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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 being 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_{EPT})) 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 (WDF) (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).

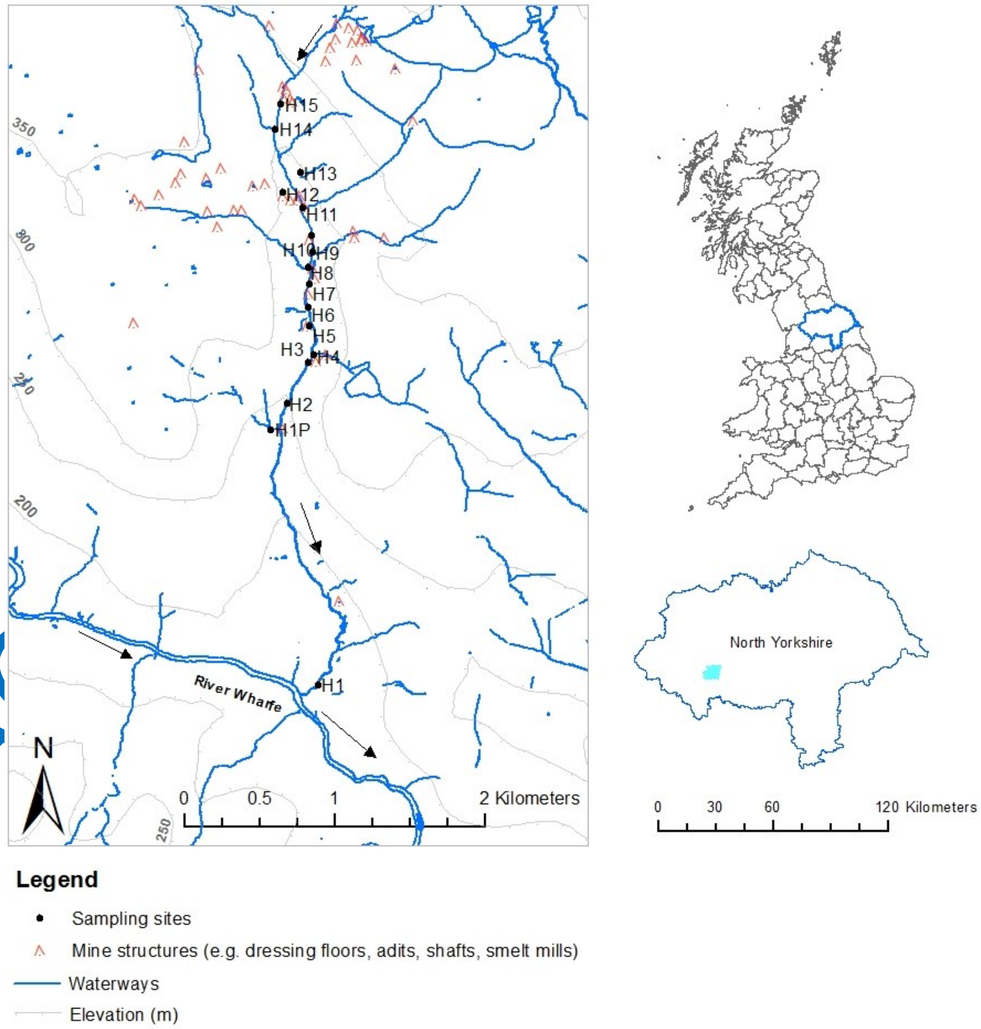

Figure 1. Hebden Beck discharging into the River Wharfe. Black dots represent sampling stations, they are ordered from upper to lower catchment, therefore from H15 to H1. Black arrows indicate the direction of flow.

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^2). 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^{2+}, Pb^{2+} Cd^{2+}, and Cu^{2+}), major anions (SO_4^{2-}, Cl^-, NO_3^-, PO_4^{3-}) 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: 5.1×10^{-7} mol/l and Pb: 2.4×10^{-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_3 (ARISTAR) then stored at 4°C.

