



Metal Removal by a Free Surface Constructed Wetland and Prediction of Metal Bioavailability and Toxicity with Diffusive Gradients in Thin Films (DGT) and Biotic Ligand Model (BLM)

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Abstract

The H-02 constructed wetland is a free water surface wetland to remove copper (Cu) and zinc (Zn) from the industrial wastewater. In this study, we evaluated the performance of the wetland from 2018 to 2019 and coupled the diffusive gradients in thin films (DGTs) and biotic ligand model (BLM) to explore metal speciation and bioavailability in wetland waters. Surface water samples were collected and piston DGTs were deployed in different sites of the wetland. The H-02 wetland functioned well during the sampling period with high removal efficiencies (Cu: $73.8 \pm 1.2\%$ and Zn: $75.2 \pm 16.0\%$). In our study, with the assumption that the combination of BLM predicted inorganic metals species, BLM Cu(II) and BLM Zn(II), were the bioavailable and toxic species, DGT-Cu did not correlate to BLM Cu(II) ($P = 0.47$), but DGT-Zn positively correlated to BLM Zn(II) ($R^2 = 0.35$, $P < 0.001$). Compared to the modeling results of BLM, DGT-indicated labile and/or bioavailable Cu included not only free Cu ions and inorganic Cu complexes but also a high percentage of Cu-labile organic matter complexes. DGT-indicated Zn included free Zn ion, inorganic Zn, and only a low percentage of Zn-labile organic matter complexes. Our findings illustrated the appropriate use of passive sampling techniques and geological modeling when biomonitoring could be substituted. The close monitoring of metal concentrations, speciation, and bioavailability helps us understand metal biogeochemistry and metal removal processes and ensure the long-term sustainability of the constructed wetland.

Keywords Constructed wetland · DGT · BLM · Metal speciation · Bioavailability

Introduction

Constructed wetlands are widely used to remove heavy metals from the wastewater in recent years because of the low cost, easy maintenance, and high metal removal efficiency (Batool and Saleh 2020; Kivaisi 2001; Vymazal 2010). Different from the natural wetlands, constructed wetlands are optionally designed to utilize soil, plants, and associated microorganisms to treat contaminants in the wastewater (Halverson 2004; Kivaisi 2001; Vymazal 2010). There are two types of

constructed wetlands based on the hydrology: free water surface (FWS) wetlands and subsurface flow wetlands (Vymazal and Kröpfelová 2008). The FWS wetland is the most widely used constructed wetlands due to its low cost and high removal efficiency of contaminants in various wastewater, such as chromium, nickel, and zinc (Maine et al. 2007; Vymazal 2010). The FWS wetland generally consists of a soil bottom, the vegetation, and a water surface above the substrate (Halverson 2004; Vymazal 2010). The function and removal efficiency of FWS wetlands are controlled by multiple processes, including sedimentation, chemical precipitation, adsorption, microbial degradation, and uptake by plants (Kadlec 2009; Watson et al. 1989).

Metal speciation shows a large effect on the metal bioavailability in the water (Morel and Hering 1993). Besides free metal ions and inorganic metal species (Spry and Wood 1985; Vink 2009), complexes between metal and labile organic matter are also bioavailable through directly acting on the biological membrane (Ahlf et al. 2009; Ferreira et al. 2008) or decomposing and releasing free metal ions (Ahlf et al. 2009;

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Worms et al. 2006). Xu and Mills (2018) found out the formation of metal-organic matter complexes in a metal treatment FWS wetland during the cold months of a year, especially the formation of metal-fulvic acid complexes, increased the overall metal bioavailability. Also, elevated metal bioavailability increases the risk of metal exposure to the surrounding ecosystem (Kadlec 2009). Therefore, it is essential to explore metal speciation in the FWS wetland to understand metal bioavailability and bioaccumulation and estimate its environmental impacts.

