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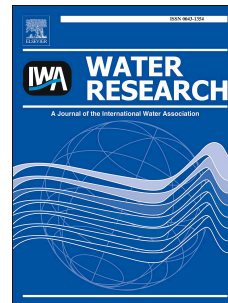


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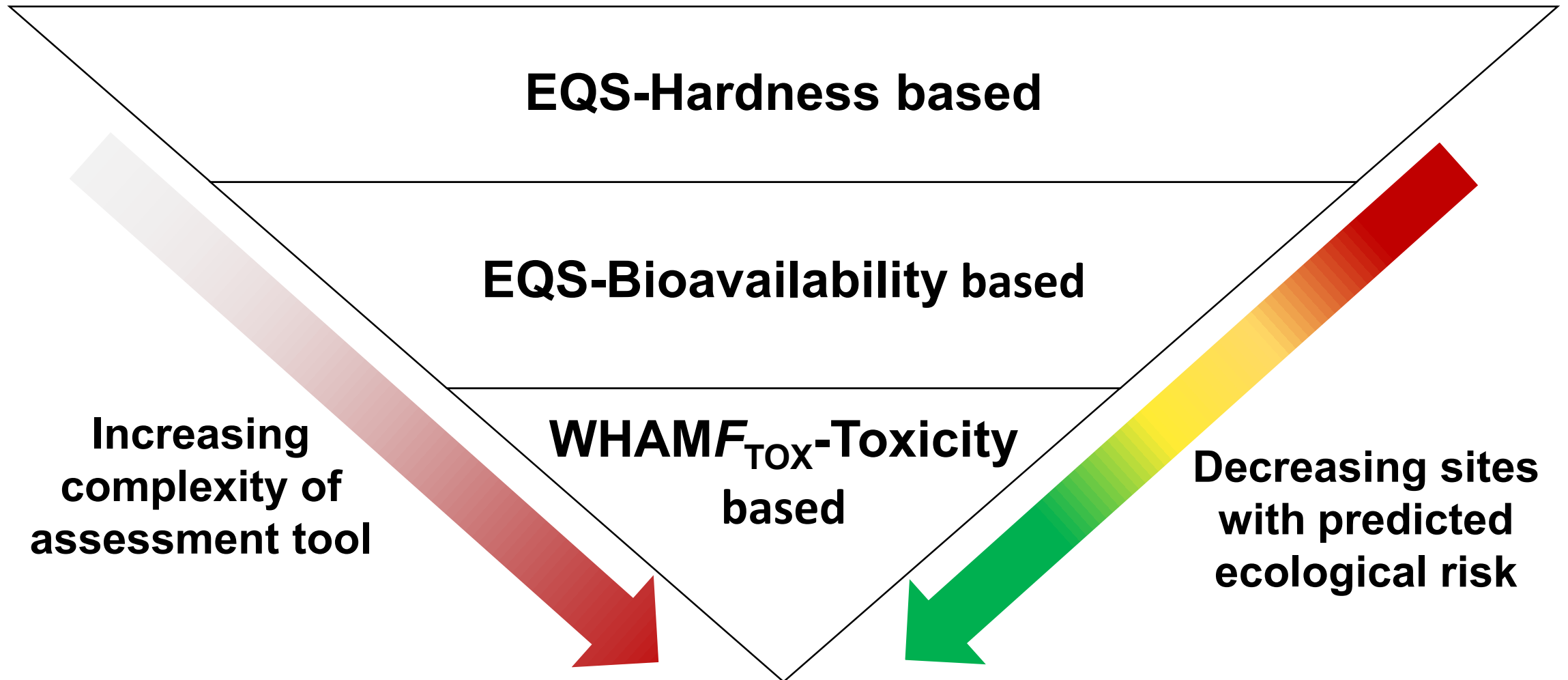
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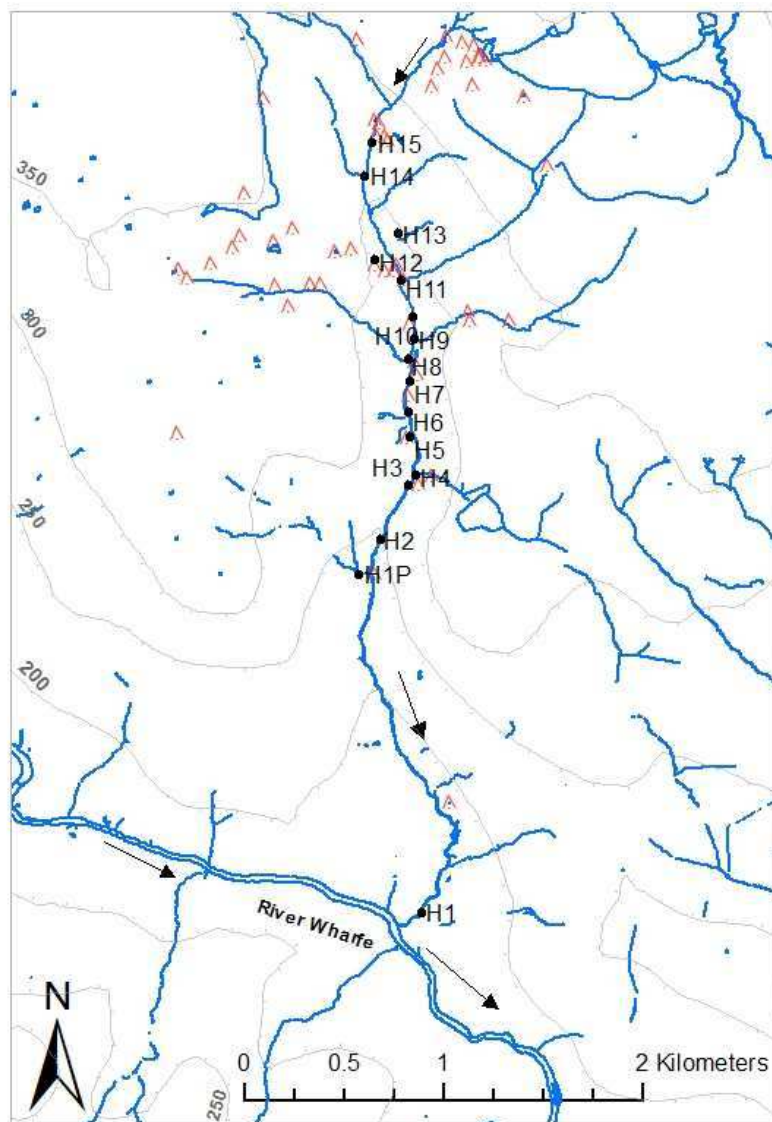
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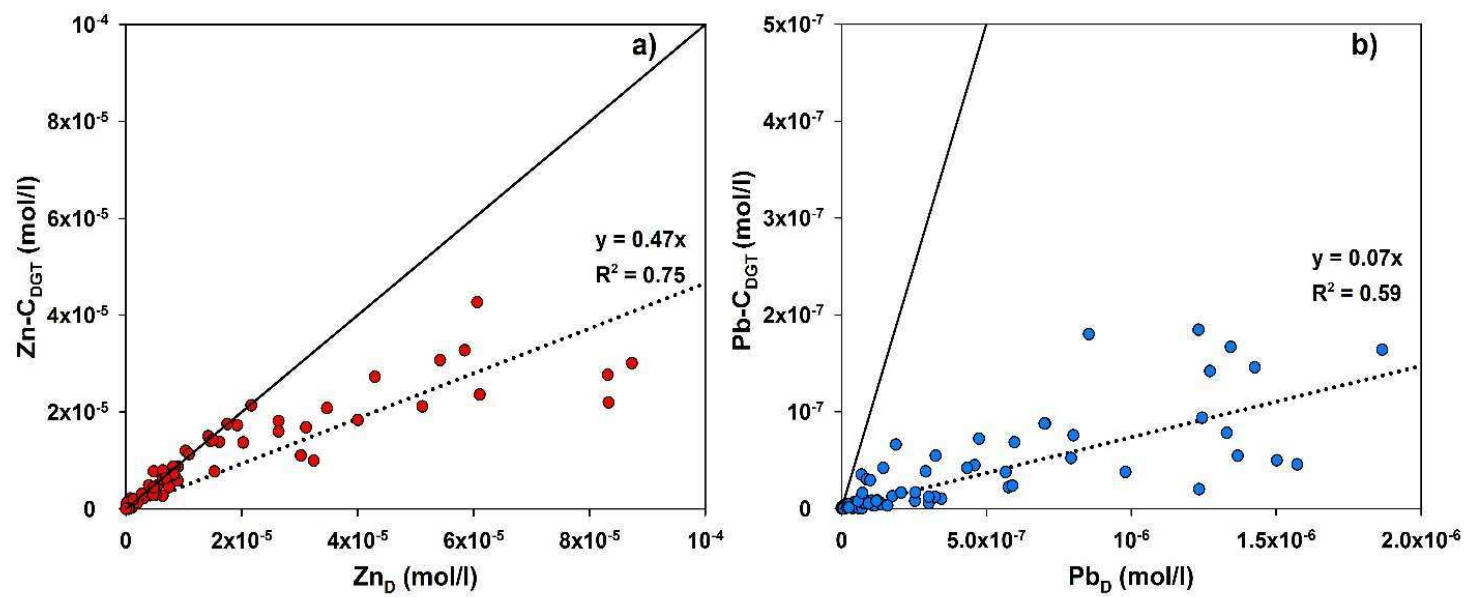