Immediately before ICPMS analysis, the eluent was diluted 10x with Milli-Q water (18.2 MΩ·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_3 elution solution (in μg/l) provided by the ICPMS analysis, V_{gel} is the volume of the resin gel, V_{HNO3} is the volume of HNO_3 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_{DGT}) can be calculated as per Zhang and Davison (1995) (Eq. 2), where, M is the mass of metal in the Chelex resin, Δg 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^2).

$$M = (C_e\,(V_{gel} + V_{HNO3}) / f_e) / 1000 \qquad (1)$$

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

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^-, SO_4^{2-}, NO_3^- and PO_4^{3-}), dissolved metals (Zn^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+}, Fe^{3+}, and Al^{3+}), 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 × 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 (ν, mmol/g) and the toxicity coefficient of each analyte (α) and i refers to each toxic cation (Eq. 3).

$$F_{TOX} = \Sigma\ \alpha_i\ \nu_i \qquad (3)$$

Concentrations of ν_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 (α_{Al}: 2.24), zinc (α_{Zn}: 2.69), lead (α_{Pb}: 2.51) and hydrogen (α_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 α_{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).

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

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

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

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 ≥ 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 ≥ 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 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 Mg^{2+}, Na^{+}, K^{+}, SO_4^{2-}, Cl^{-}, CO_3^{2-}. Thirdly, the model WHAM-F_{TOX} derived from WHAM/Model VII was applied to predict the effect of water chemistry on the maximum species richness ($SR_{EPT\text{-}max}$) 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 (1.4×10^{-6}, 4.18×10^{-7}, 6.5×10^{-5}, 3.12×10^{-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 2.8×10^{-5} to 4.8×10^{-9} mol/l and for Pb-C_{DGT} from 1.1×10^{-7} to 1.1×10^{-10} mol/l. Highest Zn-C_{DGT} identified in perennial tributaries were H7: 2.8×10^{-5} Zn mol/l and highest Pb-C_{DGT} in ephemeral tributaries H13 and H12: 1.1 × 10^{-7} Pb mol/l, while in main channel maximum concentrations were observed in H6: 7.5 × 10^{-6} Zn mol/l and H10: 2.3 × 10^{-8} Pb mol/l (Table 2).

