Relating metal bioavailability to risk assessment for aquatic species:

Daliao River Watershed, China

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16 **ABSTRACT**

17 The spatial distribution of metal bioavailability (Ni, Cu, Zn, and Pb) was first
18 evaluated within the waters of Daliao River watershed, using the diffusive gradient in
19 thin films (DGT) and chemical equilibrium models. To assess potential risks associated
20 with metal bioavailability, site-specific 95% protection levels (HC5), risk
21 characterizations ratios (RCR) and ratios of DGT-labile/HC5 were derived, using
22 species sensitivity distribution (SSD). The highest bioavailability values for metals were
23 recorded in the main channel of the Daliao River, followed by the Taizi River. Dynamic
24 concentrations predicted by WHAM 7.0 and NICA-Donnan for Cu and Zn agreed well
25 with DGT results. The estuary of the Daliao River was found to have the highest risks
26 related to Ni, Cu, and Zn. The number of sites at risk increased when considering the
27 total toxicity of Ni, Cu, and Zn.

29 **Capsule abstract:**

30 The highest bioavailability values and the highest risks of metals were found in the
31 estuary of the Daliao River.

32
33 **Keywords:** DGT (diffusive gradient in thin films); Bioavailability; Chemical
34 equilibrium models; Watertype-specific risk assessment.

1. Introduction

Given that bioavailable speciation is a good proxy for the toxicity of dissolved metals (Allen and Hansen, 1996). Therefore, the European Water Framework Directive (WFD, 2000/60/EC) suggests conducting water quality assessments based on bioavailable speciation.

Different approaches can be applied in assessing metal speciation. First, the diffusive gradient in thin films (DGT) approach is an effective in-situ tool. It integrates labile metal speciation, including free metal ions, inorganic complexes, and easily dissociated organic complexes in aquatic systems (Davison and Zhang, 1994; Zhang and Davison, 2000; Zhang, 2004). Labile speciation sampled with DGT is the same as bioavailable speciation (Martin and Goldblatt, 2007). DGT is currently used to provide integrated records of metal pollution from mining activities (Martin and Goldblatt, 2007; Warnken et al., 2009; Balistrieri and Blank, 2008; Balistrieri et al., 2012) and from municipal activities (Nagai et al., 2007), and of metal pollution in estuarine systems and coastal waters (Montero et al., 2012). The second approach considered is the use of chemical models. The Windermere Humic Aqueous Model (WHAM, versions 5.0, 6.0, and 7.0) (Tipping, 1994; Tipping, 1998; Tipping et al., 2011) and the NICA-Donnan model (Milne et al., 2001) are available for predicting the binding of protons and metal ions to humic substances (including competition) quite successfully.

The European Union has conducted realistic risk assessments, accounting for bioavailability. Toxicity data (test - normalized no observed effect concentrations (NOEC) were normalized using the biotic ligand model (BLM), and HC5 (HC5 is defined as the dissolved metal concentration that protects 95% of species in an ecosystem) or predicted no effect concentrations (PNEC) were then derived with

species sensitivity distributions (SSDs) (European Copper Institute, 2008; European Communities, 2008; Van Sprang et al., 2009). To date, geographical and temporal variation of bioavailability and species sensitivity has been studied on a small scale of isolated samples, on single species (De Schamphelaere et al., 2003), on single metals (De Laender et al., 2005), and on several metals among a range of aquatic species (Verschoor et al., 2011).

The Daliao River watershed passes through important industrial cities within Liaoning Province of China, including Tieling, Shenyang, Anshan, and Panjin. High levels of contaminants have been discharged into this watershed, the average annual outputs of Cu, Pb, and Zn were 70.3×10^3 , 40.3×10^3 and 265.9×10^3 ton, respectively, during 1990 and 2006 (Jiang et al., 2013). Levels of dissolved Cu, Pb, Zn, and Cr in the Daliao River Estuary were higher in 2010 than in 2009 (Wang et al., 2011). The sediment of the Daliao River watershed was reported to pose a risk to aquatic organisms (Deng et al., 2011; Jiang et al., 2013). To our knowledge, the only study using DGT to measure labile concentrations of Cd, Co, Fe, and Mn was conducted for the pore water of the Daliao River estuary (Wu et al., 2011). A further relevant consideration is that Chinese regulators are planning to update the current water quality standards (WQS) by incorporating bioavailability of metals (Feng et al., 2012). Given that the Liao River watershed is an important river in China, the study of the relationship between water chemistry and bioavailability of metals in the system, will thus be valuable both for risk assessment and for the development of new WQS.

The aims of this study are 1) to evaluate the spatial distributions of biologically labile concentrations of metals (Ni, Cu, Zn, and Pb) within the waters of the Daliao River watershed, applying the DGT method and chemical speciation models (WHAM 7.0 and

NICA-Donnan), 2) to evaluate the performance of DGT and chemical speciation models when water chemistry parameters vary over a spatial range, 3) to assess potential risks posed by metals, linking the sensitivities of aquatic organisms to metal speciation. Our results are expected to advance the evaluation of water quality of the Daliao River watershed.

