 5.2 \times 10^{-8}$ )	38.8	18.9 <sup>d</sup>	8.5	10	43
H14	PT	$2.1 \times 10^{-5}$ ( $\pm 5.2 \times 10^{-6}$ )	$1.5 \times 10^{-5}$ ( $\pm 4.4 \times 10^{-6}$ )	174.7	45.9	$7.6 \times 10^{-7}$ ( $\pm 3.6 \times 10^{-7}$ )	$6.1 \times 10^{-8}$ ( $\pm 1.9 \times 10^{-8}$ )	21.9	15.8 <sup>d</sup>	6.8	13	55
H13	ET	$3.2 \times 10^{-6}$ ( $\pm 6.3 \times 10^{-6}$ )	$6.1 \times 10^{-7}$ ( $\pm 3.2 \times 10^{-7}$ )	25.9	4.1 <sup>b</sup>	$9.9 \times 10^{-7}$ ( $\pm 2.6 \times 10^{-7}$ )	$1.1 \times 10^{-7}$ ( $\pm 6.6 \times 10^{-8}$ )	28.6	10.2 <sup>d</sup>	1.1 <sup>e</sup>	9	38
H12	ET	$6.5 \times 10^{-5}$ ( $\pm 3.0 \times 10^{-5}$ )	$2.7 \times 10^{-5}$ ( $\pm 4.0 \times 10^{-6}$ )	85.0	117.6	$1.4 \times 10^{-6}$ ( $\pm 4.4 \times 10^{-7}$ )	$1.1 \times 10^{-7}$ ( $\pm 4.0 \times 10^{-8}$ )	39.5	35.0 <sup>d</sup>	17.1	15	66
H11	PT	$6.3 \times 10^{-6}$ ( $\pm 2.1 \times 10^{-5}$ )	$3.3 \times 10^{-6}$ ( $\pm 7.6 \times 10^{-6}$ )	51.3	8.7	$1.5 \times 10^{-7}$ ( $\pm 4.5 \times 10^{-7}$ )	$9.2 \times 10^{-9}$ ( $\pm 1.8 \times 10^{-8}$ )	4.4	1.6 <sup>d</sup>	0.8	20	85
H10	MC	$6.8 \times 10^{-6}$ ( $\pm 2.8 \times 10^{-6}$ )	$5.2 \times 10^{-6}$ ( $\pm 2.5 \times 10^{-6}$ )	55.6	11.1	$3.8 \times 10^{-7}$ ( $\pm 2.0 \times 10^{-7}$ )	$2.3 \times 10^{-8}$ ( $\pm 1.5 \times 10^{-8}$ )	11.2	6.5 <sup>d</sup>	2.9	19	82
H9	PT	$1.5 \times 10^{-6}$ ( $\pm 1.9 \times 10^{-6}$ )	$1.3 \times 10^{-6}$ ( $\pm 5.5 \times 10^{-7}$ )	12.0	2.1 <sup>b</sup>	$1.0 \times 10^{-7}$ ( $\pm 1.1 \times 10^{-7}$ )	$4.6 \times 10^{-9}$ ( $\pm 4.4 \times 10^{-9}$ )	2.9	1.0 <sup>d</sup>	0.5	18	77
H8	PT	$4.1 \times 10^{-6}$ ( $\pm 1.5 \times 10^{-6}$ )	$3.9 \times 10^{-6}$ ( $\pm 2.3 \times 10^{-6}$ )	3.6	7.4	$9.2 \times 10^{-8}$ ( $\pm 3.2 \times 10^{-8}$ )	$1.1 \times 10^{-8}$ ( $\pm 1.1 \times 10^{-8}$ )	2.6	2.0 <sup>d</sup>	1.1	23	100
H7	PT	$4.9 \times 10^{-5}$ ( $\pm 1.3 \times 10^{-5}$ )	$2.8 \times 10^{-5}$ ( $\pm 8.5 \times 10^{-6}$ )	42.9	209.2	$2.1 \times 10^{-8}$ ( $\pm 6.1 \times 10^{-8}$ )	$1.2 \times 10^{-9}$ ( $\pm 1.1 \times 10^{-9}$ )	0.6	3.0 <sup>d</sup>	1.7	18	76
H6	MC	$7.8 \times 10^{-6}$ ( $\pm 1.5 \times 10^{-6}$ )	$7.5 \times 10^{-6}$ ( $\pm 1.0 \times 10^{-6}$ )	10.2	12.8	$2.4 \times 10^{-7}$ ( $\pm 9.9 \times 10^{-8}$ )	$8.5 \times 10^{-9}$ ( $\pm 4.4 \times 10^{-9}$ )	6.9	3.8 <sup>d</sup>	1.7	20	88
H5	PT	$1.3 \times 10^{-5}$ ( $\pm 2.9 \times 10^{-6}$ )	$1.3 \times 10^{-5}$ ( $\pm 1.5 \times 10^{-6}$ )	11.6	43.9	$2.6 \times 10^{-7}$ ( $\pm 2.2 \times 10^{-7}$ )	$4.9 \times 10^{-8}$ ( $\pm 1.8 \times 10^{-8}$ )	7.5	14.6 <sup>d</sup>	8.7	23	100
H4	ET	$5.5 \times 10^{-7}$ ( $\pm 1.4 \times 10^{-7}$ )	$2.6 \times 10^{-7}$ ( $\pm 8.9 \times 10^{-8}$ )	4.5	2.5 <sup>b,c</sup>	$2.1 \times 10^{-8}$ ( $\pm 8.2 \times 10^{-9}$ )	$3.7 \times 10^{-9}$ ( $\pm 5.4 \times 10^{-10}$ )	0.6	0.4	0.02 <sup>e</sup>	8	35
H3	PT	$< 5.1 \times 10^{-7}$	$1.2 \times 10^{-6}$ ( $\pm 5.1 \times 10^{-6}$ )	0.2	0.8	$8.0 \times 10^{-10}$ ( $\pm 9.5 \times 10^{-10}$ )	$5.4 \times 10^{-10}$ ( $\pm 4.1 \times 10^{-10}$ )	0.02	0.1	0.1	23	100
H2	MC	$7.4 \times 10^{-6}$ ( $\pm 1.1 \times 10^{-6}$ )	$7.0 \times 10^{-6}$ ( $\pm 1.0 \times 10^{-6}$ )	9.7	17.7	$1.9 \times 10^{-7}$ ( $\pm 1.4 \times 10^{-7}$ )	$1.3 \times 10^{-8}$ ( $\pm 3.9 \times 10^{-9}$ )	5.5	4.4 <sup>d</sup>	2.3	22	97
H1P	PT	$5.6 \times 10^{-7}$ ( $\pm 1.1 \times 10^{-6}$ )	$4.8 \times 10^{-9}$ ( $\pm 4.5 \times 10^{-9}$ )	0.7	2.4	$3.5 \times 10^{-9}$ ( $\pm 3.1 \times 10^{-9}$ )	$1.1 \times 10^{-10}$ ( $\pm 3.0 \times 10^{-11}$ )	0.3	0.2	0.1	23	100
H1	MC	$2.4 \times 10^{-6}$ ( $\pm 1.1 \times 10^{-6}$ )	$2.5 \times 10^{-6}$ ( $\pm 6.6 \times 10^{-7}$ )	2.1	4.2	$8.0 \times 10^{-8}$ ( $\pm 6.6 \times 10^{-8}$ )	$3.7 \times 10^{-9}$ ( $\pm 3.3 \times 10^{-9}$ )	2.3	1.8 <sup>d</sup>	1.0	23	100