Passive sampling techniques and geological modeling are always adopted among many approaches that study metal speciation. Diffusive gradients in thin films (DGT) is one of the most widespread passive samplers (Buzier et al. 2006; Yapici et al. 2008; Zhang and Davison 1995; Zhang and Davison 2015). A DGT device mimics the biological membrane, composed of a filter membrane, a diffusive gel, and an ion-exchange resin gel assembled in the housing (Zhang and Davison 1995, 2000). Free ions, inorganic metal complexes, and some metal-labile organic matter complexes can be measured by the DGT; some large-sized colloidal and particulate metals are excluded because of size restrictions of the diffusive gel (Philipps et al. 2019; Twiss and Moffett 2002; Zhang and Davison 2000). A series of studies validated the usefulness of DGT as the substitute for biomonitoring by exploring correlations between metal concentrations indicated by DGT and accumulated by organisms (Oporto et al. 2009; Philipps et al. 2019; Zhang et al. 2001). So far, DGT has been found to be an excellent prediction of the bioavailability of many metals, such as cadmium (Pérez and Anderson 2009), mercury (Gao et al. 2014), copper (Cu) (Zhang et al. 2001), lead (Philipps et al. 2019), zinc (Zn) (Cornu and Denaix 2006), though not for all elements (Amato et al. 2016).

The biotic ligand model (BLM) is one of the most well-known geochemical models relative to metal speciation and toxicity. BLM incorporates the competition of free metal ions with other cations (e.g., Ca^{2+} , Na^+ , Mg^{2+} , H^+), and meanwhile considers the influence of water chemistry (e.g., dissolved organic matter, chloride, carbonates, sulfate) on the metal speciation in the solution (Di Toro et al. 2001; Niyogi and Wood 2004; Santore et al. 2001; Santore et al. 2002). Based on the requirement of water quality parameters as input, BLM has developed different bioavailability approaches, including full or complex BLM, user-friendly BLM-based bioavailability tools, and simplified bioavailability approaches to help to quantify the species of metal in the water (Rüdel et al. 2015). In a critical review, Slaveykova and Wilkinson (2005) supported that the BLM is a useful tool for predicting the bioavailability of metals and metal complexes to aquatic biota. The application of BLM makes us further analyze the metal speciation, improving our understanding of the complicated metal biogeochemical processes in the environment (Philipps et al. 2018; Xu et al. 2019).

Recently, more and more studies apply a combination of DGT and chemical equilibrium models, e.g., ligand-exchange/DPCSV, Windermere Humic Aqueous Model (WHAM), NICA-Donnan, Stockholm Humic Model (SHM), to research the specific speciation and bioavailability of metal in the water (Balistreri and Blank 2008; Han et al. 2013; Meylan et al. 2004; Odzak et al. 2002; Yapici et al. 2008).

In this study, we combined DGT and BLM to study the speciation and bioavailability of metals in the water of the FWS wetland. Water quality parameters and metal concentrations in the water in different wetland sites were measured monthly from 2018 to 2019, coupling with DGT deployment and speciation modeling. Labile metal concentrations were indicated by DGT and metal speciation was predicted by BLM. The objectives of this study are to understand the influence of water chemistry on metal bioavailability, and to predict metal speciation and bioavailability DGT and BLM.

Materials and Methods

Sampling Sites

The H-02 wetland is an FWS wetland, composed of five source pipes, one retention basin, two wetland treatment cells, one effluent pool, and one effluent stream (Supplementary Fig. 1). Water entered and exited the wetland cells were defined as the influent and effluent. The treatment cells are half-acre, rectangular, and consist of an impermeable clay layer, 46–61 cm of soil layer, main macrophyte, *Schoenoplectus californicus*, and many other emergent and submerged algae at certain location. Water depth is about 30 m but varies slightly with location and season. The average residence time of water in each wetland cell is about 48 h (Bach et al. 2008). After being treated by the wetland cells, the water is mixed at the effluent pool and released into a stream connected to the regulatory Upper Three Runs (Xu and Mills 2018). The detailed engineer design of the H-02 wetland was illustrated by Xu and Mills (2018) and Xu et al. (2019). The influent culvert, wetland cells, effluent pool, and stream were selected as sampling sites.