Table 2. Annual averages and standard deviation (±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[a], EQS-B (Bioavailability assessment tool-BAT[b,c], Final screening tool-FST[d] and Species sensitivity distribution-SSD[e]) 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_D-measured (mol/l)**	**Zn-C_{DGT} (mol/l)**	**Zn RCR**		**Pb_D-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	3.1×10^{-5} ($\pm7.2\times10^{-6}$)	1.2×10^{-5} ($\pm6.9\times10^{-6}$)	253.5	54.0	1.3×10^{-6} ($\pm4.0\times10^{-7}$)	5.7×10^{-8} ($\pm5.2\times10^{-8}$)	38.8	18.9 [d]	8.5	10	43
H14	PT	2.1×10^{-5} ($\pm5.2\times10^{-6}$)	1.5×10^{-5} ($\pm4.4\times10^{-6}$)	174.7	45.9	7.6×10^{-7} ($\pm3.6\times10^{-7}$)	6.1×10^{-8} ($\pm1.9\times10^{-8}$)	21.9	15.8 [d]	6.8	13	55
H13	ET	3.2×10^{-6} ($\pm6.3\times10^{-6}$)	6.1×10^{-7} ($\pm3.2\times10^{-7}$)	25.9	4.1 [b]	9.9×10^{-7} ($\pm2.6\times10^{-7}$)	1.1×10^{-7} ($\pm6.6\times10^{-8}$)	28.6	10.2 [d]	1.1 [e]	9	38
H12	ET	6.5×10^{-5} ($\pm3.0\times10^{-5}$)	2.7×10^{-5} ($\pm4.0\times10^{-6}$)	85.0	117.6	1.4×10^{-6} ($\pm4.4\times10^{-7}$)	1.1×10^{-7} ($\pm4.0\times10^{-8}$)	39.5	35.0 [d]	17.1	15	66
H11	PT	6.3×10^{-6} ($\pm2.1\times10^{-5}$)	3.3×10^{-6} ($\pm7.6\times10^{-6}$)	51.3	8.7	1.5×10^{-7} ($\pm4.5\times10^{-7}$)	9.2×10^{-9} ($\pm1.8\times10^{-8}$)	4.4	1.6 [d]	0.8	20	85
H10	MC	6.8×10^{-6} ($\pm2.8\times10^{-6}$)	5.2×10^{-6} ($\pm2.5\times10^{-6}$)	55.6	11.1	3.8×10^{-7} ($\pm2.0\times10^{-7}$)	2.3×10^{-8} ($\pm1.5\times10^{-8}$)	11.2	6.5 [d]	2.9	19	82
H9	PT	1.5×10^{-6} ($\pm1.9\times10^{-6}$)	1.3×10^{-6} ($\pm5.5\times10^{-7}$)	12.0	2.1 [b]	1.0×10^{-7} ($\pm1.1\times10^{-7}$)	4.6×10^{-9} ($\pm4.4\times10^{-9}$)	2.9	1.0 [d]	0.5	18	77
H8	PT	4.1×10^{-6} ($\pm1.5\times10^{-6}$)	3.9×10^{-6} ($\pm2.3\times10^{-6}$)	3.6	7.4	9.2×10^{-8} ($\pm3.2\times10^{-8}$)	1.1×10^{-8} ($\pm1.1\times10^{-8}$)	2.6	2.0 [d]	1.1	23	100
H7	PT	4.9×10^{-5} ($\pm1.3\times10^{-5}$)	2.8×10^{-5} ($\pm8.5\times10^{-6}$)	42.9	209.2	2.1×10^{-8} ($\pm6.1\times10^{-8}$)	1.2×10^{-9} ($\pm1.1\times10^{-9}$)	0.6	3.0 [d]	1.7	18	76
H6	MC	7.8×10^{-6} ($\pm1.5\times10^{-6}$)	7.5×10^{-6} ($\pm1.0\times10^{-6}$)	10.2	12.8	2.4×10^{-7} ($\pm9.9\times10^{-8}$)	8.5×10^{-9} ($\pm4.4\times10^{-9}$)	6.9	3.8 [d]	1.7	20	88
H5	PT	1.3×10^{-5} ($\pm2.9\times10^{-6}$)	1.3×10^{-5} ($\pm1.5\times10^{-6}$)	11.6	43.9	2.6×10^{-7} ($\pm2.2\times10^{-7}$)	4.9×10^{-8} ($\pm1.8\times10^{-8}$)	7.5	14.6 [d]	8.7	23	100
H4	ET	5.5×10^{-7} ($\pm1.4\times10^{-7}$)	2.6×10^{-7} ($\pm8.9\times10^{-8}$)	4.5	2.5 [b,c]	2.1×10^{-8} ($\pm8.2\times10^{-9}$)	3.7×10^{-9} ($\pm5.4\times10^{-10}$)	0.6	0.4	0.02 [e]	8	35
H3	PT	$<5.1\times10^{-7}$	1.2×10^{-6} ($\pm5.1\times10^{-8}$)	0.2	0.8	8.0×10^{-10} ($\pm9.5\times10^{-10}$)	5.4×10^{-10} ($\pm4.1\times10^{-10}$)	0.02	0.1	0.1	23	100
H2	MC	7.4×10^{-6} ($\pm1.1\times10^{-6}$)	7.0×10^{-6} ($\pm1.0\times10^{-6}$)	9.7	17.7	1.9×10^{-7} ($\pm1.4\times10^{-7}$)	1.3×10^{-8} ($\pm3.9\times10^{-9}$)	5.5	4.4 [d]	2.3	22	97
H1P	PT	5.6×10^{-7} ($\pm1.1\times10^{-6}$)	4.8×10^{-9} ($\pm4.5\times10^{-9}$)	0.7	2.4	3.5×10^{-9} ($\pm3.1\times10^{-9}$)	1.1×10^{-10} ($\pm3.0\times10^{-11}$)	0.3	0.2	0.1	23	100
H1	MC	2.4×10^{-6} ($\pm1.1\times10^{-6}$)	2.5×10^{-6} ($\pm6.6\times10^{-7}$)	2.1	4.2	8.0×10^{-8} ($\pm6.6\times10^{-8}$)	3.7×10^{-9} ($\pm3.3\times10^{-9}$)	2.3	1.8 [d]	1.0	23	100

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

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

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

[d] Measured Pb exceeds the site specific PNEC of the Pb-FST tool.