<sup>a</sup> Limits of EQS based on site-specific hardness, Zn EQS-H=  $1.3 \times 10^{-7}$  (for sites H15, H14, H13, H11, H10, H9, H4);  $7.6 \times 10^{-7}$  (for sites H12, H6, H2, H1P) and  $1.2 \times 10^{-6}$  (for sites H8, H7, H5, H3, H1). Pb EQS-H=  $3.5 \times 10^{-8}$  for all sites.

<sup>b</sup> pH values were outside the limits of Zn-BAT tool. Default limit (pH=6) was applied for Zn EQS-B calculations.

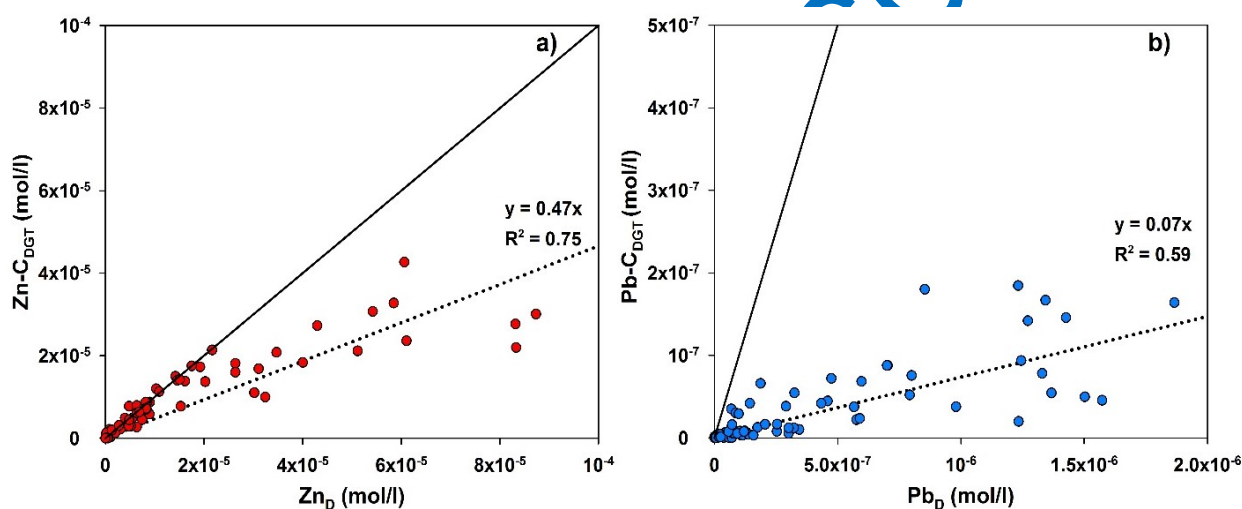
<sup>c</sup> Ca concentrations were outside the limits of Zn-BAT tool. Default limit (Ca=3 mg/l) was applied for Zn EQS-B calculations.

<sup>d</sup> Measured Pb exceeds the site specific PNEC of the Pb-FST tool.

<sup>e</sup> pH values were outside the limits of Pb-SDD tool (pH=6-8.5). Pb-PNEC should be interpreted with care.

### 10 3.2. Comparison between dissolved metal concentrations and $C_{DGT}$ measurements.

11 DGT measurements were compared with the mean of metal concentrations in water samples  
12 calculated from samples taken at both the deployment and retrieval dates. Figure 2 shows the  
13 linear correlations between measured dissolved metals in water samples and metals  
14 measured by DGT ( $M-C_{DGT}$ ). A strong relationship exists between Zn measured dissolved  
15 species and Zn- $C_{DGT}$  ( $R^2 = 0.75$ ,  $p < 0.001$ ). For Pb, a moderate relationship was observed  
16 between dissolved concentrations and  $C_{DGT}$  ( $R^2 = 0.59$ ,  $p < 0.001$ ). Whilst there is correlation  
17 between  $C_{DGT}$  and dissolved concentrations there is a lesser degree of agreement, as  
18 indicated by the slopes of 0.46 (Zn) and 0.07 (Pb) (Figure 2).



19

20 Figure 2. Measured dissolved metals versus DGT concentrations ( $C_{DGT}$ ). Panel a, shows the correlation  
21 between  $Zn_D$  and Zn- $C_{DGT}$ . Panel b, shows the correlation of  $Pb_D$  with Pb- $C_{DGT}$ . Dotted lines represent  
22 a regression line, solid lines represent the ideal 1:1 line.

23

### 24 3.3. Metal speciation calculated by WHAM/Model VII

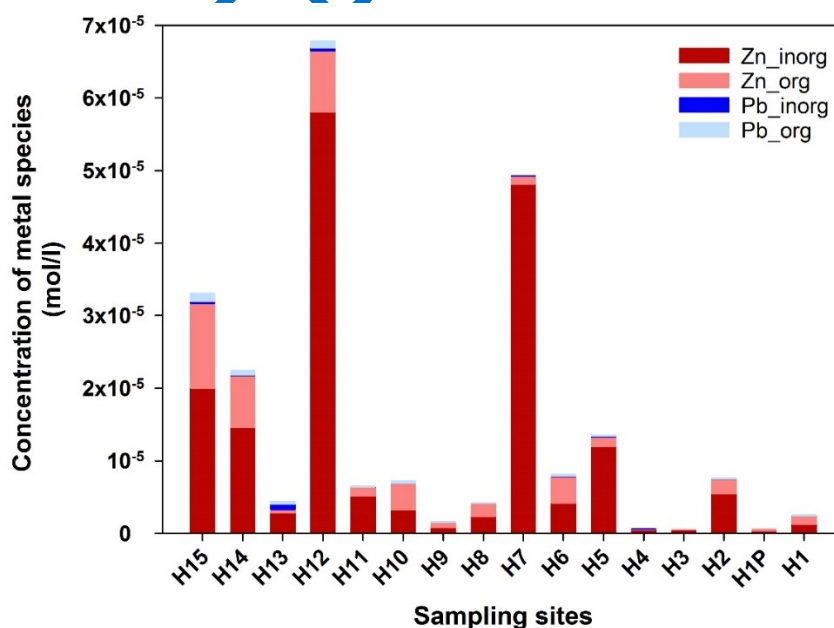
#### 25 3.3.1. Modelling where oxide precipitates are not surface active