2. Materials and Methods

2.1 Description of sampling sites

The Daliao River watershed runs from the north-eastern part of Liaoning province to Liaodong Bay in Bohai Sea, and it is composed of the main channel of the Daliao River, the Hun River, and the Taizi River. Water samples were collected during September and October, 2011. The Taizi River is 413 km long, with a catchment area of 13900 km². The source of the Taizi River lies in Hongshilizi in Xinbing County, and the river flows through the cities of Benxi, Liaoyang, and Anshan. One sampling site (Wosanxian, T-1) is located at the head of the Taizi River, and two other sampling sites (Caitun Bridge, T-2, and Xingan, T-3) lie adjacent to the industrial area and to a municipal waste treatment plant. The Hun River is 415 km long, and it has its source on the northern slope of Gunma Mountain in Qinyuan County. One sampling site along the Hun River (Wandu Park, H-1) is located at the discharge location of a municipal waste treatment plant, and another site (Sujiatun, H-2) is located about 12 km downstream of Shenyang. The Xi River is 78.2 km long and runs through the Tiexi industrial area of Shenyang. It is the largest recipient of industrial effluents and domestic wastewater from Shenyang (Guo et al., 2007). One of the sampling sites on this river (X-1, Xiannvhu Park) is located upstream of the Tiexi industrial area. Three other sites, X-2 (Zhaijia), X-3

(Zhangyi Bridge), and X-4 (Huanglatuo Bridge), lie downstream of the industrial area. Sampling sites on the main channel of the Daliao River include D-1 (Heiyingtai) and D-2 (Laodong Bridge) lying upstream of Yingkou City. D-3 (Liaohe Park) is located at a dock, while D-4 (Daliao River Estuary) is located within the industrial area of Yingkou. The locations of these sampling sites are shown in detail in **Fig. 1**.

Fig. 1. Map of sampling area: sampling locations and major potential pollution sources around.

2.2 Determination of total and dissolved metal concentrations and water chemistry parameters

The target metals of this study were Ni, Cu, Zn, and Pb. Prior to sampling, fluorine resin bottles were washed using nitric acid solution (2%). Water collection and acid digestion of unfiltered and filtered samples (filtered by 0.45- μ m hydrophilic membrane of ADVANTEC) were carried out as per the EPA method 3005A (U.S. EPA, 1992). The processes involved were outlined in detail in a previous study (Han et al., 2013). Finally,

total and dissolved concentrations of metals were determined using ICP-MS (Agilent 7700x).

Water chemistry parameters such as pH, temperature, major dissolved elements (Na, Mg, K, Ca, Cl, NO₃, SO₄, and PO₄), and dissolved organic carbon (DOC) were measured using methods similar to those used in our previous study (Han et al., 2013).

2.3 Preparation of DGT unit

DGT holders, with a 20-mm window open to the solution, open pore hydrogel, and Chelex-resin were purchased from DGT Research Ltd (<http://www.dgtresearch.com>). Protective membrane filters (0.2-μm pore size) were obtained from Millipore. The DGT units were then assembled in the laboratory, and these loaded DGT units were stored in 0.1 M NaNO₃ solution until deployment.

2.4 DGT deployment

During the field survey, multiple (2-4) DGT units were deployed at each site in order to measure the labile concentration of metals. After 24 h, the DGT units were retrieved and rinsed with Milli-Q water in situ. Simultaneously, blank DGT devices were prepared and were subjected to Milli-Q water during the deployment of DGT in situ. After transport back to the laboratory, Chelex-resin was immersed in a tube with 1 mL of nitric acid solution (1.0 mol L⁻¹) for 24 h to elute metals from the resin. Finally, the metal concentrations in the nitric acid were determined using ICP-MS.

The calculation method for labile concentration, C_{labile} is the same as that used in previous studies (Davison and Zhang, 1994; Zhang and Davison, 1995). At 20°C, the diffusion coefficients, D are $5.02 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for Ni, $5.42 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for Cu, $5.29 \times$

10⁻⁶ cm² s⁻¹ for Zn, and 6.99 × 10⁻⁶ cm² s⁻¹ for Pb (<http://www.dgtresearch.com>).

2.5 Modeling

Visual MINTEQ ver. 3.0 and WHAM 7.0 incorporate various sub-models, and they simulate different binding between metals and humic substances (HS) and between colloidal oxides and metals. The equilibrium constants of metal-inorganic complexes (OH⁻, Cl⁻, SO₄²⁻, HCO₃⁻ and CO₃²⁻) provided in the Visual MINTEQ ver. 3.0 are the same as that of WHAM 7.0 (Tipping et al., 2011). However, they differ in assumptions for the binding sites of HS available for metals: WHAM 7.0 describes ion (proton and cationic metal) binding to humic substances (HS) using a structured formulation of discrete binding site types (Tipping et al., 2011), whereas the NICA-Donnan model in the Visual MINTEQ ver. 3.0 predicts ion (proton and cationic metal) binding to HS based on continuous binding site distributions (Milne et al., 2001). In this study, NICA-Donnan model and WHAM 7.0 were used to predict binding between metals with HS. Water chemistry parameters, including pH, temperature, dissolved chemical elements (Na, Mg, K, Ca, Cl, NO₃, SO₄, and PO₄), and dissolved metals (Ni, Cu, Zn, Pb, Fe, and Al) were used as input values. Carbonate was simulated during the model calculations, with the assumption that carbonate was in equilibrium with atmospheric CO₂ (the pressure of CO₂ is 3.50 × 10⁻⁴ atm). The equilibrium constants for the carbonate were from the default database of WHAM 7.0 and Visual MINTEQ ver. 3.0. Inorganic metal complexes were simulated using default databases of the Visual MINTEQ ver. 3.0 and WHAM 7.0. Colloidal aluminum oxide had little binding with trace metals (Han et al., 2013), and dissolved aluminum speciation likely competed with trace metals for binding by HS in sampling rivers (Tipping et al., 2002; Tipping 2005).