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

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

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

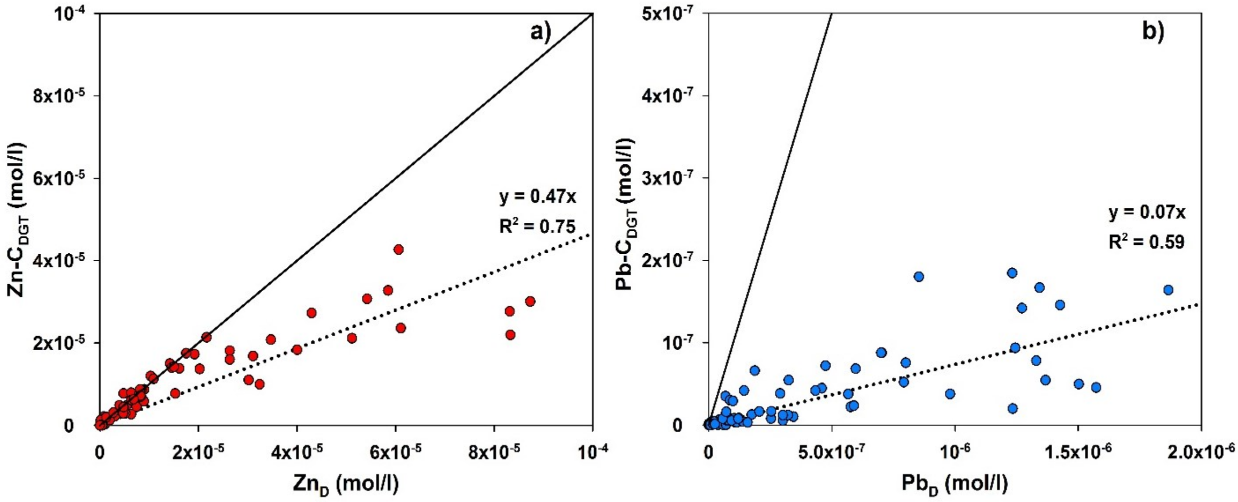

Figure 2. Measured dissolved metals versus DGT concentrations (C_{DGT}). Panel a, shows the correlation between Zn_D and Zn-C_{DGT}. Panel b, shows the correlation of Pb_D with Pb-C_{DGT}. Dotted lines represent a regression line, solid lines represent the ideal 1:1 line.

