Relating metal exposure and chemical speciation to trace metal accumulation in aquatic insects under natural field conditions

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HIGHLIGHTS

- Strong relations were observed between body burdens and free metal ion activities.
- The effect of H^+ ions on insect body burdens was most clearly revealed.
- Effects of major hardness ions on metal accumulation were rather limited.
- Insect body burdens in natural waters can be predicted using speciation modelling.

ARTICLE INFO

ABSTRACT

The present study investigated to what extent measured dissolved metal concentrations, WHAM-predicted free metal ion activity and modulating water chemistry factors can predict Ni, Cu, Zn, Cd and Pb accumulation in various aquatic insects under natural field conditions. Total dissolved concentrations and accumulated metal levels in four taxa (*Leuctra* sp., Simuliidae, *Rhithrogena* sp. and Perlodidae) were determined and free metal ion activities were calculated in 36 headwater streams located in the north-west part of England. Observed invertebrate body burdens were strongly related to free metal ion activities and competition among cations for uptake in the biota. Taking into account competitive effects generally provided better fits than considering uptake as a function of total dissolved metal levels or the free ion alone. Due to the critical importance and large range in pH (4.09 to 8.33), the H^+ ion activity was the most dominant factor influencing metal accumulation. Adding the influence of Na^+ on Cu^{2+} accumulation improved the model goodness of fit for both *Rhithrogena* sp. and Perlodidae. Effects of hardness ions on metal accumulation were limited, indicating the minor influence of Ca^{2+} and Mg^{2+} on metal accumulation in soft-water streams (0.01 to 0.94 mM Ca; 0.02 to 0.39 mM Mg). DOC levels (ranging from 0.6 to 8.9 mg L^{-1}) significantly affected Cu body burdens, however not the accumulation of the other metals.

Our results suggest that 1) uptake and accumulation of free metal ions are most dominantly influenced by competition of free H^+ ions in low-hardness headwaters and 2) invertebrate body burdens in natural waters can be predicted based on the free metal ion activity using speciation modelling and effects of H^+ competition.

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1. Introduction

Metal bioavailability and toxicity from water-borne exposure generally depend on the activity of the free metal ion, which is controlled by chemical speciation processes (binding to dissolved organic carbon (DOC), inorganic ligands (e.g. Cl^-, OH^-, CO_3^{2-}) and the effect of pH), and are largely influenced by other cations (e.g. Na^+, Ca^{2+}, Mg^{2+}) and H^+ ions competing with trace metals for uptake at particular biological uptake sites (e.g. ion-channels and other transporters in the gills of aquatic organisms) (Hare and Tessier, 1996, 1998; Bervoets and Blust,

2000). The latter concepts are integrated in the Free Ion Activity Model (FIAM), which states that the activity of the free metal ion is a good predictor of both metal availability and toxicity to aquatic organisms (Campbell, 1995), and has been used as the main rationale for the construction of the Biotic Ligand Model (BLM) in order to predict water-borne metal toxicity (Paquin et al., 2002; Niyogi and Wood, 2004).

Over the last decades efforts have been made to determine and predict trace metal speciation in natural waters. This has led to the construction of chemical speciation models such as the Windermere Humic Aqueous Model (WHAM), which enables calculation of the free metal ion concentration and activity in solution, based on water chemistry measurements and equilibrium binding interactions (e.g. pH, temperature, Ca^{2+}, Mg^{2+}, DOC) (Tipping, 1994, 1998; Tipping et al., 1998).

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Although WHAM is currently incorporated in the BLM (Paquin et al., 2002; Niyogi and Wood, 2004), the speciation model has been rarely used to predict metal accumulation in aquatic insects under natural conditions (but see Hare and Tessier, 1996, 1998; Croteau et al., 1998; Ponton and Hare, 2009; Stockdale et al., 2010). Since water chemistry and exposure scenarios can largely vary compared to conditions in the laboratory, relations between metal exposure, chemical speciation and invertebrate body burdens taking into account the influence of major ions and other metals at biological uptake sites should be assessed directly in the field. Recently, studies of Stockdale et al. (2010) and Tipping and Lofts (2013) were able to model metal levels in field-collected aquatic invertebrates using WHAM, considering organisms as humic acids, which corresponded well with measured body burdens. Since accumulated metal levels represent a time-integrated and ecologically-relevant measure of metal exposure and bioavailability, body burdens have gained increasing attention in biomonitoring studies during recent years (Hare and Tessier, 1996; Adams et al., 2011; De Jonge et al., 2013).

The aim of the current study was first to evaluate influences of chemical speciation and water chemistry (pH, DOC and major cation effects) on insect body burdens under natural field conditions, and secondly to evaluate whether WHAM-predicted free metal ion activity and other modulation factors could accurately predict the observed body burdens.

2. Material and methods

2.1. Study area and sampling design

In total 36 headwater streams of the Lake District, Ribbledale, Swaledale and the Howgill Fells, which are all located in the northwest part of England, were sampled as part of an extended field survey (Bass et al., 2008). Some of these sites have been severely metal contaminated from discharge of nearby abandoned mining sites. Samples for the determination of water chemistry were taken on four occasions (March 6–8, March 20–22, April 3–5 and April 17–19, 2006). Separate samples were taken for major solutes (one-litre, high-density polyethylene bottles), for pH (glass bottles with a ground glass stopper, completely filled) and trace metals (500 cm^3 acid-washed polyethylene bottles). All samples were kept in cool boxes at 4 °C during transport to the laboratory, where they were kept cool and dark.