Water Sampling

Surface water samples were collected per site, and water quality parameters were measured monthly from January 2018 to December 2019. The first water sample was used to measure temperature, pH, and oxidation-reduction potential in situ (ORP, Oakton pH 6+ and ORPTestr 10; Vernon Hills, IL, USA). The second sample was collected in 50 mL metal-free vials (VWR International; Radnor, PA) for measurement of metals (Cu and Zn) and anions (chloride and sulfate). The third sample was collected in a 40 mL

borosilicate glass vial (Fisher Scientific; Pittsburgh, PA) to measure dissolved organic carbon (DOC). The last water sample was collected in a 1000 mL HDPE bottle (Nalgene Nunc International Corporation; Rochester, NY) for measurement of alkalinity. Sample storage, transportation, and the process can be found in Xu and Mill's (2018) study (Xu and Mills 2018). Sterile syringe filters with 0.45 µm pore size (Millipore Corporation; Billerica, MA) were used for the preparation of dissolve metal determination and DOC measurement. Field blank samples were prepared for each sampling occasion: containers filled with Milli-Q water (Thermo Scientific, USA) were taken to the field and processed like the other samples.

DGT Deployment and Analysis

The DGTs were purchased from the DGT Research LTD (Lancaster, UK) and deployed monthly at the same sites where water samples were collected. Due to the limitations of experimental conditions, DGTs were only deployed for one year from June 2018 to May 2019. Each DGT was composed of a polyethersulphone filter membrane, an agarose crosslinked polyacrylamide diffusive gel, and a binding layer Chelex (Zhang and Davison 2000). DGTs were placed on the plastic holder with a 2 cm diameter window in surface water for four days. The deployment and retrieval time were recorded accurately to the second. After retrieval, each DGT was separated from the plastic holder, rinsed with Milli-Q water, transported back to the lab on ice, and stored in a clean plastic bag in the refrigerator (4 °C) before the process. The resin layer was separated and extracted with 1 mL 1 M HNO₃ for 24 h. The extracted solution was then diluted with Milli-Q water and analyzed. The mass of metal accumulated in the resin gel layer (*M*, ng) was calculated using Eq. (1) (Zhang and Davison 2000):

$$M = \frac{C_e * (V_{HNO_3} + V_{gel})}{f_e} \quad (1)$$

where *C_e* means the concentration of metals (Cu and Zn) in 1 M HNO₃ solution, *V_{HNO₃}* is the amount of nitric acid (1 mL), *V_{gel}* is the volume of resin (0.16 mL), and *f_e* is the elution efficiency of 0.8 (Devillers et al. 2017). Because the number of metals accumulated in the resin was assumed to be equivalent to the number of metals passing through the diffusive layer, the time-averaged metal concentrations in the environmental media *C_{DGT}* can be calculated with Eq. (2) based on Fick's first law of diffusion:

$$C_{DGT} = \frac{M * \Delta g}{D * t * A} \quad (2)$$

where *M* is the mass of metals (Cu, Zn, ng), *Δg* is the thickness of the diffusive gel and filter membrane (0.093 cm)

(Zhang and Davison 2000), *D* is the diffusion coefficient for each metal in the diffusion layer at the corresponding water temperature (cm²/s), *t* is the deployment duration (s), and *A* is the exposed area of the DGT (3.14 cm²).

Chemical Analysis

Alkalinity (expressed as CaCO₃ m/L) was determined with a Test Kit (Hach; Loveland, CO, USA). Concentrations of major anions and DOC were analyzed by the Center of Applied Isotope Studies at the University of Georgia (Athens, GA). Anions (sulfate and chloride) were measured with Dionex Ion Chromatograph (Thermo Scientific; West Columbia, SC, USA). DOC concentrations were determined by the high-temperature combustion Total Carbon Analyzer-500 (Shimadzu Inc.; Durham, NC, USA). The percent recoveries of carbon standard (SPEX CertiPrep; Metuchen, NJ, USA) was 103 ± 5% (*n* = 15). and of Dionex 7 Anion Standard II (Thermo Fisher Scientific; West Columbia, SC, USA) were 102 ± 5% for sulfate (*n* = 15) and 103 ± 13% for chloride (*n* = 15).