Previous study has shown the importance of colloidal Fe passing through filters and its role in binding of metals (Balistrieri and Blank, 2008). Therefore, by ignoring the formation of colloidal aluminum oxide, the activity of dissolved Al was assumed to be controlled by $\text{Al}(\text{OH})_3$ and dissolved Al could compete with other metals for binding to HS, with a solubility of $10^{8.5}$ (Tipping et al., 2002). For dissolved Fe(III), we could assume that colloidal iron oxide formed and absorbed trace metals. The activity of dissolved Fe(III) was determined from temperature (Liu and Millero, 1999), the Fe(III) hydroxide was assumed to precipitate and one mole of precipitated Fe(III) hydroxide forms 90 g of iron oxide (Dzombak and Morel, 1990). HS were assumed to be the main substances binding metals, concentrations of HS were assumed to be 1.4 times DOC for every site (Tao et al., 1988) (HS account for 70% of DOC and comprise 50% carbon), and HS were assumed to be composed of 90% fulvic acids (FA) and 10% humic acids (HA).

The metal speciation measured by DGT has been referred to as dynamic (Unsworth et al., 2006; Balistrieri and Blank, 2008). During deployment of DGT, if all metal speciation is assumed to dissociate rapidly and is measured by DGT (Unsworth et al., 2006), the maximum dynamic values, $C_{\text{dyn max}}$, can be obtained. In this study, $C_{\text{dyn max}}$ was calculated using the same method as used in some previous studies (Balistrieri and Blank, 2008; Han et al., 2013):

$$C_{\text{dyn max}} = C_{\text{free}} + C_{\text{inorg}} + \frac{C_{\text{FA}} D_{\text{FA}}}{D_{\text{inorg}}} + \frac{C_{\text{HA}} D_{\text{HA}}}{D_{\text{inorg}}} \quad (1)$$

where C_{free} is free ions, C_{inorg} is inorganic complexes, C_{FA} is metal complexes with fulvic acids, and C_{HA} is metal complexes with humic acids. These parameters were predicted using the WHAM 7.0 and NICA-Donnan models. The diffusion coefficients of inorganic speciation were assumed to be the same as those of free metals (20°C), and

diffusion coefficients of organic complexes with fulvic acids and humic acids were likewise assumed to be the same as those of fulvic and humic acids at 20°C (Zhang, 2004). The maximum dynamic metal concentrations calculated were compared with DGT-labile concentrations.

3. Results and discussion

3.1 pH, DOC, and major ions

The water chemistry parameters pH, DOC, and major ions for the study sites are summarized in **Table 1**.

There was wide variability of water parameters. pH varied from 7.47 ± 0.02 in the Hun River (H-1) to 8.64 ± 0.05 in the Xi River (X-1). DOC varied from $1.18 \pm 0.01 \text{ mg L}^{-1}$ in the Daliao River (D-3) to $6.19 \pm 0.05 \text{ mg L}^{-1}$ in the Daliao River (D-1). The highest DOC occurred at the estuary of the Daliao River, likely due to the large amount of effluent originating from upstream cities, approximately 2,074 million tons of industrial and domestic wastewater were discharged annually into the Liao River catchment (SEPA, China, 2004). Ca concentrations at both sites were $> 30 \text{ mg L}^{-1}$, the largest levels of Ca ($117 \pm 17 \text{ mg L}^{-1}$) occurred at D-3.

Table 1 Water parameters for the Daliao River watershed, as measured during September and October, 2011 (n≥3).