3.3. Metal speciation calculated by WHAM/Model VII

3.3.1. Modelling where oxide precipitates are not surface active

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

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

3.3.2. Modelling where oxide precipitates are surface reactive

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

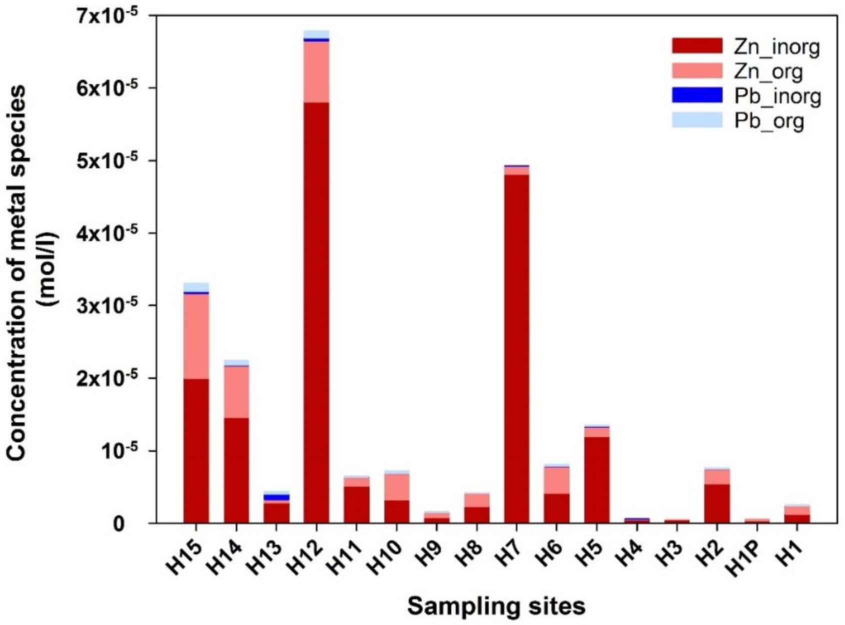

Figure 3. Calculated concentrations of metal species in the absence of surface active oxide precipitates using WHAM/Model VII.