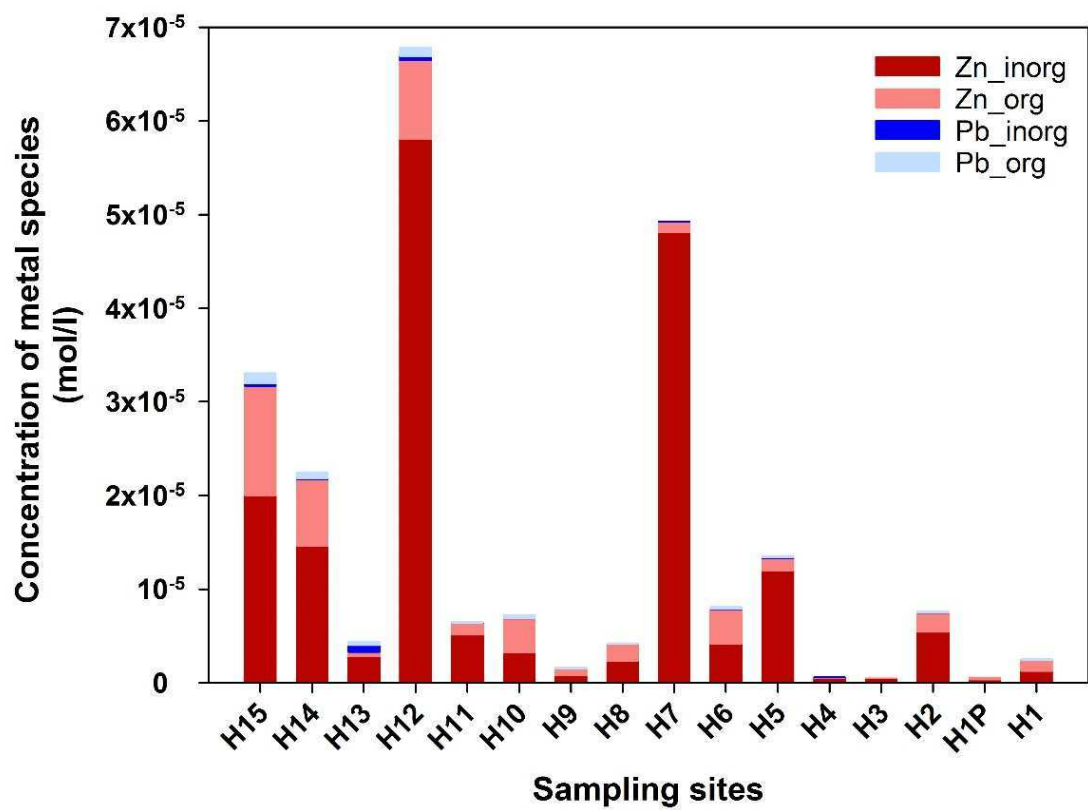
Number of sites failing standards (of 16 sampling sites)			
Metal	EQS-H	EQS-B	WHAMF _{TOX} -Toxicity SR<80%
Zinc	14	12	7
Lead	12	10	