Metal concentrations, including total metal concentrations (TCu and TZn), dissolved metal concentrations (DCu and DZn), and metal concentrations in DGT resin extracts were analyzed by the Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES, Optima 4300 DV, Perkin-Elmer) at the Savannah River Ecology Laboratory (Aiken, SC). Copper was analyzed using a wavelength of 324.752 nm and Zn using 213.857 nm. Standard curves were established with a standard reference material ICP-200.7-6 (High-Purity Standards, SC, USA). Blank, duplicates, standard reference material (SRM), and spiked samples were included to verify the precision and accuracy of the analytical system. The method detection limit was 0.06 ng/L for Cu and 0.3 ng/L for Zn. Relative percent difference of duplicate samples averaged at 3.5 ± 4.0% (*n* = 18) for Cu and 6.6 ± 10.4% (*n* = 18) for Zn. The percent recoveries of SRM 1640a (trace elements in natural water, Sigma, USA) were 98.2 ± 8.8% (*n* = 12) for Cu and 97.3 ± 8.3% (*n* = 12) for Zn. The recoveries of spiked samples (High-Purity Standards; Charleston, SC, USA) were 103.7 ± 8.7% (*n* = 19) for Cu and 103.7 ± 7.3% (*n* = 19) for Zn.

Biotic Ligand Model

In this study, BLM 3.16 (Windward Environmental LLC; Seattle, WA, USA) was adopted to calculate the chemical speciation of metals in the water. Water quality parameters, including temperature, pH, dissolved metal concentrations (DCu and DZn), alkalinity, and DOC, were included to run speciation function in BLM. Dissolved metal concentrations were used as this study focused on metal bioavailability and speciation in the water (Xu et al. 2019). The BLM-predicted

Table 1 Water quality parameters and metal concentrations in the water of each sampling site averaged for all sampling occasions. Each value was presented with mean \pm SD ($n = 24$)

Site	Influent	Cell 1	Cell 2	Effluent	Stream
Temperature ($^{\circ}$ C)	22.1 \pm 7.9	19.8 \pm 7.5	20.2 \pm 8.0	19.6 \pm 7.6	18.5 \pm 7.2
pH	8.8 \pm 0.8	6.6 \pm 0.3	6.5 \pm 0.3	6.8 \pm 0.3	7.3 \pm 0.4
ORP (mV)	76.1 \pm 46.8	91.5 \pm 54.8	106.0 \pm 42.5	106.0 \pm 38.1	128.7 \pm 37.2
Alkalinity (mg/L CaCO ₃)	51.4 \pm 15.5	52.8 \pm 14.6	52.5 \pm 14.6	53.2 \pm 15.1	51.7 \pm 16.4
DOC (mg/L)	2.5 \pm 1.3	2.9 \pm 1.4	3.4 \pm 1.8	2.9 \pm 1.4	2.7 \pm 1.2
Chloride (mg/L)	3.5 \pm 1.6	2.9 \pm 1.6	3.0 \pm 1.3	3.0 \pm 1.6	4.0 \pm 1.7
Sulfate (mg/L)	2.3 \pm 1.1	2.3 \pm 1.2	2.1 \pm 1.1	2.1 \pm 1.1	2.0 \pm 1.2
TCu (μ g/L)	20.1 \pm 6.9	5.2 \pm 2.1	6.8 \pm 3.9	5.3 \pm 1.9	5.5 \pm 1.9
DCu (μ g/L)	13.6 \pm 3.1	4.0 \pm 1.7	4.7 \pm 2.8	4.4 \pm 1.7	4.5 \pm 1.2
TZn (μ g/L)	45.4 \pm 30.1	9.2 \pm 5.9	15.3 \pm 14.8	8.6 \pm 3.6	8.2 \pm 3.6
DZn (μ g/L)	20.2 \pm 10.1	6.8 \pm 3.4	7.6 \pm 7.3	6.6 \pm 3.3	5.0 \pm 2.3
%DCu (%)	67.2 \pm 16.7	76.0 \pm 17.9	76.9 \pm 23.4	81.8 \pm 13.9	79.0 \pm 12.1
%DZn (%)	34.9 \pm 15.2	47.9 \pm 21.1	35.5 \pm 22.0	52.4 \pm 21.9	52.3 \pm 17.2