Rivers	Site No.	Temperature (°C)	pH	DOC (mg L ⁻¹)	K (mg L ⁻¹)	Na (mg L ⁻¹)	Mg (mg L ⁻¹)	Ca (mg L ⁻¹)	PO ₄ (mg L ⁻¹)	Cl (mg L ⁻¹)	NO ₃ (mg L ⁻¹)	SO ₄ (mg L ⁻¹)
Daliao River	D-1	20.6	8.63 ± 0.02	6.19 ± 0.05	7.23 ± 0.07	82.0 ± 0.00	21.4 ± 0.21	55.3 ± 5.94	ND	278 ± 0.00	14.2 ± 0.71	122 ± 3.39
	D-2	20.5	8.21 ± 0.03	2.56 ± 0.01	3.47 ± 0.00	30.7 ± 0.00	12.4 ± 0.00	52.3 ± 0.00	ND	45.0 ± 0.28	7.06 ± 0.01	54.9 ± 0.07
	D-3	19.8	7.72 ± 0.01	1.18 ± 0.01	14.8 ± 2.62	302 ± 2.55	49.5 ± 9.83	117 ± 15	ND	308 ± 0.00	1.05 ± 0.00	98.8 ± 0.00
	D-4	21.4	7.86 ± 0.05	3.12 ± 0.01	38.4 ± 0.47	832 ± 7.78	119 ± 7.71	94.7 ± 6.58	ND	427 ± 5.09	5.56 ± 0.00	175 ± 55.58
Xi River	X-1	20.8	8.64 ± 0.05	3.30 ± 0.04	2.58 ± 0.76	51.2 ± 0.78	20.9 ± 0.14	74.7 ± 1.34	ND	70.7 ± 0.07	5.53 ± 0.06	131 ± 0.21
	X-2	20.9	8.40 ± 0.02	5.15 ± 0.03	2.59 ± 0.12	2.00 ± 1.27	8.45 ± 0.57	50.4 ± 4.17	ND	37.6 ± 0.00	5.51 ± 0.04	68.5 ± 0.07
	X-3	19.1	8.00 ± 0.05	3.99 ± 0.01	4.90 ± 0.04	29.3 ± 0.14	11.4 ± 0.00	55.2 ± 2.33	ND	48.4 ± 0.14	6.54 ± 0.03	76.4 ± 0.28
	X-4	19.8	8.61 ± 0.01	4.41 ± 0.01	6.25 ± 0.03	37.3 ± 0.35	14.3 ± 0.14	58.6 ± 2.19	ND	63.8 ± 0.00	11.7 ± 0.05	92.6 ± 0.00
Hun River	H-1	20.7	7.47 ± 0.02	2.56 ± 0.02	8.28 ± 0.00	47.0 ± 0.00	17.4 ± 0.07	70.0 ± 0.92	0.04 ± 0.00	76.7 ± 1.06	2.65 ± 0.02	100 ± 0.00
	H-2	22.2	8.06 ± 0.04	2.33 ± 0.04	5.74 ± 0.05	35.6 ± 0.07	14.2 ± 0.14	62.6 ± 0.85	0.02 ± 0.00	57.8 ± 0.00	13.7 ± 0.00	89.6 ± 0.00
Taizi River	T-1	13.8	7.48 ± 0.05	1.21 ± 0.03	1.12 ± 0.00	4.03 ± 0.00	5.28 ± 0.00	37.0 ± 0.00	ND	2.89 ± 0.00	6.75 ± 0.04	3.00 ± 0.21
	T-2	16.8	8.32 ± 0.01	1.99 ± 0.01	2.35 ± 0.00	8.36 ± 0.00	9.39 ± 0.00	55.0 ± 0.00	ND	6.82 ± 0.04	11.9 ± 0.11	64.5 ± 0.14
	T-3	20.1	7.62 ± 0.05	2.58 ± 0.01	10.6 ± 0.07	19.9 ± 0.07	9.32 ± 0.01	58.9 ± 0.14	ND	25.2 ± 0.14	15.0 ± 0.06	113 ± 0.35

Arithmetic mean ± Standard deviation; ND – Not detected.

3.2 Total, dissolved, and labile metal concentrations

Total, dissolved, and labile metal concentrations are summarized in **Fig. 2**.

Total and dissolved Ni varied slightly across sampling sites. Total Ni ranged from $1.46 \pm 0.06 \mu\text{g L}^{-1}$ in the Taizi River (T-1) to $11.9 \pm 0.14 \mu\text{g L}^{-1}$ at the Daliao River estuary (D-4), and dissolved Ni ranged from $0.83 \pm 0.11 \mu\text{g L}^{-1}$ in the Taizi River (T-1) to $5.85 \pm 0.30 \mu\text{g L}^{-1}$ in the Hun River (H-1). In the cases of Cu, Zn, and Pb, total and dissolved concentrations varied spatially. Total Cu ranged from $2.88 \pm 0.72 \mu\text{g L}^{-1}$ in the Taizi River (T-1) to $87.32 \pm 0.01 \mu\text{g L}^{-1}$ at the Daliao River estuary (D-4), and dissolved Cu ranged from $1.77 \pm 0.47 \mu\text{g L}^{-1}$ in the Taizi River (T-2) to $23.79 \pm 1.01 \mu\text{g L}^{-1}$ at the Daliao River estuary (D-4). Total Zn ranged from $3.09 \pm 0.55 \mu\text{g L}^{-1}$ in the Taizi River (T-1) to $900 \pm 16.6 \mu\text{g L}^{-1}$ at the Daliao River estuary (D-4), and dissolved Zn ranged from $1.94 \pm 0.55 \mu\text{g L}^{-1}$ in the Taizi River (T-1) to $58.4 \pm 1.87 \mu\text{g L}^{-1}$ in the Daliao River (D-3). Total Pb ranged from $0.79 \pm 0.01 \mu\text{g L}^{-1}$ in the Taizi River (T-1) to $49.8 \mu\text{g L}^{-1}$ at the Daliao River estuary (D-4), and dissolved Pb ranged from $0.26 \pm 0.07 \mu\text{g L}^{-1}$ in the Taizi River (T-2) to $1.83 \mu\text{g L}^{-1}$ at the Daliao River estuary (D-4). Total concentrations of metals at site D-4 were higher than corresponding results at D-1, D-2, and D-3, a similar trend had been reported in a previous study (Montero et al., 2012). Total concentrations for Cu, Zn, and Pb exceeded the corresponding criterion continuous concentration (CCC) (U.S.EPA, 2004) and water quality standards (WQS) (SEPA, China, 2002) at D-4. Total Zn exceeded the corresponding CCC and WQS at D-3. Total Pb was higher than the CCC value at D-3, X-1, and T-2.