26 Concentrations of predicted organic and inorganic species of Zn, and Pb are shown in Figure  
27 3. Average concentrations of metal-organic complexes (i.e. metal-fulvic acid (FA) complexes)  
28 were higher for Zn than Pb, although as a function of total dissolved metal, Pb had a greater  
29 fraction present as organic complexes, consistent with a higher Pb-FA model binding constant.  
30 Values of organic species ranged for Zn from  $2.9 \times 10^{-8}$  to  $1.2 \times 10^{-5}$  mol/l and for Pb from 2.0

31  $\times 10^{-10}$  to  $1.1 \times 10^{-6}$  mol/l, where site H15 presented the highest concentrations for both metals.  
 32 For inorganic species, higher average concentrations occurred for Zn ( $3.6 \times 10^{-7}$  to  $5.8 \times 10^{-5}$   
 33 mol/l), and lower for Pb ( $6.0 \times 10^{-10}$  to  $7.2 \times 10^{-7}$  mol/l), with the highest concentrations in sites  
 34 H12 and H13, respectively. Figures 4a and 4c show linear correlation between dissolved  
 35 inorganic metal species predicted by WHAM and  $C_{DGT}$  measurements of Zn and Pb. Good  
 36 correspondence was observed for Zn ( $R^2 = 0.81$ ,  $p < 0.001$ ) and moderate relationship for Pb  
 37 ( $R^2 = 0.54$ ,  $p < 0.001$ ).

### 38 3.3.2. Modelling where oxide precipitates are surface reactive

39 Precipitation of Fe and Al oxides (with reactive surface) were simulated by using the  
 40 concentration of precipitated metal to the mass of active phase (Figures 4b and 4d). Similar  
 41 correlations were obtained when oxides were not surface reactive ( $R^2 = 0.81$ ,  $R^2 = 0.51$ ). The  
 42 presence of colloidal Pb was estimated showing more affinity to FeOx than AlOx.  
 43 Concentrations of Pb-FeOx complexes ranged from  $5.7 \times 10^{-11}$  to  $2.5 \times 10^{-7}$  mol/l, while Pb-  
 44 AlOx complexes from  $6.8 \times 10^{-12}$  to  $2.5 \times 10^{-8}$  mol/l. For both complexes the highest  
 45 concentrations were identified in H12 (Figure 5).

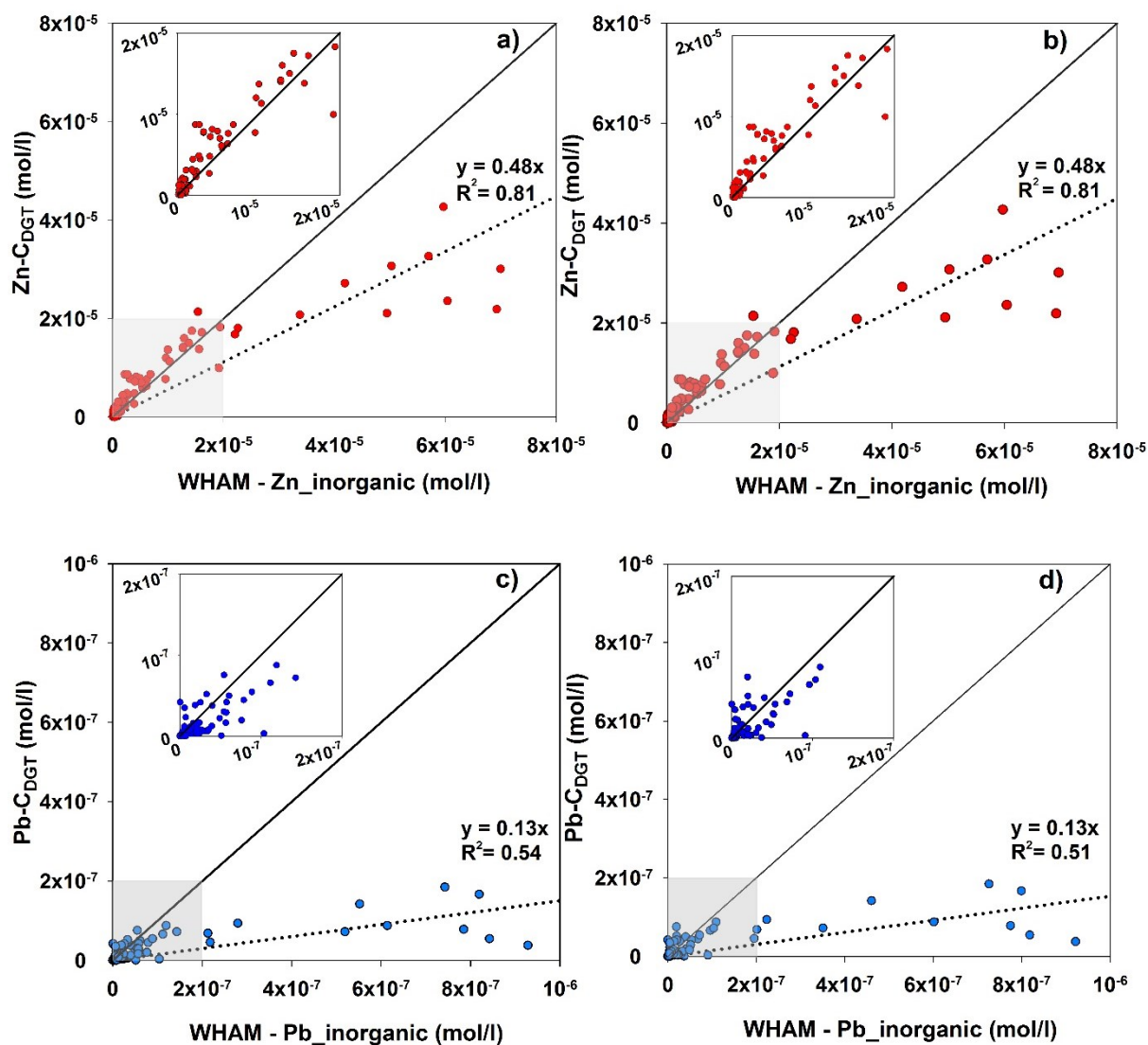


46

47 Figure 3. Calculated concentrations of metal species in the absence of surface active oxide precipitates  
 48 using WHAM/Model VII.