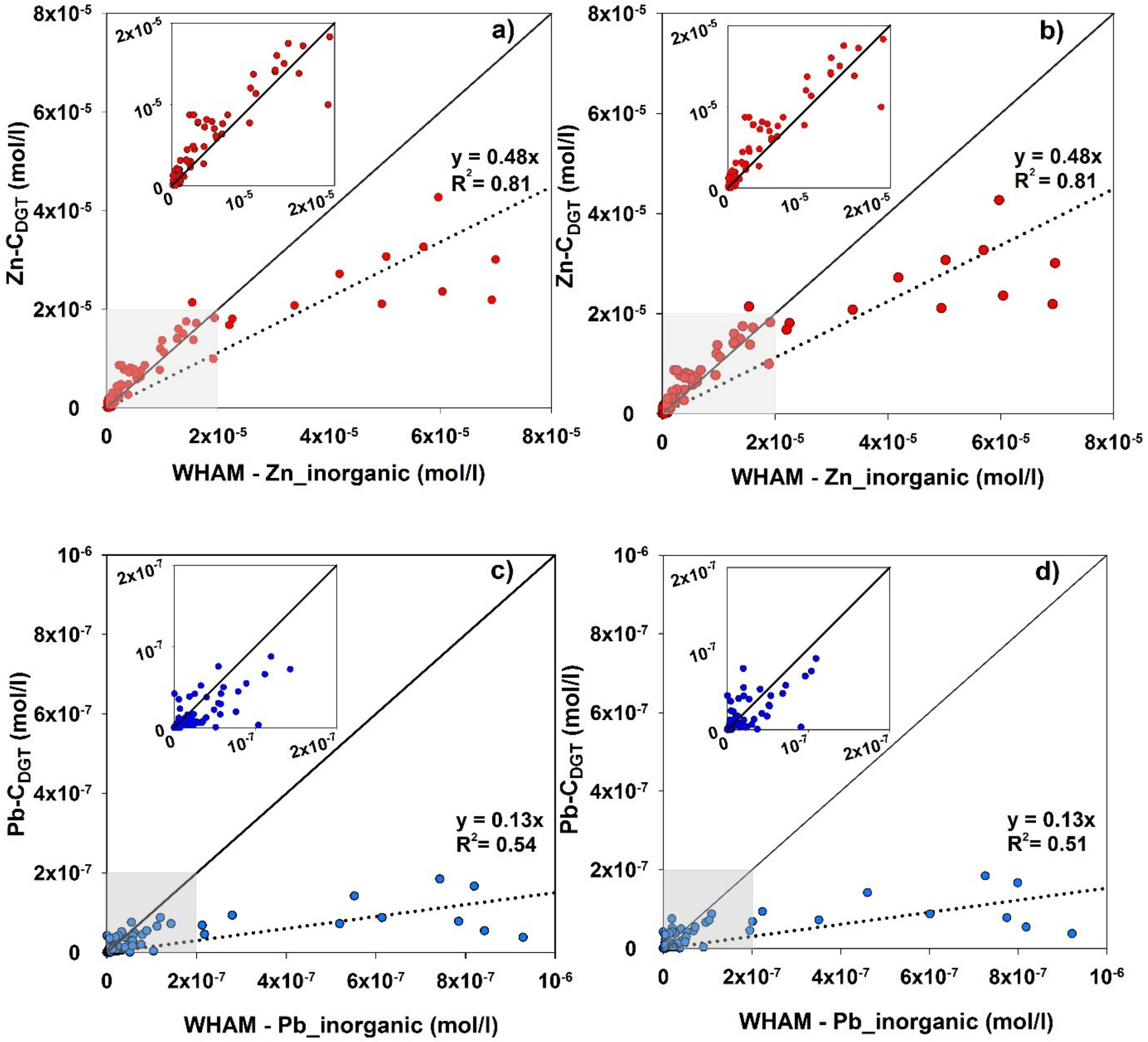

Figure 4. Relationship between inorganic metal concentrations (mol/l) calculated by WHAM and metal concentrations from C_{DGT} measurements (mol/l) for Zn (panels a and b) and Pb (panels c and d). Inorganic concentrations were predicted in WHAM in the absence (panels a and c) and presence (panels b and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

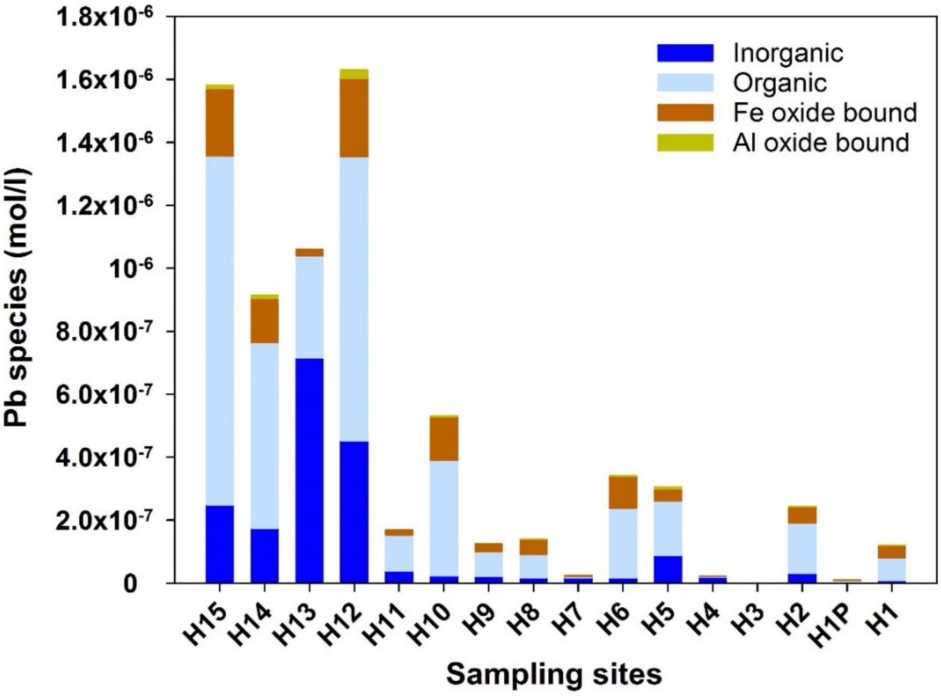

Figure 5. Calculated concentrations of Pb species in the presence of surface active oxide precipitates using WHAM/Model VII.

3.4. Water quality and ecotoxicological assessment

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

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

3.4.2. Toxicity function: WHAM-F_{TOX}

WHAM-F_{TOX} considers humic acid (HA) bound to be a plausible proxy for organism bound metal. Concentrations of metals bound to humic acid ranged for Zn (0.0013 to 0.9 mmol/g) and Pb (0.0001 to 0.09 mmol/g) (Figure 6). Reservoir (H15), perennial tributaries (H14, H7) and ephemeral tributary (H12) were characterised by high Zn concentrations (≥ 0.5 mmol/g), the same sites (except for H7) presented high Pb concentrations (≥ 0.05 mmol/g).