Fig. 2. Average total, dissolved, and DGT-labile concentration of selected metals and water quality criteria at sampling sites, as measured during September, 2011 and October, 2011. Dissolved concentrations were measured for samples filtered through membranes with 0.45 µm pore size and DGT-labile metal concentrations were determined using the diffusive gradients in thin films (DGT). The error bars represent the standard deviation of more than three replicate measurements. The vertical lines in each graph separate samples from different rivers. Water quality criteria, i.e. criterion continuous concentration (CCC) for Ni, Cu, Zn, and Pb, are based on federal regulations (U.S. EPA, 2004). Water quality standards (WQS) are based on regulations for current water quality standards in China (SEPA, China, 2002).

The highest total Cu, Zn, and Pb occurred at the estuary of the Daliao River (D-4), with a possible primary source of metals being effluent from upstream cities. Approximately 70.3×10^3 , 40.3×10^3 , and 265.9×10^3 tons of Cu, Pb, and Zn, respectively, were estimated to be discharged annually into the Liao River between 1990 to 2006 (Jiang et al., 2013). Moreover, high total Zn and total Pb were probably related to local industrial activities (such as ship building and galvanizing activities). Lastly, boating activities may also contribute to metal concentrations. According to Dunn et al. (2007), there is a clear relationship between the number of boats and Cu concentration

in an estuarine system, resulting from factors such as leaching of Cu used in antifouling paints (Montero et al., 2012). Several boats were observed at the Daliao River estuary. In the case of dissolved metals in the Daliao River, dissolved Cu (3.30 ± 0.01 – $23.79 \pm 1.01 \mu\text{g L}^{-1}$), dissolved Zn (15.68 ± 0.01 – $37.08 \pm 0.55 \mu\text{g L}^{-1}$), and dissolved Pb (0.59 – $1.83 \mu\text{g L}^{-1}$) were higher than the corresponding results from 2009 for Cu (0.09 – $2.90 \mu\text{g L}^{-1}$), Zn (2.09 – $5.89 \mu\text{g L}^{-1}$) and Pb (0.15 – $0.30 \mu\text{g L}^{-1}$) (Wang et al., 2011). Dissolved Zn and dissolved Pb were also higher than the corresponding results from 2010 for Zn (3.03 – $37.3 \mu\text{g L}^{-1}$) and Pb (0.26 – $1.88 \mu\text{g L}^{-1}$) (Wang et al., 2011).

In the case of the Taizi River, total and dissolved concentrations of Cu and Zn at T-3 were generally higher than those in the Hun and Xi Rivers (except for Zn at X-1). A possible pollution source is the steel smelting industry in Benxi City. In the case of the Hun River in Shenyang City, total and dissolved concentrations for Cu, Zn, and Pb at site H-1 were higher than corresponding results at H-2, with the main reason being that most of Shenyang's urban wastewater is biologically treated, with effluent subsequently discharged into the Hun River (Guo et al., 2011).

In the case of the Xi River, dissolved Cu was 1.86 ± 0.62 – $4.61 \pm 0.81 \mu\text{g L}^{-1}$, dissolved Zn was 2.54 ± 0.83 – $22.83 \pm 0.72 \mu\text{g L}^{-1}$, and dissolved Pb was 0.22 ± 0.11 – $1.52 \pm 0.06 \mu\text{g L}^{-1}$. These values were lower than results obtained by Wu et al. (2011), where dissolved Cu was 1.97 – $39.2 \mu\text{g L}^{-1}$ in 2007, and 1.4 – $25.8 \mu\text{g L}^{-1}$ in 2008, dissolved Zn was 18.3 – $65.5 \mu\text{g L}^{-1}$ in 2007, and 18.8 – $58.5 \mu\text{g L}^{-1}$ in 2008, and dissolved Pb was 0.29 – $7.4 \mu\text{g L}^{-1}$ in 2007, and 0.76 – $4.64 \mu\text{g L}^{-1}$ in 2008.

DGT-labile for Ni ranged from $0.28 \pm 0.02 \mu\text{g L}^{-1}$ (T-1) to $4.92 \pm 0.04 \mu\text{g L}^{-1}$ (D-3).

DGT-labile for Cu ranged from $0.34 \pm 0.04 \mu\text{g L}^{-1}$ (T-1) to $4.93 \pm 0.11 \mu\text{g L}^{-1}$ (D-4).