49





50

51 Figure 4. Relationship between inorganic metal concentrations (mol/l) calculated by WHAM and metal  
 52 concentrations from  $C_{DGT}$  measurements (mol/l) for Zn (panels a and b) and Pb (panels c and d).  
 53 Inorganic concentrations were predicted in WHAM in the absence (panels a and c) and presence  
 54 (panels b and d) of active oxides. Dotted lines represent a regression line and solid lines represent the  
 55 ideal 1:1 line.

56

57

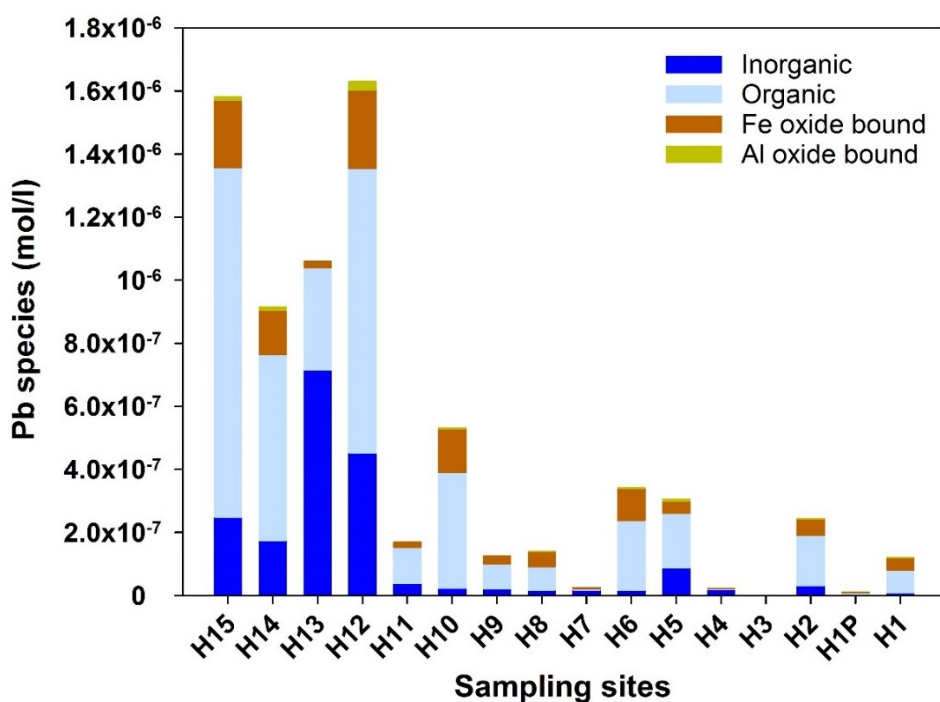
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63

64 Figure 5. Calculated concentrations of Pb species in the presence of surface active oxide precipitates  
 65 using WHAM/Model VII.  
 66

67 **3.4. Water quality and ecotoxicological assessment**

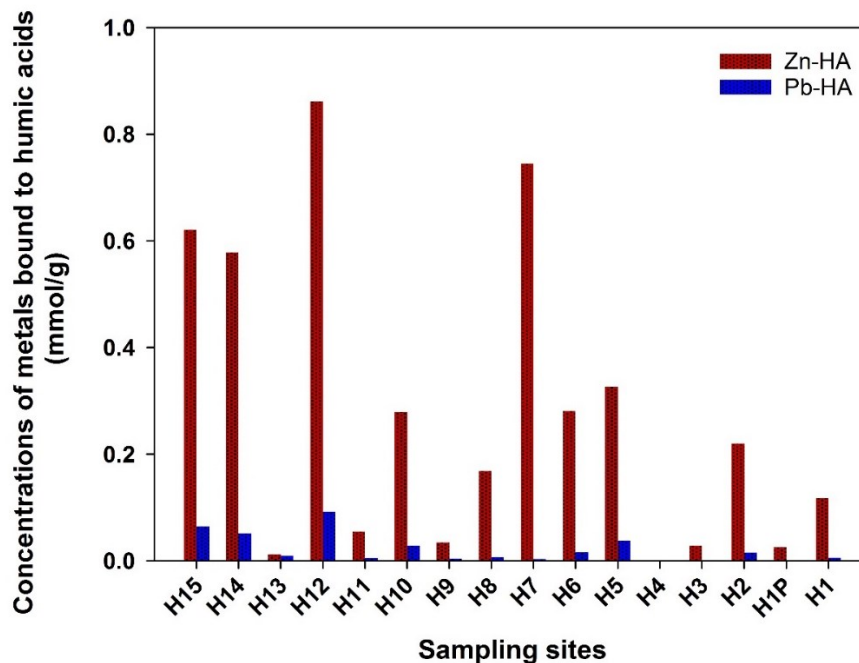
68 **3.4.1. Environmental Quality Standards: EQS-H and EQS-B**

69 Annual averages concentrations were used for assessing EQS-H and EQS-B. The EQS-H  
 70 standards were exceeded for Zn from 0.2 to 254-fold and for Pb from 0.02 to 40-fold (Table  
 71 2). Highest Zn exceedances (> 60-fold) occurred in upstream sites surrounded by spoil mine  
 72 wastes (H15, H14 and H12). These sites and H13, also presented highest Pb exceedances  
 73 (> 20-fold). The EQS-B standards were exceeded for Zn from 0.8 to 209-fold and for Pb from  
 74 0.02 to 17-fold. Highest Zn exceedances (> 50-fold) appeared in sites H7 > H12 > H15 which  
 75 represent a mine adit, an ephemeral tributary flowing through spoil mine wastes and a  
 76 reservoir next to a smelting mill. For Pb, highest levels (> 6-fold) were present in H12 > H5,  
 77 H15 > H14 (Table 2).

78 **3.4.2. Toxicity function: WHAM-F<sub>TOX</sub>**

79 WHAM-F<sub>TOX</sub> considers humic acid (HA) bound to be a plausible proxy for organism bound  
 80 metal. Concentrations of metals bound to humic acid ranged for Zn (0.0013 to 0.9 mmol/g)

81 and Pb (0.0001 to 0.09 mmol/g) (Figure 6). Reservoir (H15), perennial tributaries (H14, H7)  
 82 and ephemeral tributary (H12) were characterised by high Zn concentrations ( $\geq 0.5$  mmol/g),  
 83 the same sites (except for H7) presented high Pb concentrations ( $\geq 0.05$  mmol/g).