2.2. Water chemistry analysis and chemical speciation

Within one day after collecting, samples were analysed for pH using a glass electrode while taking care to avoid de-gassing of the samples. Total concentrations of Na, Mg, Al, K, Ca, Mn and Fe were measured after one week using inductively coupled plasma-optical emission spectrometry (ICP-OES). Chloride (Cl), nitrate (NO$_3$-N) and sulphate (SO$_4$-S) were determined by ion chromatography; alkalinity was measured by Gran titration and dissolved organic carbon (DOC) by combustion. Ammonia-N (NH$_4$-N), phosphorus (P) and silica (SiO$_2$) were determined colorimetrically and suspended particulate matter (SPM) was determined gravimetrically. Water samples intended for trace metal analysis were filtered over a 0.45 μm polypropylene filter, acidified with 1% nitric acid (HNO$_3$; 69%) and total levels of Ni, Cu, Zn, Cd and Pb were quantified using inductively coupled plasma-mass spectrometry (ICP-MS). All analyses made use of International Quality Control standards, with verification by the Proficiency Testing scheme. Furthermore, a Certified Reference Material (CRM) was used in the determination of trace metals in surface waters.

Free ion activity (FIA) calculations of the measured trace metals were performed using the Windermere Humic Aqueous Model (WHAM) (Tipping, 1994), incorporating the Humic Ion-Binding Model VI (Tipping, 1998). To calculate the chemical speciation, the concentrations of Na, K, Ca, Mg, Cl, NO$_3$, and SO$_4$ and concentrations

of filterable trace metals (Ni, Cu, Zn, Cd and Pb) were assumed to represent truly dissolved components (i.e. the inorganic ionic species and complexes and/or metals bound to dissolved organic matter (DOM)). The filterable fraction may also include some metal in association with mineral and organic colloids, however these species are neglected in the present analysis. The cation-binding properties of DOM were expressed in terms of isolated fulvic acid, which is thought to be the most active DOM fraction in natural waters (Vincent et al., 2001). DOM concentrations were estimated based on measured DOC, assuming DOM to be 50% carbon and that 65% of the DOM behaves like isolated FA and is thus active regarding cation binding (Tipping et al., 2008). Ionic strength effects on the inorganic reactions were taken into account using the extended Debye–Hückel equation.

2.3. Determination of invertebrate body burdens

Aquatic insect samples were taken using a pond net (April 17–19, 2006). At the laboratory each sample was carefully sorted through and the collected organisms were identified to family or genus level. Not all taxa were equally well presented in the sample collection and the current analysis is confined to the taxa that were present in more than 18 of the sampled sites, including *Leuctra* sp. (O. Plecoptera; 33 sites), Perlodidae (O. Plecoptera; 25 sites), Simuliidae (O. Diptera; 24 sites) and *Rhithrogena* sp. (O. Ephemeroptera; 18 sites). All samples were placed into 1.5 mL polypropylene sampling vials and stored at −20 °C. For each 50 sample vials with invertebrates also 5 empty vials were included to be used as process controls and reference material. Samples were dried until constant dry weight at 60 °C in a laboratory furnace. Subsequently they were weighed on a Sartorius SE2 ultra microbalance (accuracy of 0.1 μg) and transferred to acid-cleaned and pre-weighed 0.5 or 1.5 mL polypropylene vials.

Invertebrate samples were microwave digested in HNO$_3$ — hydrogen peroxide (H$_2$O$_2$; 30%) solution (3:1, v/v) by a step-wise method in which samples were microwave treated four times, each time increasing the microwave power by 10% (Blust et al., 1988). For each series of 50 samples also 5 control samples were processed and 5 samples of invertebrate reference material (mussel BCR-668) were included for quality control. After the digestion procedure the digest was diluted with ultra-pure water (Milli-Q) to obtain a solution of 5% acid and the vials were reweighed to accurately determine the final sample volume. Trace metal concentrations in invertebrate tissue were analysed using a quadrupole inductively coupled plasma mass spectrometer (ICP-MS; Varian UltraMass 700, Victoria, Australia). Results regarding invertebrate body burdens have been partly incorporated in the studies of Stockdale et al. (2010) and De Jonge et al. (2013).

2.4. Data treatment and statistical analysis

According to the FIAM, metal concentrations in aquatic organisms ([M]$_{organism}$), can be described by the activity of the free metal ion ({M^{2+}}) based on the following equations, depending on competition between metal ions and other ions (e.g. H$^+$, Na$^+$, Ca^{2+}) for biological uptake sites (Hare and Tessier, 1996, 1998; Croteau et al., 1998):

$$[M]_{organism} = F\{M^{2+}\} \tag{1}$$

$$[M]_{organism} = \frac{F\{M^{2+}\}}{(\{H^+\} + K_a)} \tag{2}$$

$$[M]_{organism} = \frac{F\{M^{2+}\}}{(1 + K_{M2}\{M2^{2+}\})} \tag{3}$$