Note: *ORP* oxidation-reduction potential, *DOC* dissolved organic carbon, *TCu/TZn* total copper/zinc concentration, *DCu/DZn* dissolved copper/zinc concentration, %DCu and %DZn indicate the percentage of DCu and DZn in TCu and TZn, respectively

metal speciation included free metal ions (Cu²⁺ and Zn²⁺), inorganic metals (Cu_{inorg} and Zn_{inorg}, the sum of all inorganic complexes), and metal-DOC complexes (Cu-DOC and Zn-DOC). In this study, free ions and inorganic complexes were combined and defined as BLM-indicated inorganic metal species (BLM-Cu(II) and BLM-Zn(II)).

Statistical Analysis

All data in this study was expressed with mean, standard deviation (SD), and sample size (n). All tables and figures were generated by Excel 2010 (Microsoft Corporation, USA) and Origin 9.7.0.188 (Origin Lab Corporation, USA). The method of comparison of means was *t* Test at a 5% significance level. Equality of variance and normality was assessed prior to the use of the *t* Test. Linear regression modeling was employed to evaluate the correlation of dissolved metal concentrations versus DGT-indicated metal concentrations or BLM-predicted inorganic metal concentrations, and DGT-indicated metal concentrations versus BLM-predicted metal concentrations. The metal removal efficiencies of the H-02 constructed wetland were calculated by Eqs. (3) and (4) (Xu et al. 2019):

$$\text{Cu removal efficiency (\%)} = \frac{([TCu_{Influent}] - [TCu_{Effluent}]) * 100}{[TCu_{Influent}]} \quad (3)$$

$$\text{Zn removal efficiency (\%)} = \frac{([TZn_{Influent}] - [TZn_{Effluent}]) * 100}{[TZn_{Influent}]} \quad (4)$$

Results

Water Chemistry

All water quality parameters averaged for sampling occasions, including temperature, pH, ORP, alkalinity, DOC, chloride, and sulfate in each sampling site were shown in Table 1 and Supplementary Fig. 2. The pH values changed with sampling sites: pH in the influent (8.8 \pm 0.8) was highest among all sites ($P < 0.001$) and decreased dramatically in cell 1 (6.6 \pm 0.3) and cell 2 (6.5 \pm 0.3). From influent to stream, the average ORP increased from 76.1 mV to 128.7 mV ($P = 0.002$). The other water quality parameters, temperature, alkalinity, DOC, chloride, and sulfate did not indicate any difference among sites ($P > 0.05$). The temporal change of water quality parameters was shown in Supplementary Fig. 3. Temperature and DOC concentration showed similar and noticeable fluctuation with time, which increased in the warm season (April to September) and decreased in the cold season (October to next March). Sulfate concentrations were relatively high from January to June of 2018 (2.9–4.9 mg/L) and suddenly decreased by almost 90% in the following two months (July and August 2018); sulfate concentrations then slowly increased (1.3–2.5 mg/L) in the rest months of 2018 and 2019. In addition, it was noted that sulfate concentrations in the stream water collected from May of 2019 presented a surprisingly low value, which was not discovered in other sites.