84

85 Figure 6. Calculated concentrations of Zn and Pb bound to humic acids (mmol/g).  
 86

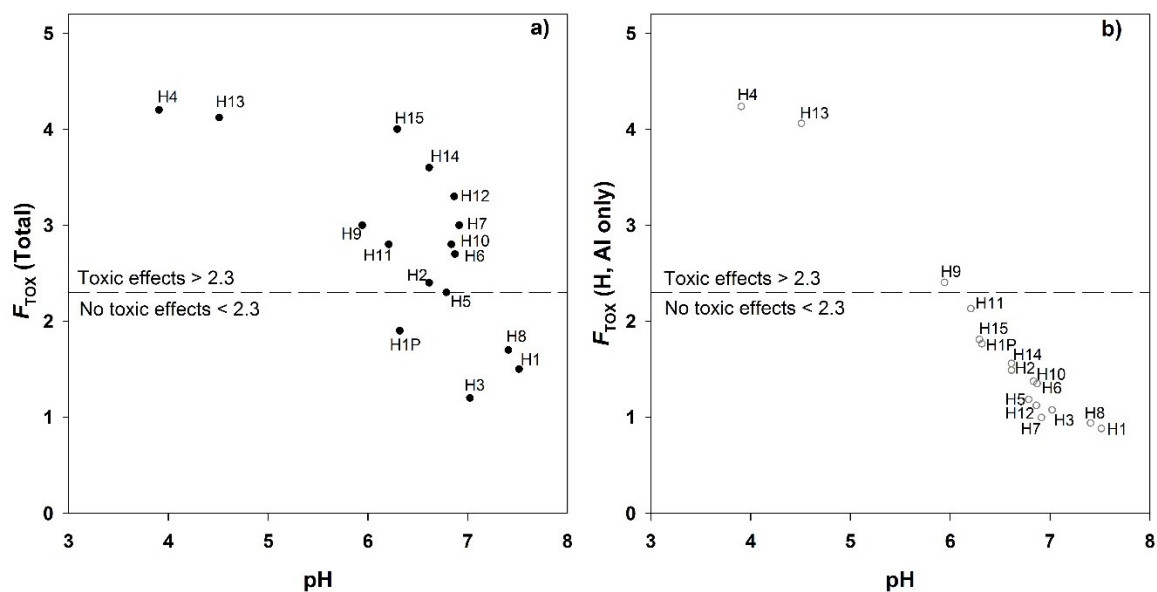
87 Toxicity contributions ( $F_{TOX,M}$ ) for each element (Zn, Pb, H and Al) were calculated from  
 88 Equation 3 (Methods Section 2.5.) and summed to produce a total toxicity function value  
 89 (Total\_ $F_{TOX}$ ). Equations 4 and 6 were applied to convert Total\_ $F_{TOX}$  to a prediction of the  
 90 maximum species richness that could be expected based on the chemistry of each stream.  
 91 Effects of metal mixtures on predicted species richness are shown in Table 3. Reduction of  
 92 predicted species richness ( $SR_{EPT} < 23$ ) was identified at several sites. In the main river  
 93 channel (H10, H6 and H2 ) and perennial tributary (H11) a slight decrease was presented  
 94 ( $SR_{EPT}$ : 22 to 19) associated with the combined moderate toxicity of H, Zn and Al. A more  
 95 significant decrease ( $SR_{EPT}$ : 18 to 9) was shown in sites H15, H14, H13, H12, H9 and H7 due  
 96 to higher contributions from Zn, contributions of H and Al and additionally small contributions  
 97 from Pb; while the lowest value ( $SR_{EPT}$ : 8) occurred in site H4 related to the influence of H and  
 98 Al and not metals.

99 Table 3. Predicted species richness of macroinvertebrates orders (Ephemeroptera, Plecoptera and  
 100 Trichoptera) ( $SR_{EPT}$ ), where  $SR_{EPT} = 23$  indicates no toxic effects and  $SR_{EPT} < 23$  denotes toxic effects.  
 101 Toxicity values ( $F_{TOX}$ ) obtained by WHAM- $F_{TOX}$  were calculated from monthly data. Values are  
 102 expressed as mean and standard deviation ( $\pm SD$ ).

Sites	$SR_{(EPT)}$	Total_ $F_{TOX}$	$F_{TOX-Zn}$	$F_{TOX-Pb}$	$F_{TOX-H}$	$F_{TOX-Al}$
H15	10	4.0	1.7( $\pm 0.7$ )	0.2( $\pm 0.1$ )	1.4( $\pm 0.4$ )	0.4( $\pm 0.5$ )
H14	13	3.6	1.6( $\pm 0.5$ )	0.1( $\pm 0.0$ )	1.2( $\pm 0.3$ )	0.3( $\pm 0.5$ )
H13	9	4.1	0.0( $\pm 0.1$ )	0.0( $\pm 0.0$ )	2.6( $\pm 0.3$ )	1.4( $\pm 0.3$ )
H12	15	3.3	2.3( $\pm 0.9$ )	0.2( $\pm 0.1$ )	1.0( $\pm 0.3$ )	0.2( $\pm 0.5$ )
H11	20	2.8	0.1( $\pm 0.3$ )	0.0( $\pm 0.0$ )	1.8( $\pm 0.6$ )	0.3( $\pm 0.2$ )
H10	19	2.8	0.7( $\pm 0.3$ )	0.1( $\pm 0.0$ )	1.2( $\pm 0.4$ )	0.1( $\pm 0.1$ )
H9	18	3.0	0.1( $\pm 0.1$ )	0.0( $\pm 0.0$ )	2.0( $\pm 0.7$ )	0.4( $\pm 0.2$ )
H8	23	1.7	0.5( $\pm 0.2$ )	0.0( $\pm 0.0$ )	0.9( $\pm 0.3$ )	0.1( $\pm 0.1$ )
H7	18	3.0	2.0( $\pm 0.3$ )	0.0( $\pm 0.0$ )	1.0( $\pm 0.2$ )	0.0( $\pm 0.1$ )
H6	20	2.7	0.8( $\pm 0.2$ )	0.0( $\pm 0.0$ )	1.2( $\pm 0.3$ )	0.2( $\pm 0.3$ )
H5	23	2.3	0.9( $\pm 0.2$ )	0.1( $\pm 0.1$ )	1.1( $\pm 0.2$ )	0.1( $\pm 0.2$ )
H4	8	4.2	0.0( $\pm 0.0$ )	0.0( $\pm 0.0$ )	3.3( $\pm 0.4$ )	0.9( $\pm 0.3$ )
H3	23	1.2	0.1( $\pm 0.0$ )	0.0( $\pm 0.0$ )	1.0( $\pm 0.3$ )	0.0( $\pm 0.1$ )
H2	22	2.4	0.6( $\pm 0.2$ )	0.0( $\pm 0.0$ )	1.3( $\pm 0.4$ )	0.2( $\pm 0.3$ )
H1P	23	1.9	0.1( $\pm 0.0$ )	0.0( $\pm 0.0$ )	1.5( $\pm 0.6$ )	0.2( $\pm 0.1$ )
H1	23	1.5	0.3( $\pm 0.0$ )	0.0( $\pm 0.1$ )	0.8( $\pm 0.2$ )	0.0( $\pm 0.1$ )