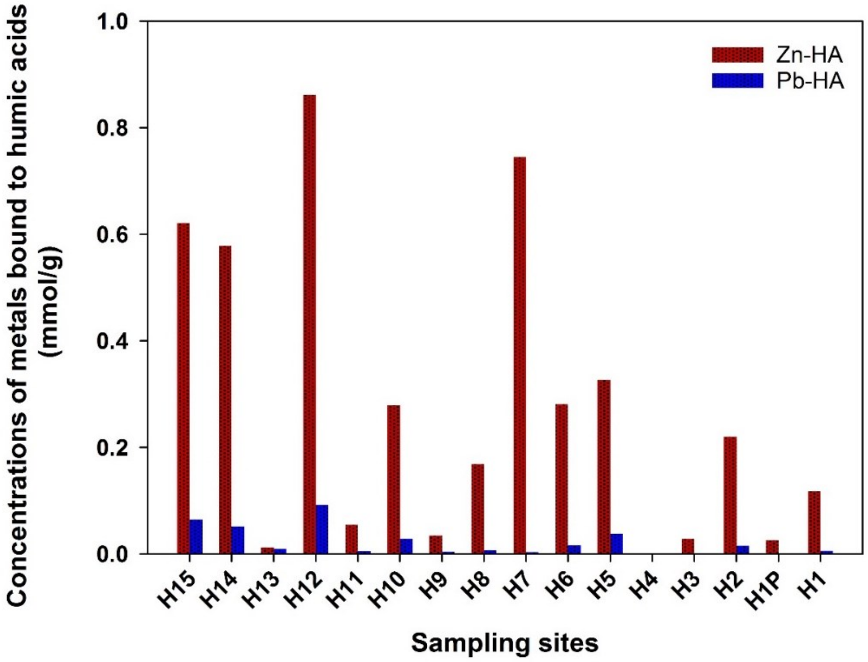

Figure 6. Calculated concentrations of Zn and Pb bound to humic acids (mmol/g).

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

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

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

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

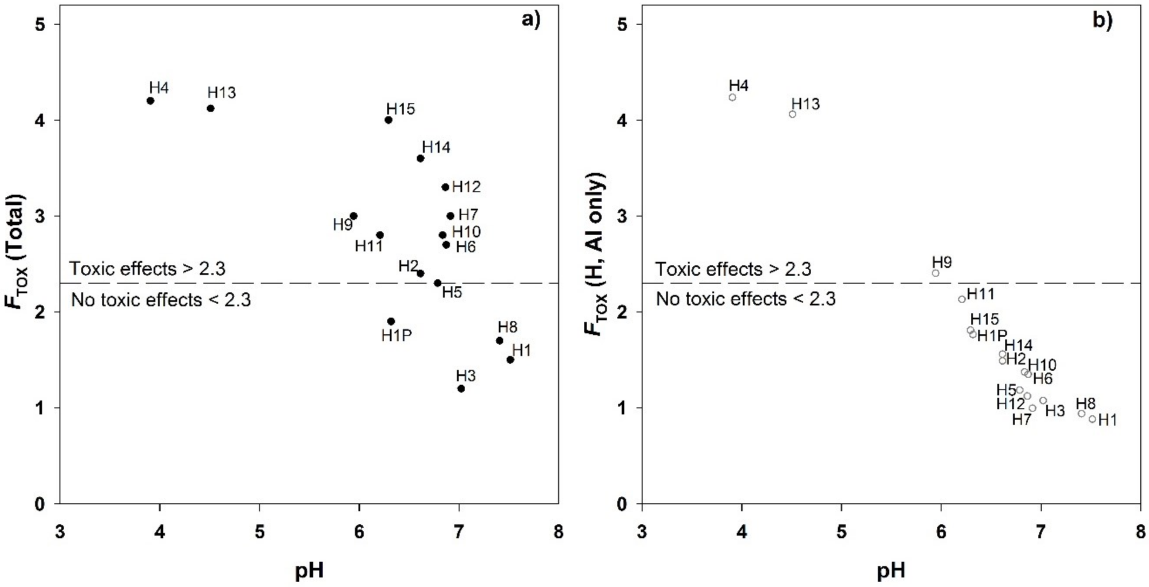

Figure 7. Variations of toxicity function (F_{TOX}) with pH. Panel a shows total F_{TOX}, which includes the effect of metals derived from mine-affected areas and acid contribution from peat soils. Panel b shows the contribution of only H and Al to F_{TOX}. The horizontal dashed line represents the F_{TOX}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