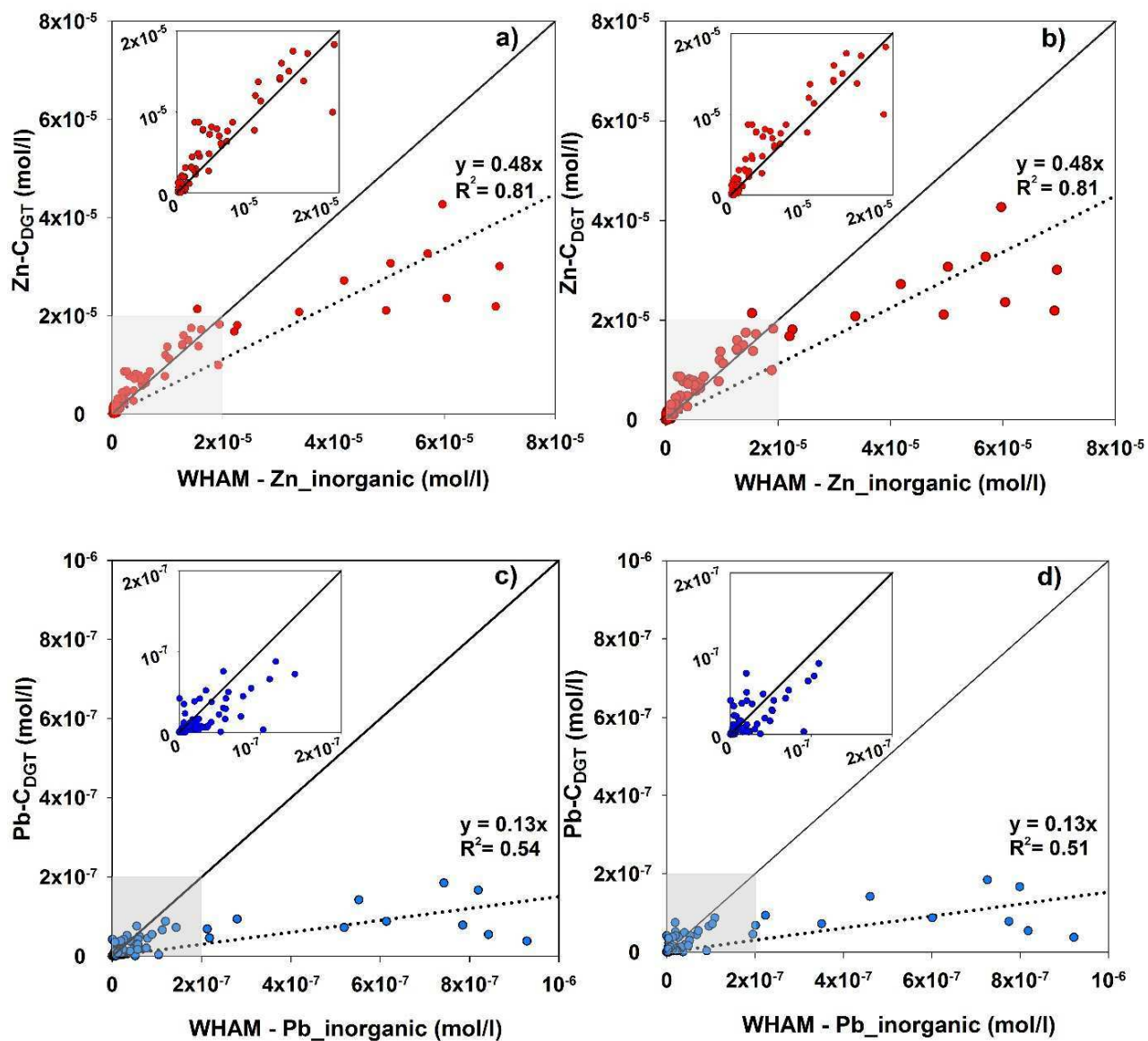


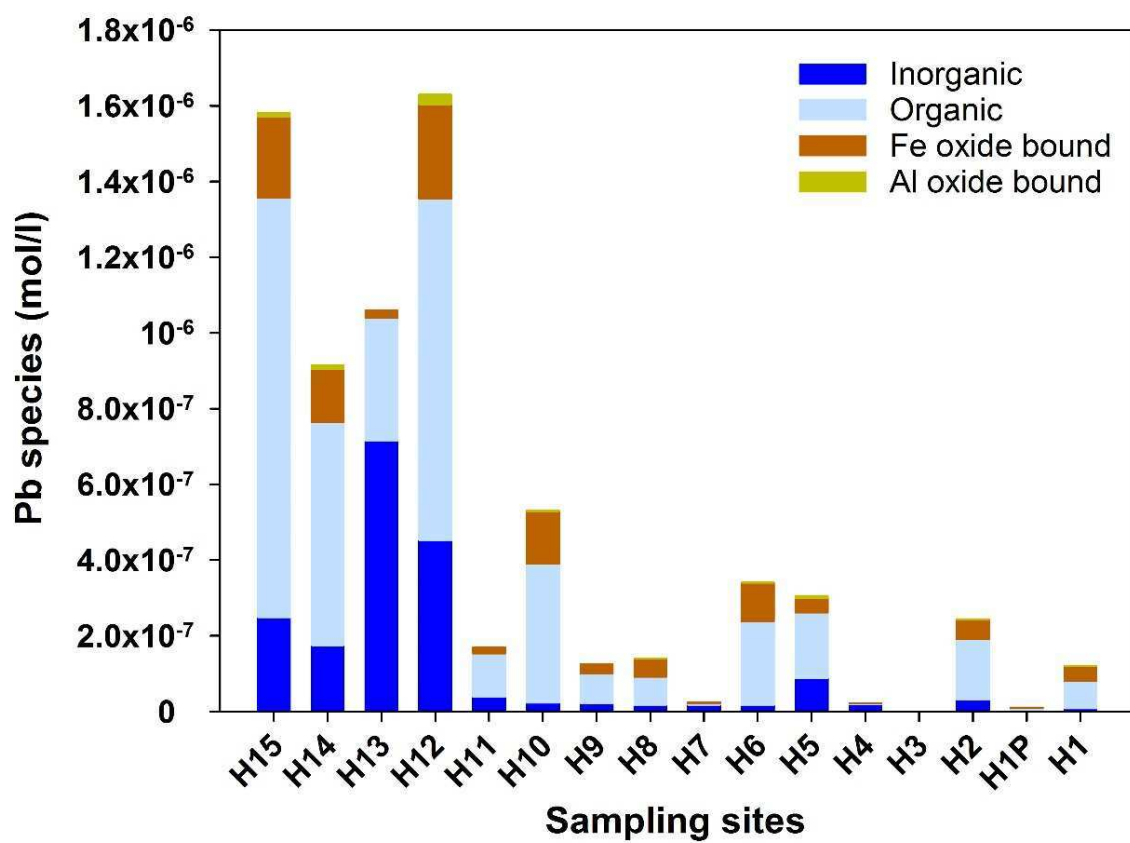
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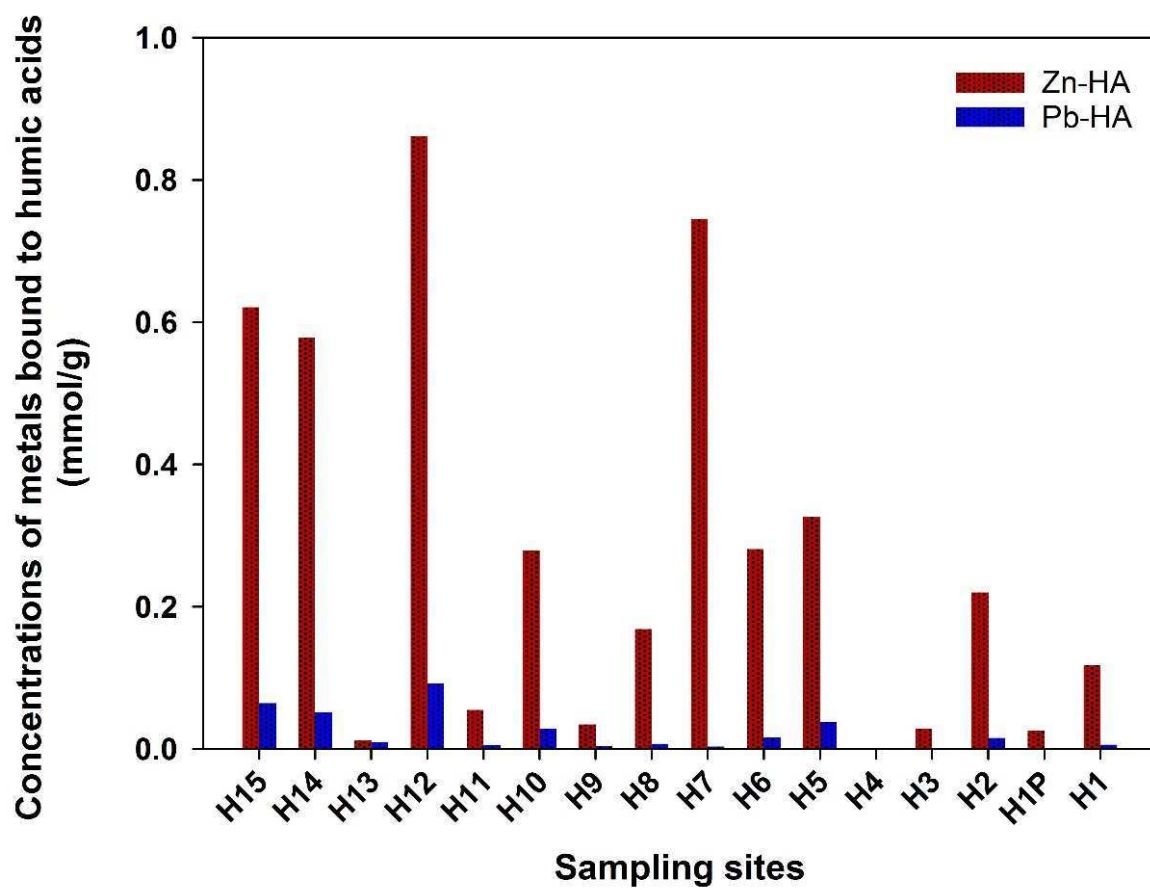
- Sampling sites
- ▲ Mine structures (e.g. dressing floors, adits, shafts, smelt mills)
- Waterways
- Elevation (m)

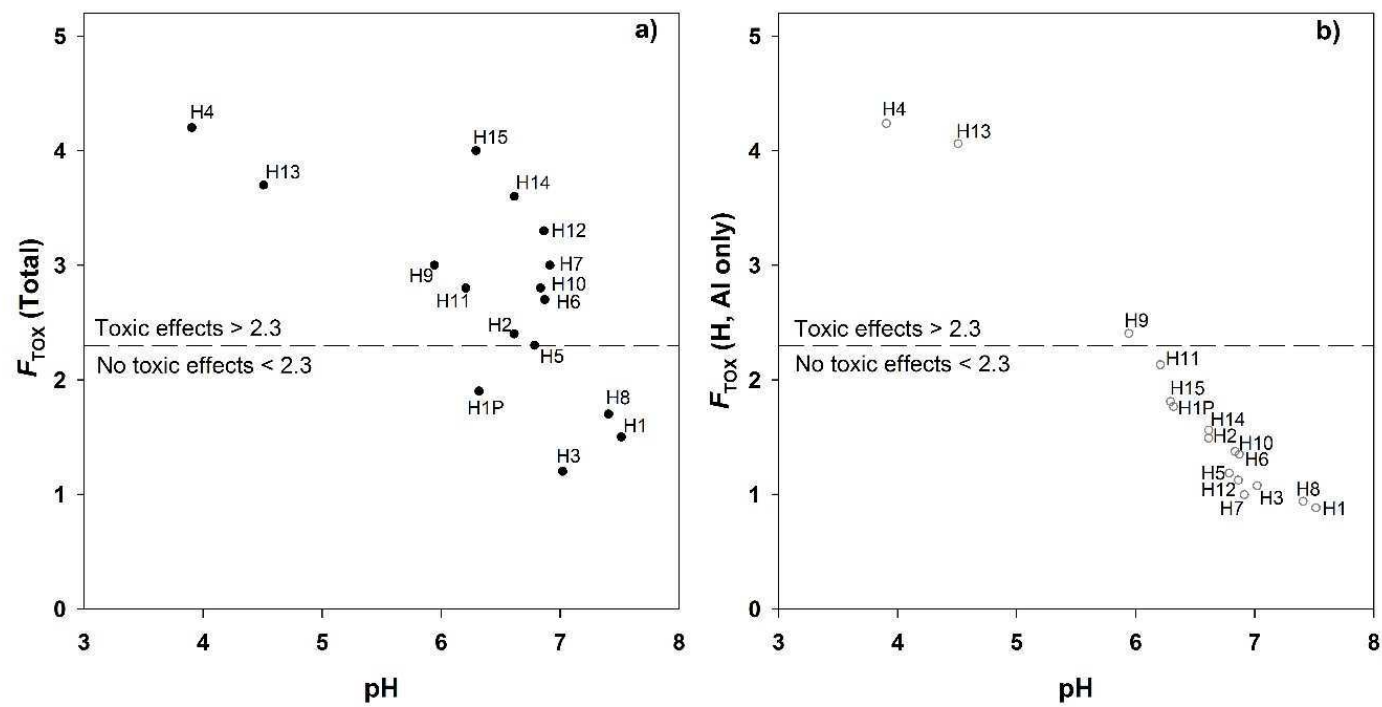












Evaluating water quality and ecotoxicology assessment techniques using data from a lead and zinc effected upland limestone catchment