103  
 104 Contributions of metal toxic effects ( $F_{TOX}$ ) at different pH conditions were predicted by WHAM-  
 105  $F_{TOX}$  (Figure 7). Figure 7a demonstrates the effects of heavy metals at spoil runoff or adit sites.  
 106 Higher toxicity ( $> 2.3$ ) is related to acidic waters (H4 and H13), while adits or water draining  
 107 through spoil sites are rich in Zn and Pb (H15, H14, H12, H7). Fig 7b highlights the lack of  
 108 metal contribution of metals at the lower pH sites ( $pH < 5$ ) due low metal-humic binding as a  
 109 result of enhanced competition for binding sites from the elevated H and Al concentrations  
 110 (and possible lower metal too).

111  
 112  
 113  
 114



115

116 Figure 7. Variations of toxicity function ( $F_{TOX}$ ) with pH. Panel a shows total  $F_{TOX}$ , which includes the  
 117 effect of metals derived from mine-affected areas and acid contribution from peat soils. Panel b shows  
 118 the contribution of only H and Al to  $F_{TOX}$ . The horizontal dashed line represents the  $F_{TOX}$  threshold (2.3),  
 119 above which toxic effect are predicted to occur. The upper limit of the plot represents the upper threshold  
 120 (5.2).

121

### 122 3.6. Evaluation of metal assessment tools

123 EQS-H, EQS-B and WHAM- $F_{TOX}$  were compared to evaluate their relative assessment of  
 124 potential impact with respect to metal toxicity in aquatic ecosystems (Table 2). Multiple sites  
 125 are necessary as WFD applies to lower order streams than previous legislation, meaning all  
 126 perennial tributaries may be important from a compliance viewpoint. Each of these sites  
 127 represents a length of stream therefore an ecosystem. Ephemeral tributaries are assessed as  
 128 they can represent high input sites into other streams, although these sites may not be  
 129 significant as aquatic ecosystems as they are more likely to be dry during the productive  
 130 summer months. From the comparison, EQS-H are more conservative than EQS-B. EQS-H  
 131 showed wider ranges of exceedance for Zn (0.2 to 254-fold) and for Pb (0.02 to 40-fold), while  
 132 compared to EQS-B for Zn (0.8 to 209-fold) and Pb (0.02 to 17-fold). For both tools, the highest  
 133 Zn failures occurred in reservoir (H15), ephemeral tributary (H12) and perennial tributaries  
 134 (H14 and H7). For Pb, the same sites, except H7 showed significant failures, in addition, using  
 135 EQS-H an ephemeral tributary (H13) showed exceedance, likewise the perennial tributary

136 (H5) applying the EQS-B approach. For the Pb bioavailability tools (FST and SSD), results  
137 showed that the FST approach with fewer chemistry inputs is more conservative than SSD.  
138 By using FST, values ranged from 0.1 to 35-fold, while SSD presented ranges from 0.1 to 17-  
139 fold. Calculations from WHAM- $F_{TOX}$  showed that at twelve sites the water chemistry may  
140 reduce the maximum species diversity of macroinvertebrates by between 3 and 65%. All  
141 ephemeral tributaries (H12, H13, and H4) together with a perennial tributary (H14) and  
142 reservoir (H15) showed the largest predicted reductions in species richness of 34% to 65%.

### 143 *3.7. In situ macroinvertebrate survey*

144 A total of twenty four macroinvertebrate families were identified across all of the sites. The  
145 BMWP scores based on the presence and absence of macroinvertebrates families indicated  
146 a moderately impacted water quality (BMWP > 50) for sites H14, H10, H9, H6, H5, H3, H2,  
147 H1P and H1 representing mainly main channel sites. Lower scores (BMWP < 25) in sites  
148 representing source waters and tributaries (H15, H13, H12, H11, H8, H7 and H4) indicated  
149 pollution deriving poor water quality (Figure S1), while ASTP scores (< 2) showed sites H13  
150 and H12 as the most polluted (Figure S2), although ephemeral tributaries may expected to  
151 have reduced or poor biological diversity due to periodic dry periods, particularly in the summer  
152 months.

153 Four macroinvertebrate orders were predominant in the catchment (Figure S3). Based on  
154 numbers of total individuals counted, the order Diptera showed 40% represented by the  
155 families Psychodidae and Simuliidae (Figure S4), followed by the order Ephemeroptera  
156 (mayfly) with 18% where Baetidae was the most prevalent (Figure S5), Trichoptera (caddisfly)  
157 presented 28% with Hydropsychidae as the dominant family (Figure S6), and the order  
158 Plecoptera (stonefly) with 11% represented by the family Perlodidae (Figure S7).

## 159 **4. Discussion**

160 For aquatic environments, metal assessment has evolved as bioavailability is increasingly  
161 considered a critical measure for improving water quality, e.g. under the EU Water Framework

162 Directive. A preliminary water quality assessment was carried out where dissolved  
163 concentrations of Zn and Pb were assumed to be bioavailable depending on ranges of water  
164 hardness conditions, and exceedances were calculated from previous-established EQS-H.  
165 Concentrations of Zn and Pb exceeded the limits by 175-fold and 40-fold respectively,  
166 however, little consideration is given to their bioavailable forms. Merrington (2016) has  
167 indicated that hardness limits have been derived from ecotoxicological tests mainly from  
168 crustaceans and fish, where water chemical parameters do not necessarily reflect field  
169 conditions. Thus, the use of hardness-based standards may be overly conservative as they  
170 might under-represent other important taxa. In addition, they fail to consider chemical  
171 speciation in distinguishing sites with potential environmental risk associated with metals.