Values of DGT-labile for Zn varied from $1.51 \pm 0.32 \mu\text{g L}^{-1}$ (T-1) to $59.06 \pm 3.88 \mu\text{g L}^{-1}$

(D-3), while values of DGT-labile for Pb were $<0.84 \pm 0.17 \mu\text{g L}^{-1}$ (D-3). DGT-labile concentrations for metals in the main stream of Daliao River were generally higher than corresponding concentrations in the Taizi River (T-3).

3.3 Dissolved metal speciation

Fractions of free ions, inorganic metal complexes, and metal complexes with fulvic and humic acids are summarized in **Fig. 3**. Free ions and inorganic species for Ni and Zn accounted for most of the dissolved concentrations. From WHAM 7.0 results, free Ni and inorganic Ni were 4.4–75.2% and 20.0–94.6%, respectively, while free Zn and inorganic Zn were 15.8–73.6% and 13.4–66.7%, respectively. According to NICA-Donnan, free ions and inorganic species for Ni were 34.4–90.2% and 1.24–83.9%, and free Zn and inorganic Zn were 18.9–83.4% and 5.8–71.8%. According to WHAM 7.0, carbonate species were dominating inorganic species for Ni and Zn, carbonate Ni and carbonate Zn accounted for 67.2–99.5% of inorganic Ni, and 43.8–86.1% of inorganic Zn. NICA-Donnan showed that carbonate Ni and carbonate Zn accounted for 34.2–93.1% of inorganic Ni, and 10.9–76.5% of inorganic Zn. Similarly, Unsworth et al. (2006) reported that, carbonate Ni was dominant inorganic species in hard waters. Most dissolved Cu occurred in complexes with humic substances. In the case of Cu, fulvic and humic species accounted for 65.7–87.4% and 6.8–10.2% of dissolved Cu, as predicted by WHAM 7.0. NICA-Donnan predicted a lower fraction of Cu complexes with fulvic acids (15.1–45.7%), and a higher fraction of Cu complexes with humic acids 38.5–76.2%. Most of dissolved Pb comprised colloidal speciation with iron oxide (80.6–97.2% with WHAM 7.0 and 81.2–98.5% with NICA-Donnan).

Fig. 3. The fractions of free ions, inorganic metal species, metal complexes with fulvic acid (FA) and metal complexes with humic acid (HA), calculated using equilibrium computer programs WHAM 7.0 and NICA-Donnan for Ni, Cu, Zn, and Pb in the Daliao River watershed. Concentrations of humic substances were assumed to be 1.4 times DOC, and they consisted of 90% fulvic acid and 10% humic acid. By ignoring the formation of colloidal aluminum oxide, the activity of free iron(III) was simulated to be controlled by colloidal Fe(III) oxide, the solubility dissolved Fe(III) was determined from temperature (Liu and Millero, 1999). The Fe(III) hydroxide was assumed to precipitate, and one mole of precipitated Fe(III) hydroxide forms 90 g of iron oxide (Dzombak and Morel, 1990). The activity of dissolved Al(III) was assumed to be controlled by $\text{Al}(\text{OH})_3$ with a solubility of $10^{8.5}$ (Tipping et al., 2002).

3.4 Dynamic metal concentrations and labile metal concentrations determined by DGT

Fig. 4 presents a comparison between the calculated dynamic concentrations and DGT-labile concentrations of metals. In the cases of Ni, Cu, and Zn, there were little differences between the results predicted by different equilibrium models (WHAM 7.0 and NICA-Donnan models). For Ni and Cu, dynamic concentrations predicted by WHAM 7.0 were a little higher than those predicted by NICA-Donnan, whereas dynamic Pb predicted by NICA-Donnan was higher than that predicted by WHAM 7.0. Similarly, the dynamic concentrations were calculated using different equilibrium

models (WHAM 6.0 and NICA-Donnan models) in previous studies (Balistrieri and Blank, 2008; Unsworth et al., 2006), dynamic Ni and Cu predicted by WHAM 6.0 were higher than those predicted by NICA-Donnan, and dynamic Pb values predicted by NICA-Donnan was higher than the corresponding results predicted by WHAM 6.0.

Fig. 4. Comparison of calculated maximum dynamic metal concentrations and labile metal concentrations using the diffusive gradients in thin films (DGT) method, for Ni, Cu, Zn, and Pb in the Daliao River watershed. Dynamic metal concentrations were calculated using chemical models that incorporated different metal/organic-matter complexes models (WHAM 7.0 and NICA-Donnan).

The DGT-labile concentration for Ni ($[\text{Ni}]_{\text{DGT}}$) was lower than the dynamic concentration ($[\text{Ni}]_{\text{max dynamic}}$) at all sites ($[\text{Ni}]_{\text{max dynamic}} = 1.530[\text{Ni}]_{\text{DGT}}$ using WHAM 7.0 and $[\text{Ni}]_{\text{max dynamic}} = 1.404[\text{Ni}]_{\text{DGT}}$ using NICA-Donnan). The reason for $[\text{Ni}]_{\text{max dynamic}}$ being higher than $[\text{Ni}]_{\text{DGT}}$ was that dissociation kinetics of Ni were slower, compared to other metals (Warnken et al., 2007; Fafous et al., 2004).