3.6. Evaluation of metal assessment tools

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

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

3.7. In situ macroinvertebrate survey

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

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

4. Discussion

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

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

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

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

Metal speciation and bioavailability are controlled by a range of different factors such as pH, ionic strength, DOC, hardness, pH and inorganic or organic colloids. Given these factors, the quantity of metal available for organism uptake is not reflected by the total dissolved concentration (Hart, 1981; Luoma, 2008). This study correlated M-C_{DGT} measurements with inorganic metal complexes predicted from dissolved measured concentrations under the absence and presence of active inorganic colloids (e.g. FeOx and AlOx) as they can compete for Zn and Pb binding with DOM. If active oxide colloids are excluded from the modelling, Zn is predicted to be largely present as inorganic (e.g. Zn^{2+}, $Zn(OH)^{+}$, etc.) rather than organic complexes, and highest predicted inorganic concentration was at site H12 (5.8×10^{-5} mol/l). Conversely Pb had a greater affinity for organic binding, with highest organic concentration at H15 (10^{-6} mol/l) (Figure 5). Both metals showed good linear correlations between inorganic complexes and C_{DGT} for Zn (R^2 = 0.81), and Pb (R^2 = 0.54), reflecting a modest improvement in the correlation of Zn-C_{DGT} with predicted Zn inorganic complexes than previous correlation between Zn-C_{DGT} with Zn-measured, which can be attributable to the fact that dissolved Zn is present primarily as inorganic and free ions, reflecting the absence or low level of Zn-organic complexes in the correlation analysis. The agreement observed for Zn confirms that the total dissolved Zn (mainly inorganic) is approximately equivalent to the dynamic Zn-C_{DGT} (except at high Zn loading, as previously discussed). For Pb, there is a discrepancy between Pb-C_{DGT} and dissolved Pb, likely due to larger fractions in other less labile phases (Pb-organic complexes) (Warnken et al., 2008; Han et al., 2013). Outcomes from modelling conditions where oxide precipitates are surface reactive showed similar behaviours for Zn (R^2 = 0.81) and Pb (R^2 = 0.51) to those obtained in the absence of surface activity. The lack of improved agreement to the Pb-inorganic in presence of oxides with the Pb-C_{DGT} (dynamic) concentration implies that the model may be under-predicting the binding to these forms. Results from DGT measurements provided useful information about metal dynamics and speciation which is valuable for understanding metal uptake mechanisms. Metal bioavailability studies from Bradac et al. (2009); Yin et al. (2014); (Balistrieri et al., 2012) suggest DGTs as useful tool for understanding the accumulation of metals in freshwater ecosystems (e.g. algae, snails and fish). However, results from this study indicated that DGT is not a suitable substitute for direct water measurements due to complex uptake kinetics of different forms and at higher metal concentrations. These limitations make DGT a potentially useful bioavailability assessment tool, but further work is needed to compare DGT measurements in the field with actual in situ organism metal burdens. Zhang and Davison (2015) have indicated that given the complexity of biological system and chemistry of environmental media, the ability of DGT to mimic metal uptake and predict bioavailability is variable. Further research based on the comparison of field data and laboratory experiments under controlled temperature, deployment time and flow rates are suggested for evaluating the applicability of DGTs regarding metal bioavailability in freshwaters. Evidence of marked diurnal ranges as well as flow-dependent variations in metal concentrations reported by Jones et al. (2015), also suggests that DGT measurements should be complemented by frequent measurements of metal concentrations (e.g. with auto samplers) to identify the actual variation experienced by DGTs.

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

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

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

5. Conclusions

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

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

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

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