Metal Concentration in the Water

Metal concentrations (TCu, DCu, TZn, and DZn) and the percentages of dissolved metal in total metal (%DCu and

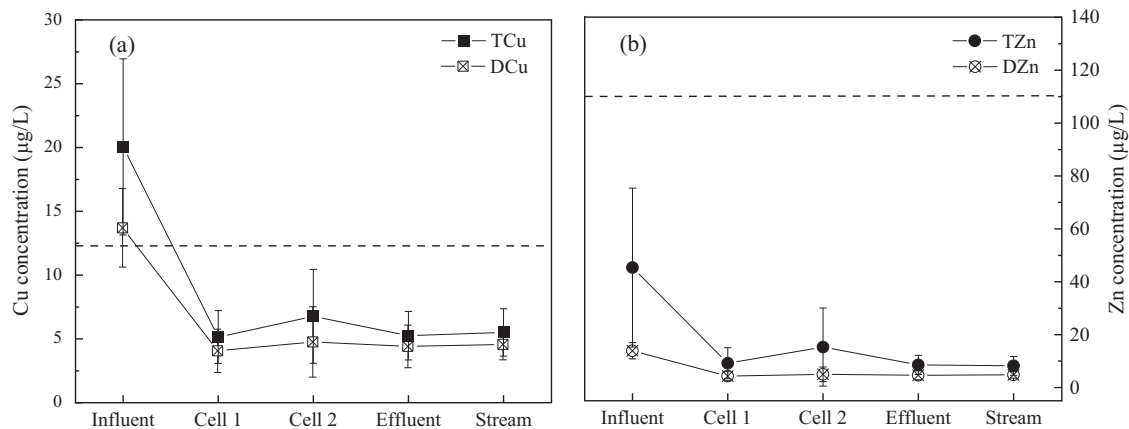


Fig. 1 Total and dissolved Cu (a) and Zn (b) concentration in the water of each sampling site averaged for all sampling occasions. Broken lines indicate the National Pollutant Discharge Elimination System

limit (12.3 µg/L of Cu and 110 µg/L of Zn). The circles and error bars indicate means and SD

Table 2 Metal concentrations (µg/L) of different species in the water of each sampling site averaged for sampling occasions from June 2018 to May 2019. Each value was presented with mean and SD ($n = 12$)

Speciation	Influent		Cell 1		Cell 2		Effluent		Stream	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
DGT DGT-Cu	2.2	1.9	0.5	0.2	0.4	0.2	0.7	0.3	0.8	0.4
DGT DGT-Zn	12.5	9.1	4.6	2.6	4.3	3.1	5.7	3.4	3.9	1.8
BLM Cu-DOC	13.2	2.0	3.4	1.1	4.2	2.0	4.0	1.3	4.1	0.9
Cu _{inorg}	3.7E-1	3.7E-1	3.8E-3	1.7E-3	4.5E-3	2.9E-3	4.4E-3	2.1E-3	5.8E-3	3.6E-3
Cu ²⁺	2.8E-3	3.3E-3	9.6E-3	6.7E-3	1.2E-2	7.5E-3	7.3E-3	4.2E-3	3.4E-3	1.5E-3
Zn-DOC	15.2	10.8	2.6	1.4	3.5	3.6	3.6	2.6	3.8	1.9
Zn _{inorg}	5.0	4.5	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.1
Zn ²⁺	2.7	3.0	2.9	1.5	3.5	2.6	3.0	2.0	1.6	0.8

%DZn) were shown in Table 1, Fig. 1, Table S1. Total concentrations in the influent were the highest (TCu: 20.1 ± 6.9 µg/L, $P < 0.001$; TZn: 45.4 ± 30.1 µg/L, $P < 0.001$) among all sites. After flowing through the treatment cells, total concentrations in the effluent were much lower (TCu: 5.3 ± 1.9 µg/L, $P < 0.001$; TZn: 8.6 ± 3.6 µg/L, $P < 0.001$). Dissolved metal concentrations showed similar trends as total metals and decreased notably after flowing through the wetland treatment cells. DCu accounted for most TCu with the average %DCu of $74.9 \pm 17.6\%$ for all water samples. However, DZn only accounted for $43.8 \pm 20.5\%$ in TZn. The temporal change of metal concentrations in each sampling site was shown in Fig. S5. The fluctuations of both total and dissolved metal concentrations in the influent were higher than those in other sites. The temporal changes of dissolved metal concentrations in all sample sites did not correspond to those of the total metal concentrations.