172 In complex environments such as freshwaters, understanding of the underlying conditions  
173 controlling the dynamics and speciation of metals is critical for assessing and managing the  
174 risk they pose to aquatic life (Han et al., 2013). This study evaluated the utility of DGT for  
175 yielding both time averaged and dynamic Zn and Pb concentrations (potentially a proxy for  
176 organism availability) (Diviš et al., 2007; Degryse et al., 2009; Amato et al., 2014; Søndergaard  
177 et al., 2011; 2014; Omanović et al., 2015). By comparing, DGT measurements ( $C_{DGT}$ ) with  
178 direct water measurements (M-measured), results indicated that  $C_{DGT}$  concentrations were  
179 generally lower than the directly measured values. For Zn- $C_{DGT}$  and Zn-measured a strong  
180 linear correlation ( $R^2 = 0.75$ ) was present suggesting the presence of Zn mainly as free ions  
181 and readily labile complexes, a slope of 0.47 revealed that DGT measurements at higher  
182 concentrations failed to reflect dissolved measured concentrations. The better agreement  
183 presented at lower concentrations might be caused by the kinetic rate of Zn forms, which will  
184 determine its accumulation into the Chelex-resin layer (Warnken et al., 2007). Free  $Zn^{2+}$   
185 seems to be rapidly absorbed by the Chelex-resin layer, while the supply of labile complexed  
186 forms will depend on their degree of lability (Puy et al., 2012). Zhang and Davison (2015)  
187 indicated that fully labile complexes might not be completely dissociated within the diffusion  
188 layer, thus their uptake in the Chelex-resin could be reduced or not occur. Correlation between

189 Pb- $C_{DGT}$  and Pb-measured was moderate ( $R^2 = 0.59$ ), with a slope of 0.07 indicating poor  
190 agreement between the data, attributable to the presence of less labile Pb forms (e.g. Pb-  
191 FeOx, Pb-DOM) that need more time for diffusion or presence of bigger particles that could  
192 be excluded from DGT membranes (Han et al., 2013). Considering correlations for both  
193 metals,  $C_{DGT}$  measurements can be affected by the amount of total metal dissolved  
194 concentrations present as free ions and their complexation with inorganic or organic  
195 substances (Zhang and Davison, 2015). Furthermore, long deployments (> 15 days) might  
196 expose DGTs to biological factors like biofouling, and flow fluctuations that might decrease  
197 the stirring rates, reducing DGT's performance (Davison and Zhang, 1994; Webb and Keough,  
198 2002; Turner et al., 2014).

199 Metal speciation and bioavailability are controlled by a range of different factors such as pH,  
200 ionic strength, DOC, hardness, pH and inorganic or organic colloids. Given these factors, the  
201 quantity of metal available for organism uptake is not reflected by the total dissolved  
202 concentration (Hart, 1981; Luoma, 2008). This study correlated M- $C_{DGT}$  measurements with  
203 inorganic metal complexes predicted from dissolved measured concentrations under the  
204 absence and presence of active inorganic colloids (e.g. FeOx and AlOx) as they can compete  
205 for Zn and Pb binding with DOM. If active oxide colloids are excluded from the modelling, Zn  
206 is predicted to be largely present as inorganic (e.g.  $Zn^{2+}$ ,  $Zn(OH)^+$ , etc.) rather than organic  
207 complexes, and highest predicted inorganic concentration was at site H12 ( $5.8 \times 10^{-5}$  mol/l).  
208 Conversely Pb had a greater affinity for organic binding, with highest organic concentration at  
209 H15 ( $10^{-6}$  mol/l) (Figure 5). Both metals showed good linear correlations between inorganic  
210 complexes and  $C_{DGT}$  for Zn ( $R^2 = 0.81$ ), and Pb ( $R^2 = 0.54$ ), reflecting a modest improvement  
211 in the correlation of Zn- $C_{DGT}$  with predicted Zn inorganic complexes than previous correlation  
212 between Zn- $C_{DGT}$  with Zn-measured, which can be attributable to the fact that dissolved Zn is  
213 present primarily as inorganic and free ions, reflecting the absence or low level of Zn-organic  
214 complexes in the correlation analysis. The agreement observed for Zn confirms that the total  
215 dissolved Zn (mainly inorganic) is approximately equivalent to the dynamic Zn- $C_{DGT}$  (except at



216 high Zn loading, as previously discussed). For Pb, there is a discrepancy between Pb- $C_{DGT}$   
217 and dissolved Pb, likely due to larger fractions in other less labile phases (Pb-organic  
218 complexes) (Warnken et al., 2008; Han et al., 2013). Outcomes from modelling conditions  
219 where oxide precipitates are surface reactive showed similar behaviours for Zn ( $R^2 = 0.81$ )  
220 and Pb ( $R^2 = 0.51$ ) to those obtained in the absence of surface activity. The lack of improved  
221 agreement to the Pb-inorganic in presence of oxides with the Pb- $C_{DGT}$  (dynamic) concentration  
222 implies that the model may be under-predicting the binding to these forms. Results from DGT  
223 measurements provided useful information about metal dynamics and speciation which is  
224 valuable for understanding metal uptake mechanisms. Metal bioavailability studies from  
225 Bradac et al. (2009); Yin et al. (2014); (Balistrieri et al., 2012) suggest DGTs as useful tool for  
226 understanding the accumulation of metals in freshwater ecosystems (e.g. algae, snails and  
227 fish). However, results from this study indicated that DGT is not a suitable substitute for direct  
228 water measurements due to complex uptake kinetics of different forms and at higher metal  
229 concentrations. These limitations make DGT a potentially useful bioavailability assessment  
230 tool, but further work is needed to compare DGT measurements in the field with actual in situ  
231 organism metal burdens. Zhang and Davison (2015) have indicated that given the complexity  
232 of biological system and chemistry of environmental media, the ability of DGT to mimic metal  
233 uptake and predict bioavailability is variable. Further research based on the comparison of  
234 field data and laboratory experiments under controlled temperature, deployment time and flow  
235 rates are suggested for evaluating the applicability of DGTs regarding metal bioavailability in  
236 freshwaters. Evidence of marked diurnal ranges as well as flow-dependent variations in metal  
237 concentrations reported by Jones et al. (2015), also suggests that DGT measurements should  
238 be complemented by frequent measurements of metal concentrations (e.g. with auto  
239 samplers) to identify the actual variation experienced by DGTs.