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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

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

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

33

34 **1. Introduction**

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

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

56 In the last few decades, a variety of in situ analytical approaches have been developed to
57 understand the dynamics of metals in natural waters (Buffle, 2000). One such technique is
58 Diffusive Gradients in Thin-films (DGT) for assessing a range of active species, depending
59 upon their mobility (diffusion coefficients) and kinetics (dissociation rates) across a thin film
60 of polyacrylamide gel (Zhang and Davison, 2015). This technique has been used as a
61 monitoring tool for providing kinetic information on labile metal species in rivers, soil or
62 sediment impacted by mines and coastal waters (Unsworth et al., 2006; Warnken et al.,
63 2009). In addition, DGT has been applied to investigate potential metal availability in
64 freshwater ecosystems, for instance the accumulation of cadmium in communities of algae
65 (periphyton) has been evaluated by Bradac et al. (2009).

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

82 aquatic organisms and support the implementation of Environmental Quality Standards
83 (EQS).

84 Metal bioavailability and toxicity have long been recognized to be a function of water
85 chemistry (Paquin et al., 2002). Until recently environmental regulations considered only
86 hardness-based conditions to derive EQS (Beane et al., 2016). However, latest research
87 has increased understanding of the influence of physicochemical variables in metal
88 speciation, and ecotoxicological studies have extended our knowledge of metal effects on
89 biota. Consequently, bioavailability-based approaches such as the Biotic Ligand Models
90 (BLMs) have been implemented within EQS for chemical and ecological assessment, to
91 evaluate the overall quality of a given waterbody (Niyogi and Wood, 2004). In the UK, the
92 BLMs have been simplified to create a user-friendly method known as metal bioavailability
93 assessment tool (M-BAT), which use pH, DOC and calcium as input data to account for
94 toxicity of single metals in freshwaters within a regulatory context (e.g. Zn-BLM, Cu-BLM,
95 Mn-BLM and Ni-BLM) (WFD-UKTAG, 2014; De Schamphelaere and Janssen, 2002;
96 Steenbergen et al., 2005; Lock et al., 2007). The M-BAT tools parameterised to date include
97 those for Zn, Cu, Mn, Ni and Pb. Some of these tools have been incorporated into a tiered-
98 approach monitoring scheme to implement the bioavailability-based water quality guidelines.
99 However, they have limitations for assessing metal mixture effects since they are based on
100 BLMs for specific metal-organism, and they do not include all dissolved chemical species. To
101 overcome this issue, an alternative bioavailability-based model (WHAM- F_{TOX}) developed by
102 Stockdale et al. (2010) offers a plausible option for quantifying mixture toxicity and its
103 potential effects on aquatic organisms. WHAM- F_{TOX} , in common with the BLM, uses
104 organisms as reactants. However, WHAM- F_{TOX} assumes that toxicity is related to non-
105 specific binding to organism surfaces rather than specific biotic ligands (Stockdale et al.,
106 2010). Several studies have applied WHAM- F_{TOX} for predicting metal toxicity to aquatic biota
107 in laboratory experiments and linking the effects of chemical speciation of metals and
108 protons to species richness of freshwater macroinvertebrates (e.g. Ephemeroptera,

109 Plecoptera and Trichoptera (SR_{EPT}) and zooplankton species diversity (Tipping and Lofts,
110 2013; 2015; Stockdale et al., 2010; 2014; Qiu et al., 2015).

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

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

136 2. Methods

137 2.1. Site description

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

145 2.2. Water sampling

146 Monthly field surveys were conducted from November 2013 to December 2014 at sixteen
147 sampling sites. Sampling locations (Figure 1) include a source pool, tributaries and the main
148 river channel, covering the most mining impacted area (5 km²). The detailed sampling
149 strategy and analytical procedures are described in Valencia-Avellan et al. (2017).
150 Parameters recorded *in situ* such as temperature, pH, dissolved oxygen, conductivity and
151 flow, together with measured concentrations of dissolved metals (defined as filterable with a
152 0.45 µm filter) (Zn²⁺, Pb²⁺, Cd²⁺, and Cu²⁺), major anions (SO₄²⁻, Cl⁻, NO₃²⁻, PO₄²⁻) and
153 dissolved inorganic and organic carbon (DIC, DOC) were considered as model input
154 parameters for metal speciation assessment. Concentrations of dissolved metals were also
155 used to evaluate their association with the presence of labile-inorganic species in the river
156 water. Detection limits for metals in water samples were Zn: 5.1 × 10⁻⁷ mol/l and Pb: 2.4 ×
157 10⁻¹³ mol/l. For statistical analysis, annual average concentrations were calculated for each
158 site.

159 2.3. Determination of C_{DGT} in river water

160 Diffusive Gradients in Thin-films (DGT) devices (DGT Research Ltd, Lancaster, UK)
161 incorporated a Chelex metal binding layer, a 0.8 mm thick diffusive hydrogel (polyacrylamide

162 gel crossed by agarose cross-linker) and a polyethersulphone filter membrane. DGT devices
163 were subject to minimal handling and transported to and from the site in acid cleaned zip-
164 lock bags. A total of 121 DGT measurements were performed bimonthly from December
165 2013 to November 2014 across the sampling sites as part of the water sampling surveys.
166 Deployment time averaged approximately one month but varied from 18 days to 43 days.
167 Duplicate devices were deployed at two of the sixteen sites and a field blank was processed
168 in the field once per visit. All DGTs were processed in the laboratory within 24 hours: this
169 involved separating the layers of the probe using acid-washed Teflon tweezers. Each resin
170 gel (Chelex) layer was placed in a 2 ml polypropylene tube with 0.7 ml of 1 M HNO₃
171 (ARISTAR) then stored at 4°C. Immediately before ICPMS analysis, the eluent was diluted
172 10x with Milli-Q water (18.2 MΩ·cm).

173 Labile metal concentrations were determined following the method of Zhang and Davison
174 (1995). First, the mass of metal (*M*) in the Chelex resin is calculated (Eq. 1), where *C_e* is the
175 concentration of metals in the 1 M HNO₃ elution solution (in µg/l) provided by the ICPMS
176 analysis, *V_{gel}* is the volume of the resin gel, *V_{HNO3}* is the volume of HNO₃ added to the resin
177 gel, and *f_e* is the elution factor for each metal, typically 0.8. Once mass is determined, the
178 concentration of labile metals (*C_{DGT}*) can be calculated as per Zhang and Davison (1995)
179 (Eq. 2), where, *M* is the mass of metal in the Chelex resin, Δg is the thickness of the diffusive
180 gel (0.8 mm) plus the thickness of the filter membrane (0.13 mm), *D* is the diffusion
181 coefficient of metal in the gel reported at <http://www.dgtresearch.com/>, *t* is deployment time
182 and *A* is the exposure area (3.14 cm²).

$$183 \quad M = (C_e (V_{gel} + V_{HNO3}) / f_e) / 1000 \quad (1)$$

$$184 \quad C_{DGT} = (M\Delta g / (DtA)) / 1000 \quad (2)$$

185 2.4. Speciation modelling using Windermere Humic Aqueous Model (WHAM/Model
186 VII)

187 WHAM/Model VII calculates the chemical speciation of metals using humic (HA) and fulvic
188 acids (FA) as active DOM components. In this study, default model parameters were used
189 together with the measurements for the following inputs; temperature, pH, dissolved anions
190 (Cl^- , SO_4^{2-} , NO_3^- and PO_4^{2-}), dissolved metals (Zn^{2+} , Pb^{2+} , Cu^{2+} , Cd^{2+} , Fe^{3+} , and Al^{3+}),
191 dissolved inorganic carbon (DIC; input as carbonate) and DOC. For the modelling DOM was
192 assumed to be composed of 50% carbon and the active fraction considered to be 65% fulvic
193 acid, and hence was calculated from DOC values ($\text{FA} = \text{DOC} \times 1.3$) (Tipping et al., 2008).
194 The activity of both Al and Fe was calculated from the measured total filtered concentration
195 and from the equations derived for Al by Tipping (2005) and for Fe by Lofts and Tipping
196 (2011), with the lower of the two values being adopted. This avoids over-estimation of Al or
197 Fe activity in filtrates containing colloidal forms of the metals. Two different scenarios were
198 considered for modelling. Firstly, Al and Fe oxide precipitates were not considered to be
199 active with respect to surface chemistry. In a second scenario it was considered that the
200 oxides contain active binding sites and that one mole of oxide has 90 g/mol for Fe (Dzombak
201 and Morel, 1990), and 61 g/mol for Al (Lofts, 2012) of active phase. Concentrations of metals
202 bound to oxides were calculated from the output data. These scenarios were applied to allow
203 comparison with the DGT results.