For Cu, the DGT-labile concentration ($[\text{Cu}]_{\text{DGT}}$) was similar to the dynamic concentration ($[\text{Cu}]_{\text{max dynamic}}$) calculated by NICA-Donnan ($[\text{Cu}]_{\text{max dynamic}} =$

1.041[Cu]_{DGT} - 0.315). [Cu]_{max dynamic} predicted using WHAM 7.0 was also closer to
 [Cu]_{DGT} ([Cu]_{max dynamic} = 0.968[Cu]_{DGT} - 0.055), except for site D-4. In this study,
 dynamic Cu concentration calculated using NICA-Donnan and WHAM 7.0 was the
 same as DGT-labile concentration. This was the same as previous results which had
 shown that, in hard waters, dynamic Cu concentrations predicted by WHAM 6.0 and
 NICA-Donnan agreed well with each other (Unsworth et al., 2006). The DGT-labile
 concentration for Cu was lower than the dynamic concentration at D-4, a possible
 primary reason being that Cu-HA complexes likely accounted for most of Cu complexes
 with humic substances (**Fig. 3**), and the dissociation rate of Cu-HA complexes
 (dissociation of Cu-HA complexes was assumed to be the same as that of humic acids,
 and D_{HA} is $0.58 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ at 20°C) was lower than that of Cu-FA complexes
 (dissociation of Cu-FA complexes was assumed to be the same as that of fulvic acids,
 and D_{FA} is $1.12 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ at 20°C) in the diffusive layer of DGT (Zhang, 2004). If
 the fraction of Cu-HA complexes increases, dissociation of Cu complexes with humic
 substances becomes slow (Warnken et al., 2007; Warnken et al., 2009), and measured
 labile Cu is therefore lower than predicted dynamic concentration. The DGT-labile
 concentration for Zn ([Zn]_{DGT}) agreed well with the dynamic concentration ([Zn]_{max}
 dynamic) ([Zn]_{max dynamic} = 1.041[Zn]_{DGT} with WHAM 7.0 and [Zn]_{max dynamic} =
 0.974[Zn]_{DGT} with NICA-Donnan). In a previous study that, using WHAM 6.0 and
 NICA-Donnan, [Zn]_{DGT} was found to be the same as [Zn]_{max dynamic} (Balistrieri and
 Blank, 2008). [Zn]_{DGT} was equal to [Zn]_{max dynamic}, with a possible reason being that free
 ion and inorganic species accounted for large fractions of dissolved Zn (Balistrieri et al.,
 2012) (**Fig. 4**). Another possible reason was that the dissociation rate of Zn complexes
 with humic substances was very high (Warnken et al., 2007).

Predicted dynamic Pb concentrations ($[\text{Pb}]_{\text{max dynamic}}$) using NICA-Donnan and WHAM 7.0 were both lower than the DGT-labile concentration ($[\text{Pb}]_{\text{DGT}}$) ($[\text{Pb}]_{\text{max dynamic}} = 0.202[\text{Pb}]_{\text{DGT}}$ for WHAM 7.0 and $[\text{Pb}]_{\text{max dynamic}} = 0.115[\text{Pb}]_{\text{DGT}}$ for NICA-Donnan). In a previous study, $[\text{Pb}]_{\text{max dynamic}}$ predicated by WHAM 6.0 and NICA-Donnan were also lower than measured DGT-labile Pb (Balistrieri and Blank, 2008).

3.5 Watertype-specific risk assessment of species sensitivity distributions

To determine site-specific risks of Ni, Cu, and Zn, linking bioavailability speciation, we constructed SSDs based on normalized NOEC for a range of aquatic species (**Fig. 5**). The detailed processes for the normalization of NOEC and the construction of SSDs are given in **Supporting Information**. We also derived the 95% protection level (designated as “HC5”), risk characterizations ratios (RCR) (RCR is the ratio of dissolved metal concentrations over HC5), and ratios of DGT-labile/HC5 for Ni, Cu, and Zn across sampling sites (**Table 2**).

In the case of the Ni-SSD (**Fig. 5**), the most sensitive species included crustacean (*Ceriodaphnia dubia*, at 12 sampling sites), mollusks (*Lymnea stagnalis*, at 1 sampling site), and algae (*Ankistrodesmus falcatus*, at 1 sampling site). For Cu-SSD, the most sensitive species was a mollusk (*Juga plicifera*, at all sampling sites). For Zn-SSD, the most sensitive species were a fish (*Jordanella floridae*, at 1 sampling site) and several species of algae (*Chlorella* sp., at 12 sampling sites).