240 Outcomes from EQS-H, EQS-B and WHAM- $F_{TOX}$  provided different levels of prediction  
241 regarding metal bioavailability and toxicity. In comparing EQS-H and EQS-B, the simpler  
242 approach (EQS-H) is more conservative than the standard that considered more water

243 chemistry parameters and metal-organism interactions (EQS-B) (Merrington, 2016). However,  
244 by contrasting EQS-B and WHAM- $F_{TOX}$ , results suggest that the latter approach is the least  
245 conservative of the three approaches. This was clearly identified in sites which exceeded  
246 EQS-B like H7 (Zn: 209-fold and Pb: 2-fold) and H5 (Zn: 44-fold and Pb: 9-fold), but with  $F_{TOX}$ -  
247 SR showing 100% for predicted species richness for both sites. WHAM- $F_{TOX}$  also revealed  
248 that major toxic effects can occur at circumneutral pH mostly due to Zn and Pb. Moreover, a  
249 limited biological survey agreed with this chemical assessment. The most impoverished sites  
250 presented low predicted macroinvertebrate richness ( $SR_{EPT} \leq 34\%$ ) correlating with high  
251 concentrations of metals (H12, H13 and H14) or low-pH values (H4) (Table 2). Overall, the  
252 orders Diptera and Trichoptera were dominant (40% and 28%) with Chironomidae and  
253 Hydropsychidae as the more abundant families. Responses of macroinvertebrates and  
254 diatoms to different metal levels in Hebden Beck have been previously evaluated by the  
255 Environment Agency (2014). The ecological assessment revealed a relationship between  
256 increasing Zn concentrations and reduced biodiversity. Likewise invertebrate data showed  
257 that responses of species diversity and composition could be related to the influence of  
258 complex interactions between metals and acid scenarios. In early and current assessment,  
259 the family Baetidae (O: Ephemeroptera) was dominant, possibly due to its tolerance to  
260 different levels of pollution. A study by Mebane et al. (2015), indicated that the species most  
261 sensitive to pollution are the last to re-colonise after recovery from Cu contamination. In our  
262 study less sensitive families (Perlodidae, Nemouridae, Baetidae) are widespread throughout  
263 the catchment. More sensitive families like Glossomidae, Ephemerellidae and Heptageniidae  
264 are mainly confined to main channel sites (H2, H6, H10), although Heptageniidae was also  
265 present in a metal impacted site H14 (predicted species richness of 55% of the theoretical  
266 maximum), another metal sensitive family, Psychodidae was present in less metal impacted  
267 sites, such as H1P, showing a predicted species richness of 100%. These results are  
268 consistent with the  $F_{TOX}$  species predictions, however, current interpretations are generalised  
269 as we only identified macroinvertebrates to family level, and species sensitivity is complex,  
270 where differences in pollution tolerance occur within different taxa (Clements et al., 2013).

271 From a regulatory perspective, bioavailability based standards (EQS-B) offer a more  
272 ecologically-based approach to address water quality failures than traditional hardness  
273 corrections (EQS-H) (Peters et al., 2016), due to their parameterisation with organism toxicity  
274 data or field species observations. In this study, both standards facilitated the screening of  
275 sites with potential metal risk, however, EQS-B identified sites where dissolved metal  
276 concentrations and speciation mechanisms ( $M-C_{DGT}$ ) were related. Although, bioavailability-  
277 based models have been developed on field data and applied in the establishment of water  
278 quality standards for metals, their applicability for routine regulatory purposes need to be  
279 tested, especially for sites where physicochemical conditions (e.g. pH, hardness, DOC) are  
280 near or outside of the boundaries of the model (Rüdel et al., 2015; WFD-UKTAG, 2009a). In  
281 this study, some sites presented pH and Ca values below Zn-BLM limits, thus default limits of  
282 this tool (pH = 6 and Ca = 3mg/l) were used in Zn assessment, consequently the use of  
283 complementary models like WHAM and  $WHAM_{TOX}$  can offer more complete toxicity  
284 predictions under original physico-chemical conditions. Because  $WHAM_{TOX}$  considers  
285 protons within its formulation, it can be applied to any combination of water chemistry and  
286 metal mixtures. Bioavailability based EQS were implemented for England and Wales in 2014  
287 (DEFRA, 2014). For a practical implementation of these tools a compliance scheme was  
288 suggested by the UK Technical Advisory Group, which includes: i) comparison between metal  
289 concentrations with EQS-H, ii) application of BLM tools (e.g. Zn-BLM, Pb-BLM) to predict site-  
290 specific bioavailability (EQS-B), and iii) local refinement by using models that require larger  
291 number of input parameters (e.g. full M-BLM or  $WHAM_{TOX}$ ) (Bio-met, 2015; WFD-UKTAG,  
292 2009b). Further research is desirable, especially in the generation of toxicity data to improve  
293 the significance of toxicity coefficients (e.g.  $\alpha_{Pb}$ ). Nevertheless, the combination of EQS-B and  
294  $WHAM_{TOX}$  could provide an improved rational assessment which is essential for the  
295 purposes of considering remediation activities. Vulnerable sites can be prioritised under a  
296 holistic scenario (water chemistry, metal concentrations and interactions between metal and  
297 organism) where remedial actions can be focused on reducing metal bioavailability for the  
298 purpose of improving ecological endpoints.

299 This study has evaluated different methods for the assessment of the chemical and ecological  
300 status of Hebden catchment regarding metal pollution. The first method (EQS-H) was a simple  
301 and well-established technique, which provided a very conservative regulatory limit that may  
302 be overprotective as seen when calculations are performed with tools designed specifically to  
303 account for speciation and organisms interactions. The second (EQS-B) and third (WHAM-  
304  $F_{TOX}$ ) method based on more rigorous bioavailability tools (e.g. Zn-BLM and Pb-SSD),  
305 provided less conservative limits but supported by the normalisation of the ecotoxicity data to  
306 the site specific water chemistry. Through the evaluation of these approaches a better  
307 understanding of metal speciation and dynamics was possible, supporting the prediction of  
308 toxic effects from metal mixtures and the identification of sites with potential biological risks.  
309 Furthermore, the biological survey provided a context for relating the response of aquatic  
310 organisms to river water chemistry and pollutants, which are useful for future ecological  
311 assessments.

## 312 5. Conclusions

313 The use of bioavailability-based standards is needed to fully assess the impacts of metal  
314 pollution and implement regulatory actions for delivering good ecological status.

- 315 • In carboniferous catchments, metal toxic effects occur at circumneutral pH. Zn is mainly  
316 present as free ions and readily labile complexes while Pb is present as less labile forms  
317 (e.g. organic complexes and colloids).
- 318 • Metal assessment through the EQS-H approach is more conservative than EQS-B and  
319 WHAM- $F_{TOX}$ . The incorporation of bioavailability will better address some of the processes  
320 that control toxic metals effects. In addition, catchment management will be improved as  
321 the bioavailability approach will aid in the identification of key reaches where remediation  
322 may be required.
- 323 • Metal concentrations estimated from DGT provided useful information for the assessment  
324 of Zn and Pb speciation and for understanding metal uptake mechanisms. However, results

325 suggest that further research is required for evaluating the applicability of this tool in the  
326 assessment of metal bioavailability under field conditions.

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