204 2.5. Estimating metal mixture toxicity using WHAM- F_{TOX}

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

$$210 F_{\text{TOX}} = \sum \alpha_i v_i \quad (3)$$

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

219 For these calculations, the following conditions were applied: i) if F_{TOX} is lower than 2.33
 220 (F_{TOX-LT}) the maximum SR_{EPT} will be 23 and no toxicity occurs (Eq. 4), ii) a graded linear
 221 toxic response occurs as F_{TOX} increases (Eq. 5), and iii) if F_{TOX} is higher than 5.20 (F_{TOX-UT})
 222 no species are predicted to be present (Eq. 6). Obtained values were converted to a
 223 percentage of the maximum SR_{EPT} (90% percentile). A detailed description of these
 224 calculations is provided by Stockdale et al. (2010).

$$225 \text{ If } F_{TOX} \leq F_{TOX-LT}, \text{ then } SR_{EPT} = SR_{EPT-max} \quad (4)$$

$$226 \text{ If } F_{TOX} > F_{TOX-LT}, \text{ then } SR_{EPT} = SR_{EPT-max} - \left(SR_{EPT-max} \times \frac{F_{TOX} - F_{TOX-LT}}{F_{TOX-UT} - F_{TOX-LT}} \right) \quad (5)$$

$$227 \text{ If } F_{TOX} > F_{TOX-UT}, \text{ then } SR_{EPT} = 0 \quad (6)$$

229 2.6. Water quality assessment tools

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

238 Secondly, assessment tools based on the BLM were applied. One tool, known as the Metal
239 Bioavailability Assessment Tool (M-BAT) was used for Zn (and is also parameterised for Cu,
240 Mn and Ni) and the “Final Pb Screening Tool” was used for Pb, these are both available from
241 the UK Water Framework Directive Technical Advisory Group (WFD-UKTAG, 2014). An
242 additional Pb tool is also available from the International Lead Association that combines
243 bioavailability models with detailed species sensitivity distribution (SSD) analyses
244 (International Lead Association, 2017). These two Pb tools are referred to as Pb-FST and
245 Pb-SSD for the UK-TAG and ILA versions, respectively. For these BLM based tools, an
246 alternate risk characterisation ratio (RCR) was calculated, where PEC values are the
247 bioavailable metals based on dissolved concentrations, and PNEC are the site-specific
248 values obtained from the tools, if $RCR \geq 1$ water quality standards were exceeded
249 (Merrington and Peters, 2013; WFD-UKTAG, 2014). For these calculations, Zn-BAT
250 considered dissolved concentrations and the water chemical parameters Ca^{2+} , pH and DOC
251 as input data, Pb-FST only required concentrations for Pb and DOC. In the case of the Pb-
252 SSD, it required additional parameters such as Mg^{2+} , Na^+ , K^+ , SO_4^{2-} , Cl^- , CO_3^{2-} . Thirdly, the
253 model WHAM- F_{TOX} derived from WHAM/Model VII was applied to predict the effect of water
254 chemistry on the maximum species richness ($SR_{EPT-max}$) as described above.

255 *2.7. Biological survey*

256 A single macroinvertebrate survey was carried out at all sites in August 2014. Samples were
257 collected using a standard kick sampling technique and modified from the Water Framework
258 Directive compliant methodologies (EU-STAR project, 2004). Given the small size of
259 watercourses in the survey, one-minute kick samples were taken at thirteen sites, 30 second
260 kick samples were taken at 2 narrow sites and one sweep sample was taken in a pond at the
261 top of the catchment. Kick samples were followed by a stone search to collect organisms
262 attached to the substrate. Specimens were analysed live at each site. Several were
263 preserved and taken back to the lab for identification confirmation. Invertebrates were
264 analysed to family level. All relevant families within the sample were scored on a presence or

265 absence basis and frequency scale, using the Biological Monitoring Working Party (BMWP)
266 index which rates invertebrates based on their tolerance to organic pollution. BMWPs
267 greater than 100 are associated with unpolluted streams, while the scores of heavily polluted
268 streams are less than 10. In addition, the average sensitivity of the macroinvertebrate
269 families known as the Average Score Per Taxon (ASPT) was determined by dividing the
270 BMWP scores by the number of taxa present. Scores for ASPT are considered from 0 to 10,
271 where 10 indicates a unpolluted site containing large numbers of high scoring taxa (Armitage
272 et al., 1983) (Table 1). Impacts of metal pollution might not be reflected by the BMWP and
273 associated average score per taxon (ASPT) scoring systems as they were originally
274 developed as indicators of organic pollution (Tipping et al., 2009). Despite this caveat,
275 BMWP scores, number of taxa and ASPT values were compared with metal concentrations
276 and outcomes from water quality assessment tools.

277 **3. Results**

278 General water chemistry for Hebden Beck has been reported in detail by Valencia-Avellan et
279 al. (2017). It is dominated by the underlying geology of the catchment (i.e. buffered with high
280 Ca and circumneutral pH values), whilst metal levels are associated with the weathering and
281 erosion of Pb and Zn minerals exposed by past mining. Mine-impacted water is
282 characterised by circumneutral pH (6.3-7.5) and elevated metal concentrations. Maximum
283 concentrations of Pb, Cd, Zn, and Cu were measured in site H12 corresponding to an
284 ephemeral tributary flowing through spoil mine wastes (1.4×10^{-6} , 4.18×10^{-7} , 6.5×10^{-5} ,
285 3.12×10^{-7} mol/l, respectively) (Table S1). Full chemistry data are included in Tables S1-S3.
286 Considering the significantly higher concentrations of Zn and Pb, we focus primarily on these
287 metals. Associations between DIC/DOC, sulfate and metals were identified. DIC was
288 strongly correlated with Ca and Mg ($R^2 = 0.9$), whilst DOC was more closely correlated with
289 levels of Fe, Al, Pb and Cu ($0.1 \geq r \leq 0.7$). Sulfate correlated strongly with Zn and Cd ($R^2 =$
290 $0.6, 0.7$; $p < 0.001$), possibly associated with the dissolution of sulfide minerals (Table S4).

291 3.1. Dynamic metal (C_{DGT}) concentrations

292 From DGT measurements at each site, annual average Zn- C_{DGT} concentrations ranged from
293 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-
294 C_{DGT} identified in perennial tributaries were H7: 2.8×10^{-5} Zn mol/l and highest Pb- C_{DGT} in
295 ephemeral tributaries H13 and H12: 1.1×10^{-7} Pb mol/l, while in main channel maximum
296 concentrations were observed in H6: 7.5×10^{-6} Zn mol/l and H10: 2.3×10^{-8} Pb mol/l (Table
297 2).

298 3.2. Comparison between dissolved metal concentrations and C_{DGT} measurements.

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

307 3.3. Metal speciation calculated by WHAM/Model VII

308 3.3.1. Modelling where oxide precipitates are not surface active

309 Concentrations of predicted organic and inorganic species of Zn, and Pb are shown in
310 Figure 3. Average concentrations of metal-organic complexes (i.e. metal-fulvic acid (FA)
311 complexes) were higher for Zn than Pb, although as a function of total dissolved metal, Pb
312 had a greater fraction present as organic complexes, consistent with a higher Pb-FA model
313 binding constant. Values of organic species ranged for Zn from 2.9×10^{-8} to 1.2×10^{-5} mol/l
314 and for Pb from 2.0×10^{-10} to 1.1×10^{-6} mol/l, where site H15 presented the highest
315 concentrations for both metals. For inorganic species, higher average concentrations
316 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}

317 mol/l), with the highest concentrations in sites H12 and H13, respectively. Figures 4a and 4c
318 show linear correlation between dissolved inorganic metal species predicted by WHAM and
319 C_{DGT} measurements of Zn and Pb. Good correspondence was observed for Zn ($R^2 = 0.81$, p
320 < 0.001) and moderate relationship for Pb ($R^2 = 0.54$, $p < 0.001$).