Table 2 shows HC5 predicted from SSDs, based on normalized NOEC for individual samples, sample-specific HC5 was 3.43–11.2 $\mu\text{g L}^{-1}$ for Cu, 4.70–14.5 $\mu\text{g L}^{-1}$ for Ni, and 12.3–42.8 $\mu\text{g L}^{-1}$ for Zn. Dissolved Ni was generally lower than HC5, whereas dissolved Cu exceeded HC5-Cu at T-3, D-3, and D-4, and dissolved Zn exceeded

HC5-Zn in the Daliao River. Seven new sites were at risk (X-1, X-2, X-4, H-1, H-2, T-1, and T-2) when combining the effects of Ni, Cu, and Zn, and total RCR values became significantly higher than single RCR values in the Daliao River. Assuming that the effects of these metals were additive, our results imply that the combined risk of Ni, Cu, and Zn to aquatic species might be significant at some sites (total RCR was above 1), although considered individually, single metal were not considered to pose a risk to aquatic species. From the ratios of DGT-labile concentrations over HC5 (DGT-labile/HC5), maximum values for Ni, Cu, and Zn occurred in the estuary of Daliao River, the values were respectively 0.75 for Ni, 0.55 for Cu, and 1.75 for Zn. For Ni and Cu, DGT-labile/HC5 was <1 at any site, this indicates that there were likely no risks to aquatic species. However, for Zn, DGT-labile/HC5 was >1 in the estuary of Daliao River (D-3 and D-4), this indicates that there was likely high risk of Zn to aquatic species. Finally, the results of RCR values and DGT-labile/HC5 likely resulted in the high risks to aquatic species in the estuary of Daliao River.

Table 2 HC5, Risk Characterization Ratios (RCR), and DGT-labile/HC5

	HC5 ($\mu\text{g L}^{-1}$)			RCR			Sum RCR	DGT-labile/HC5		
	Ni	Cu	Zn	Ni	Cu	Zn		Ni	Cu	Zn
D-1	9.27	11.2	19.8	0.45	0.75	0.99	2.11	0.19	0.14	0.88
D-2	5.18	6.04	12.3	0.57	0.55	1.28	2.39	0.15	0.17	0.78
D-3	6.58	4.65	33.7	0.81	1.36	1.73	4.63	0.75	0.33	1.75
D-4	11.1	9.04	35.7	0.28	2.63	1.04	4.35	0.25	0.55	1.08
X-1	6.21	4.18	27.8	0.45	0.45	0.82	1.44	0.14	0.16	0.45
X-2	8.08	4.53	42.8	0.44	0.69	0.20	1.20	0.31	0.36	0.16
X-3	9.39	9.58	36.2	0.47	0.34	0.07	0.78	0.16	0.06	0.05
X-4	6.74	5.74	24.2	0.64	0.80	0.16	1.24	0.15	0.15	0.17
H-1	14.5	5.76	24.0	0.40	0.45	0.65	1.56	0.18	0.18	0.49
H-2	7.98	3.43	22.0	0.64	0.75	0.28	1.84	0.34	0.19	0.25
T-1	12.6	3.73	44.9	0.07	0.78	0.04	1.06	0.04	0.36	0.03
T-2	4.70	5.11	27.1	0.46	0.35	0.19	1.09	0.34	0.07	0.17
T-3	10.3	6.42	29.1	0.32	1.42	0.66	2.08	0.20	0.32	0.71

RCR is a ratio of dissolved metal concentration over HC5. If RCR is above 1 (indicated in bold letters), risk to aquatic organisms is indicate.

Fig. 5. SSD for Ni, Cu, and Zn based on normalized NOEC at sites within the Daliao River watershed. Colors indicate different species groups. Species sensitivity distribution (SSD) is a cumulative normal distribution (often a log-normal distribution) of no observed effect concentrations (NOEC) for various species, and it describes the variation in sensitivity of species to metals. Using the biotic ligand model (BLM), NOEC were normalized from test-NOEC in chronic toxicity experiments, and were used for the construction of the SSD.

4. Conclusions

In this work, spatial distributions of metal bioavailability were evaluated, using the DGT method and chemical equilibrium models (WHAM 7.0 and NICA-Donnan). Risk variations associated with site-specific bioavailability were also predicted. Results demonstrated that bioavailability assessment and site-specific risk assessment could provide for an integrated evaluation of water quality within the Daliao River watershed.

Bioavailability levels of Cu, Zn, and Pb were highest in the waters of the Daliao River. The dynamic concentrations predicted by WHAM 7.0 and NICA-Donnan for Cu, Zn agreed well with DGT results, dynamic concentration for Ni was higher than DGT results, and dynamic concentration for Pb was lower than DGT results, because most of dissolved Pb presented as colloidal speciation with iron oxide.

Site-specific risk assessment using the SSD approach links the sensitivities of a wide range of aquatic organisms with metal speciation. When metals were considered in isolation, the maximum risk (dissolved metals over HC5) for Ni, Cu, and Zn occurred in the estuary of the Daliao River, with values being 0.81, 2.63, and 1.73, respectively. Seven additional sites were found to be at risk when RCR of Ni, Cu, and Zn were assumed to be additive. The maximum ratios of DGT-labile concentrations over HC5 for Ni, Cu, and Zn also occurred in the estuary of Daliao River (the values were respectively 0.75 for Ni, 0.55 for Cu, and 1.75 for Zn). Both bioavailability assessment and site-specific risk assessment for metals were in accordance with the characteristics of water quality. Of the rivers studied, the Daliao River was likely found to be at risk.

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