321 3.3.2. Modelling where oxide precipitates are surface reactive

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

329 3.4. Water quality and ecotoxicological assessment

330 3.4.1. Environmental Quality Standards: EQS-H and EQS-B

331 Annual averages concentrations were used for assessing EQS-H and EQS-B. The EQS-H
332 standards were exceeded for Zn from 0.2 to 254-fold and for Pb from 0.02 to 40-fold (Table
333 2). Highest Zn exceedances (> 60 -fold) occurred in upstream sites surrounded by spoil mine
334 wastes (H15, H14 and H12). These sites and H13, also presented highest Pb exceedances
335 (> 20 -fold). The EQS-B standards were exceeded for Zn from 0.8 to 209-fold and for Pb from
336 0.02 to 17-fold. Highest Zn exceedances (> 50 -fold) appeared in sites H7 $>$ H12 $>$ H15
337 which represent a mine adit, an ephemeral tributary flowing through spoil mine wastes and a
338 reservoir next to a smelting mill. For Pb, highest levels (> 6 -fold) were presented in H12 $>$
339 H5, H15 $>$ H14 (Table 2).

340 3.4.2. Toxicity function: WHAM- F_{TOX}

341 WHAM- F_{TOX} considers humic acid (HA) bound to be a plausible proxy for organism bound
342 metal. Concentrations of metals bound to humic acid ranged for Zn (0.0013 to 0.9 mmol/g)

343 and Pb (0.0001 to 0.09 mmol/g) (Figure 6). Reservoir (H15), perennial tributaries (H14, H7)
344 and ephemeral tributary (H12) were characterised by high Zn concentrations (≥ 0.5 mmol/g),
345 the same sites (except for H7) presented high Pb concentrations (≥ 0.05 mmol/g).

346 Toxicity contributions ($F_{\text{TOX-M}}$) for each element (Zn, Pb, H and Al) were calculated from
347 Equation 3 (Methods Section 2.5.) and summed to produce a total toxicity function value
348 (Total_ F_{TOX}). Equations 4 and 6 were applied to convert Total_ F_{TOX} to a prediction of the
349 maximum species richness that could be expected based on the chemistry of each stream.
350 Effects of metal mixtures on predicted species richness are shown in Table 3. Reduction of
351 predicted species richness ($\text{SR}_{\text{EPT}} < 23$) was identified at several sites. In the main river
352 channel (H10, H6 and H2) and perennial tributary (H11) a slight decrease was presented
353 (SR_{EPT} : 22 to 19) associated with the combined moderate toxicity of H, Zn and Al. A more
354 significant decrease (SR_{EPT} : 18 to 10) was shown in sites H15, H14, H13, H12, H9 and H7
355 due to higher contributions from Zn, contributions of H and Al and additionally small
356 contributions from Pb; while the lowest value (SR_{EPT} : 8) occurred in site H4 related to the
357 influence of H and Al and not metals.

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

365 3.6. Evaluation of metal assessment tools

366 EQS-H, EQS-B and WHAM- F_{TOX} were compared to evaluate their relative assessment of
367 potential impact with respect to metal toxicity in aquatic ecosystems (Table2). Multiple sites
368 are necessary as WFD applies to lower order streams than previous legislation, meaning all
369 perennial tributaries may be important from a compliance viewpoint. Each of these sites

370 represents a length of stream therefore an ecosystem. Ephemeral tributaries are assessed
371 as they can represent high input sites into other streams, although these sites may not be
372 significant as aquatic ecosystems as they are more likely to be dry during the productive
373 summer months. From the comparison, EQS-H are more conservative than EQS-B. EQS-H
374 showed wider ranges of exceedance for Zn (0.2 to 254-fold) and for Pb (0.02 to 40-fold),
375 while compared to EQS-B for Zn (0.8 to 209-fold) and Pb (0.02 to 17-fold). For both tools,
376 the highest Zn failures occurred in reservoir (H15), ephemeral tributary (H12) and perennial
377 tributaries (H14 and H7). For Pb, the same sites, except H7 showed significant failures, in
378 addition, using EQS-H an ephemeral tributary (H13) showed exceedance, likewise the
379 perennial tributary (H5) applying the EQS-B approach. For the Pb bioavailability tools (FST
380 and SSD), results showed that the FST approach with fewer chemistry inputs is more
381 conservative than SSD. By using FST, values ranged from 0.1 to 35-fold, while SSD
382 presented ranges from 0.1 to 17-fold. Calculations from WHAM- F_{TOX} showed that at twelve
383 sites the water chemistry may reduce the maximum species diversity of macroinvertebrates
384 by between 3 and 65%. All ephemeral tributaries (H12, H13, and H4) together with a
385 perennial tributary (H14) and reservoir (H15) showed the largest predicted reductions in
386 species richness of 34% to 65%.

387 *3.7. In situ macroinvertebrate survey*

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

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

403 **4. Discussion**

404 For aquatic environments, metal assessment has evolved as bioavailability is increasingly
405 considered a critical measure for improving water quality, e.g. under the EU Water
406 Framework Directive. A preliminary water quality assessment was carried out where
407 dissolved concentrations of Zn and Pb were assumed to be bioavailable depending on
408 ranges of water hardness conditions, and exceedances were calculated from previous-
409 established EQS-H. Concentrations of Zn and Pb exceeded the limits by 175-fold and 40-fold
410 respectively, however, little consideration is given to their bioavailable forms. Merrington
411 (2016) has indicated that hardness limits have been derived from ecotoxicological tests
412 mainly from crustaceans and fish, where water chemical parameters do not necessarily
413 reflect field conditions. Thus, the use of hardness-based standards may be overly
414 conservative as they might under-represent other important taxa. In addition, they fail to
415 consider chemical speciation in distinguishing sites with potential environmental risk
416 associated with metals.

417 In complex environments such as freshwaters, understanding of the underlying conditions
418 controlling the dynamics and speciation of metals is critical for assessing and managing the
419 risk they pose to aquatic life (Han et al., 2013). This study evaluated the utility of DGT for
420 yielding both time averaged and dynamic Zn and Pb concentrations (potentially a proxy for
421 organism availability) (Diviš et al., 2007; Degryse et al., 2009; Amato et al., 2014;
422 Søndergaard et al., 2011; 2014; Omanović et al., 2015). By comparing, DGT measurements
423 (C_{DGT}) with direct water measurements (M-measured), results indicated that C_{DGT}

424 concentrations were generally lower than the directly measured values. For Zn- C_{DGT} and
425 Zn-measured a strong linear correlation ($R^2 = 0.75$) was present suggesting the presence of
426 Zn mainly as free ions and readily labile complexes, a slope of 0.47 revealed that DGT
427 measurements at higher concentrations failed to reflect dissolved measured concentrations.
428 The better agreement presented at lower concentrations might be caused by the kinetic rate
429 of Zn forms, which will determine its accumulation into the Chelex-resin layer (Warnken et
430 al., 2007). Free Zn^{2+} seems to be rapidly absorbed by the Chelex-resin layer, while the
431 supply of labile complexed forms will depend on their degree of lability (Puy et al., 2012).
432 Zhang and Davison (2015) indicated that fully labile complexes might not be completely
433 dissociated within the diffusion layer, thus their uptake in the Chelex-resin could be reduced
434 or not occur. Correlation between Pb- C_{DGT} and Pb-measured was moderate ($R^2 = 0.59$),
435 with a slope of 0.07 indicating poor agreement between the data, possibly attributable to the
436 presence of less labile Pb forms (e.g. Pb-FeOx, Pb-DOM) that need more time for diffusion
437 or presence of bigger particles that could be excluded from DGT membranes (Han et al.,
438 2013). Considering correlations for both metals, C_{DGT} measurements can be affected by the
439 amount of total metal dissolved concentrations present as free ions and their complexation
440 with inorganic or organic substances (Zhang and Davison, 2015). Furthermore, long
441 deployments (> 15 days) might expose DGTs to biological factors like biofouling, and flow
442 fluctuations that might decrease the stirring rates, reducing DGT's performance (Davison
443 and Zhang, 1994; Webb and Keough, 2002; Turner et al., 2014).

444 Metals speciation and bioavailability are controlled by a range of different factors such as
445 pH, ionic strength, DOC, hardness, pH and inorganic or organic colloids. Given these
446 factors, the quantity of metal available for organism uptake is not reflected by the total
447 dissolved concentration (Hart, 1981; Luoma, 2008). This study correlated M- C_{DGT}
448 measurements with inorganic metal complexes predicted from dissolved measured
449 concentrations under the absence and presence of active inorganic colloids (e.g. FeOx and
450 AlOx) as they can compete for Zn and Pb binding with DOM. If active oxide colloids are

451 excluded from the modelling, Zn is predicted to be largely present as inorganic (e.g. Zn^{2+} ,
452 $Zn(OH)^+$, etc.) rather than organic complexes, and highest predicted inorganic concentration
453 was at site H12 (5.8×10^{-5} mol/l). Conversely Pb had a greater affinity for organic binding,
454 with highest organic concentration at H15 (10^{-6} mol/l) (Figure 5). Both metals showed good
455 linear correlations between inorganic complexes and C_{DGT} for Zn ($R^2 = 0.81$), and Pb ($R^2 =$
456 0.54), reflecting a modest improvement in the correlation of Zn- C_{DGT} with predicted Zn
457 inorganic complexes than previous correlation between Zn- C_{DGT} with Zn-measured, which
458 can be attributable to the fact that dissolved Zn is present primarily as inorganic and free
459 ions, reflecting the absence or low level of Zn-organic complexes in the correlation analysis.
460 The agreement observed for Zn confirms that the total dissolved Zn (mainly inorganic) is
461 approximately equivalent to the dynamic Zn- C_{DGT} (except at high Zn loading, as previously
462 discussed). For Pb, there is a discrepancy between Pb- C_{DGT} and dissolved Pb, likely due to
463 larger fractions in other less labile phases (Pb-organic complexes) (Warnken et al., 2008;
464 Han et al., 2013). Outcomes from modelling conditions where oxide precipitates are surface
465 reactive showed similar behaviours for Zn ($R^2 = 0.81$) and Pb ($R^2 = 0.51$) to those obtained
466 in the absence of surface activity. The lack of improved agreement to the Pb-inorganic in
467 presence of oxides with the Pb- C_{DGT} (dynamic) concentration implies that the model may be
468 under-predicting the binding to these forms. Results from DGT measurements provided
469 useful information about metal dynamics and speciation which is valuable for understanding
470 metal uptake mechanisms. Metal bioavailability studies from Bradac et al. (2009); Yin et al.
471 (2014); (Balistrieri et al., 2012) suggest DGTs as useful tool for understanding the
472 accumulation of metals in freshwater ecosystems (e.g. algae, snails and fish). However,
473 results from this study indicated that DGT is not a suitable substitute for direct water
474 measurements due to complex uptake kinetics of different forms and at higher metal
475 concentrations. These limitations make DGT a potentially useful bioavailability assessment
476 tool, but further work is needed to compare DGT measurements in the field with actual in situ
477 organism metal burdens. Zhang and Davison (2015) have indicated that given the
478 complexity of biological system and chemistry of environmental media, the ability of DGT to

479 mimic metal uptake and predict bioavailability is variable. Further research based on the
480 comparison of field data and laboratory experiments under controlled temperature,
481 deployment time and flow rates are suggested for evaluating the applicability of DGTs
482 regarding metal bioavailability in freshwaters. Evidence of marked diurnal ranges as well as
483 flow-dependent variations in metal concentrations reported by Jones et al. (2015), also
484 suggests that DGT measurements should be complemented by frequent measurements of
485 metal concentrations (e.g. with auto samplers) to identify the actual variation experienced by
486 DGTs.

487 Outcomes from EQS-H, EQS-B and WHAM- F_{TOX} provided different levels of prediction
488 regarding metal bioavailability and toxicity. In comparing EQS-H and EQS-B, the simpler
489 approach (EQS-H) is more conservative than the standard that considered more water
490 chemistry parameters and metal-organism interactions (EQS-B) (Merrington, 2016).
491 However, by contrasting EQS-B and WHAM- F_{TOX} , results suggest that the latter approach is
492 the least conservative of the three approaches. This was clearly identified in sites which
493 exceeded EQS-B like H7 (Zn: 209-fold and Pb: 2-fold) and H5 (Zn: 44-fold and Pb: 9-fold),
494 but with F_{TOX} -SR showing 100% for predicted species richness for both sites. WHAM- F_{TOX}
495 also revealed that major toxic effects can occur at circumneutral pH mostly due to Zn and
496 Pb. Moreover, a limited biological survey agreed with this chemical assessment. The most
497 impoverished sites presented low predicted macroinvertebrate richness ($\text{SR}_{\text{EPT}} \leq 34\%$)
498 correlating with high concentrations of metals (H12, H13 and H14) or low-pH values (H4)
499 (Table 2). Overall, the orders Diptera and Trichoptera were dominant (40% and 28%) with
500 Chironomidae and Hydropsychidae as the more abundant families. Responses of
501 macroinvertebrates and diatoms to different metal levels in Hebden Beck have been
502 previously evaluated by the Environment Agency (2014). The ecological assessment
503 revealed a relationship between increasing Zn concentrations and reduced biodiversity.
504 Likewise invertebrate data showed that responses of species diversity and composition
505 could be related to the influence of complex interactions between metals and acid scenarios.

506 In early and current assessment, the family Baetidae (O: Ephemeroptera) was dominant,
507 possibly due to its tolerance to different levels of pollution. A study by Mebane et al. (2015),
508 indicated that the species most sensitive to pollution are the last to re-colonise after recovery
509 from Cu contamination. In our study less sensitive families (Perlodidae, Nemouridae,
510 Baetidae) are widespread throughout the catchment. More sensitive families like
511 Glossomidae, Ephemerellidae and Heptageniidae are mainly confined to main channel sites
512 (H2, H6, H10), although Heptageniidae was also present in a metal impacted site H14
513 (predicted species richness of 55% of the theoretical maximum), another metal sensitive
514 family, Psychodidae was present in less metal impacted sites, such as H1P, showing a
515 predicted species richness of 100%. These results are consistent with the F_{TOX} species
516 predictions, however, current interpretations are generalised as we only identified
517 macroinvertebrates to family level, and species sensitivity is complex, where differences in
518 pollution tolerance occur within different taxa (Clements et al., 2013).

519 From a regulatory perspective, bioavailability based standards (EQS-B) offer a more
520 ecologically-based approach to address water quality failures than traditional hardness
521 corrections (EQS-H) (Peters et al., 2016), due to their parameterisation with organism
522 toxicity data or field species observations. In this study, both standards facilitated the
523 screening of sites with potential metal risk, however, EQS-B identified sites where dissolved
524 metal concentrations and speciation mechanisms ($M-C_{\text{DGT}}$) were related. Although,
525 bioavailability-based models have been developed on field data and applied in the
526 establishment of water quality standards for metals, their applicability for routine regulatory
527 purposes need to be tested, especially for sites where physicochemical conditions (e.g. pH,
528 hardness, DOC) are near or outside of the boundaries of the model (Rüdel et al., 2015;
529 WFD-UKTAG, 2009a). In this study, some sites presented pH and Ca values below Zn-BLM
530 limits, thus default limits of this tool (pH = 6 and Ca = 3mg/l) were used in Zn assessment,
531 consequently the use of complementary models like WHAM and $\text{WHAM}_{F_{\text{TOX}}}$ can offer more
532 complete toxicity predictions under original physico-chemical conditions. Because

533 WHAM F_{TOX} considers protons within its formulation, it can be applied to any combination of
534 water chemistry and metal mixtures. Bioavailability based EQS were implemented for
535 England and Wales in 2014 (DEFRA, 2014). For a practical implementation of these tools a
536 compliance scheme was suggested by the UK Technical Advisory Group, which includes: i)
537 comparison between metal concentrations with EQS-H, ii) application of BLM tools (e.g. Zn-
538 BLM, Pb-BLM) to predict site-specific bioavailability (EQS-B), and iii) local refinement by
539 using models that require larger number of input parameters (e.g. full M-BLM or WHAM-VII)
540 (Bio-met, 2015; WFD-UKTAG, 2009b). Further research is desirable, especially in the
541 generation of toxicity data to improve the significance of toxicity coefficients (e.g. α_{Pb}).
542 Nevertheless, the combination of EQS-B and WHAM- F_{TOX} could provide an improved
543 rational assessment which is essential for the purposes of considering remediation activities.
544 Vulnerable sites can be prioritised under a holistic scenario (water chemistry, metal
545 concentrations and interactions between metal and organism) where remedial actions can
546 be focused on reducing metal bioavailability for the purpose of improving ecological
547 endpoints.

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

559 relating the response of aquatic organisms to river water chemistry and pollutants, which are
560 useful for future ecological assessments.

561 **5. Conclusions**

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

- 564 • In carboniferous catchments, metal toxic effects occur at circumneutral pH. Zn is mainly
565 present as free ions and readily labile complexes while Pb is present as less labile forms
566 (e.g. organic complexes and colloids).
- 567 • Metal assessment through the EQS-H approach is more conservative than EQS-B and
568 WHAM- F_{TOX} . The incorporation of bioavailability will better address some of the
569 processes that control toxic metals effects. In addition, catchment management will be
570 improved as the bioavailability approach will aid in the identification of key reaches where
571 remediation may be required.
- 572 • Metal concentrations estimated from DGT provided useful information for the assessment
573 of Zn and Pb speciation and for understanding metal uptake mechanisms. However,
574 results suggest that further research is required for evaluating the applicability of this tool
575 in the assessment of metal bioavailability under field conditions.

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ACCEPTED MANUSCRIPT

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

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Tables

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

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 (a), EQS-B (Bioavailability assessment tool-BAT, Final screening tool-FST and Species sensitivity distribution-SSD) and WHAM- F_{TOX} ($SR_{EPT} = 23$ no toxic effects, $SR_{EPT} < 23$ toxic effects, and reduction of 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 (±7.2×10 ⁻⁶)	1.2×10 ⁻⁵ (±6.9×10 ⁻⁶)	253.5	54.0	1.3×10 ⁻⁶ (±4.0×10 ⁻⁷)	5.7×10 ⁻⁸ (±5.2×10 ⁻⁸)	38.8	18.9 (d)	8.5	10	43
H14	PT	2.1×10 ⁻⁵ (±5.2×10 ⁻⁶)	1.5×10 ⁻⁵ (±4.4×10 ⁻⁶)	174.7	45.9	7.6×10 ⁻⁷ (±3.6×10 ⁻⁷)	6.1×10 ⁻⁸ (±1.9×10 ⁻⁸)	21.9	15.8 (d)	6.8	13	55
H13	ET	3.2×10 ⁻⁶ (±6.3×10 ⁻⁶)	6.1×10 ⁻⁷ (±3.2×10 ⁻⁷)	25.9	4.1 (b)	9.9×10 ⁻⁷ (±2.6×10 ⁻⁷)	1.1×10 ⁻⁷ (±6.6×10 ⁻⁸)	28.6	10.2 (d)	1.1 (e)	12	52
H12	ET	6.5×10 ⁻⁵ (±3.0×10 ⁻⁵)	2.7×10 ⁻⁵ (±4.0×10 ⁻⁶)	85.0	117.6	1.4×10 ⁻⁶ (±4.4×10 ⁻⁷)	1.1×10 ⁻⁷ (±4.0×10 ⁻⁸)	39.5	35.0 (d)	17.1	15	66
H11	PT	6.3×10 ⁻⁶ (±2.1×10 ⁻⁵)	3.3×10 ⁻⁶ (±7.6×10 ⁻⁶)	51.3	8.7	1.5×10 ⁻⁷ (±4.5×10 ⁻⁷)	9.2×10 ⁻⁹ (±1.8×10 ⁻⁸)	4.4	1.6 (d)	0.8	20	85
H10	MC	6.8×10 ⁻⁶ (±2.8×10 ⁻⁶)	5.2×10 ⁻⁶ (±2.5×10 ⁻⁶)	55.6	11.1	3.8×10 ⁻⁷ (±2.0×10 ⁻⁷)	2.3×10 ⁻⁸ (±1.5×10 ⁻⁸)	11.2	6.5 (d)	2.9	19	82
H9	PT	1.5×10 ⁻⁶ (±1.9×10 ⁻⁶)	1.3×10 ⁻⁶ (±5.5×10 ⁻⁷)	12.0	2.1 (b)	1.0×10 ⁻⁷ (±1.1×10 ⁻⁷)	4.6×10 ⁻⁹ (±4.4×10 ⁻⁹)	2.9	1.0 (d)	0.5	18	77
H8	PT	4.1×10 ⁻⁶ (±1.5×10 ⁻⁶)	3.9×10 ⁻⁶ (±2.3×10 ⁻⁶)	3.6	7.4	9.2×10 ⁻⁸ (±3.2×10 ⁻⁸)	1.1×10 ⁻⁸ (±1.1×10 ⁻⁸)	2.6	2.0 (d)	1.1	23	100
H7	PT	4.9×10 ⁻⁵ (±1.3×10 ⁻⁵)	2.8×10 ⁻⁵ (±8.5×10 ⁻⁶)	42.9	209.2	2.1×10 ⁻⁸ (±6.1×10 ⁻⁸)	1.2×10 ⁻⁹ (±1.1×10 ⁻⁹)	0.6	3.0 (d)	1.7	18	76
H6	MC	7.8×10 ⁻⁶ (±1.5×10 ⁻⁶)	7.5×10 ⁻⁶ (±1.0×10 ⁻⁶)	10.2	12.8	2.4×10 ⁻⁷ (±9.9×10 ⁻⁸)	8.5×10 ⁻⁹ (±4.4×10 ⁻⁹)	6.9	3.8 (d)	1.7	20	88
H5	PT	1.3×10 ⁻⁵ (±2.9×10 ⁻⁶)	1.3×10 ⁻⁵ (±1.5×10 ⁻⁶)	11.6	43.9	2.6×10 ⁻⁷ (±2.2×10 ⁻⁷)	4.9×10 ⁻⁸ (±1.8×10 ⁻⁸)	7.5	14.6 (d)	8.7	23	100
H4	ET	5.5×10 ⁻⁷ (±1.4×10 ⁻⁷)	2.6×10 ⁻⁷ (±8.9×10 ⁻⁸)	4.5	2.5 (b,c)	2.1×10 ⁻⁸ (±8.2×10 ⁻⁹)	3.7×10 ⁻⁹ (±5.4×10 ⁻¹⁰)	0.6	0.4	0.02 (e)	8	35
H3	PT	<5.1×10 ⁻⁷	1.2×10 ⁻⁶ (±5.1×10 ⁻⁸)	0.2	0.8	8.0×10 ⁻¹⁰ (±9.5×10 ⁻¹⁰)	5.4×10 ⁻¹⁰ (±4.1×10 ⁻¹⁰)	0.02	0.1	0.1	23	100
H2	MC	7.4×10 ⁻⁶ (±1.1×10 ⁻⁶)	7.0×10 ⁻⁶ (±1.0×10 ⁻⁶)	9.7	17.7	1.9×10 ⁻⁷ (±1.4×10 ⁻⁷)	1.3×10 ⁻⁸ (±3.9×10 ⁻⁹)	5.5	4.4 (d)	2.3	22	97
H1P	PT	5.6×10 ⁻⁷ (±1.1×10 ⁻⁶)	4.8×10 ⁻⁹ (±4.5×10 ⁻⁹)	0.7	2.4	3.5×10 ⁻⁹ (±3.1×10 ⁻⁹)	1.1×10 ⁻¹⁰ (±3.0×10 ⁻¹¹)	0.3	0.2	0.1	23	100
H1	MC	2.4×10 ⁻⁶ (±1.1×10 ⁻⁶)	2.5×10 ⁻⁶ (±6.6×10 ⁻⁷)	2.1	4.2	8.0×10 ⁻⁸ (±6.6×10 ⁻⁸)	3.7×10 ⁻⁹ (±3.3×10 ⁻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.

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 ($\pm SD$).

Sites	$SR_{(EPT)}$	Total_ F_{TOX}	F_{TOX-Zn}	F_{TOX-Pb}	F_{TOX-H}	F_{TOX-AI}
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	12	3.7	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)

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

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Figures Legends

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.

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.

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.

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

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).

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Highlights

- In carboniferous catchments, Zn and Pb toxic effects occur at circumneutral pH.
- Metal assessment with EQS-H tools is more conservative than EQS-B and WHAM- F_{Tox} .
- Bioavailability-based tools support remediation to reach good ecological status.