The removal efficiencies of Cu ($73.8 \pm 10.2\%$) and Zn ($75.2 \pm 16.0\%$) calculated by Eqs. (3) and (4) were relatively consistent in 2018 and 2019 (Fig. S3 and Table S1).

DGT-indicated Metal Concentrations

DGT-indicated Cu and Zn concentrations (DGT-Cu and DGT-Zn) in the water of each sampling site were shown in Table 2 and Table S2. The highest values of DGT-Cu (2.2 ± 1.9 µg/L, $P < 0.001$) and DGT-Zn (12.5 ± 9.1 µg/L, $p < 0.001$) were observed in the influent, which were almost 2 to 3 times higher compared to other sites. The percentages of DGT-Cu and DGT-Zn in dissolved metal (%DGT-Cu and %DGT-Zn) were shown in Fig. 3a, c, respectively. The %DGT-Zn ($71.0 \pm 20.9\%$) was generally higher than those of %DGT-Cu ($15.5 \pm 8.1\%$) for all sampling sites ($P < 0.001$). There were no statistical differences of %DGT-Cu among sites ($P = 0.13$), except for the cell 2 and the effluent ($P = 0.02$). The %DGT-Zn of the influent was lower than the others ($P < 0.05$). The temporal changes of DGT-indicated in all sample sites did not show noticeable seasonal variation (Fig. S5). Linear regression indicated that DGT-Zn and DZn showed a strong positive relationship ($y = 0.55x + 0.99$, $R^2 = 0.80$, $P < 0.001$), while DGT-Cu and DCu showed a relatively weak relationship ($y = 0.17x - 0.12$, $R^2 = 0.45$, $P < 0.001$, Fig. 2).

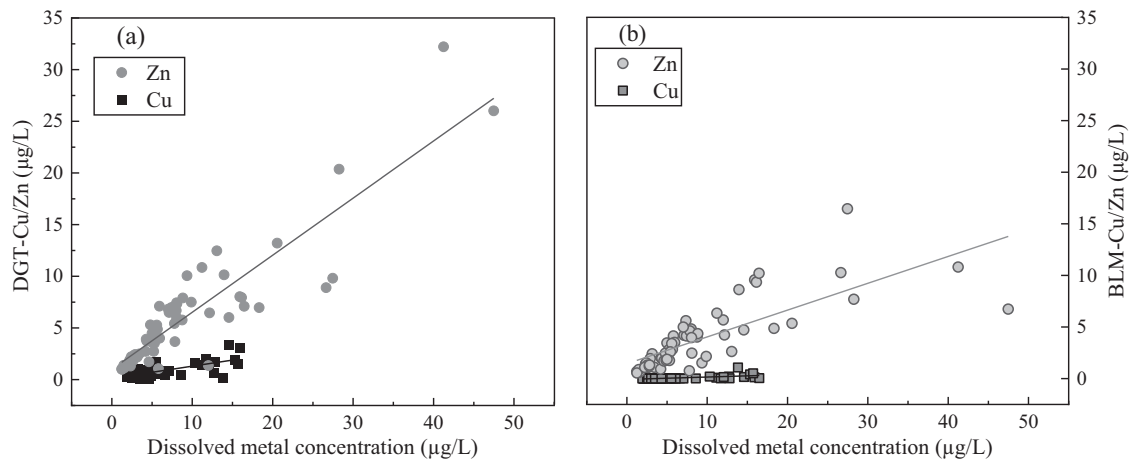


Fig. 2 Linear regressions between dissolved metal concentration and DGT-indicated metal concentration (a) or BLM-predicted inorganic metal concentration (b) in all water samples. For DGT-Zn vs. DZn (a): $y = 0.55x + 0.99$, $R^2 = 0.80$, $P < 0.001$; DGT-Cu vs. DCu (a):

$y = 0.17x - 0.12$, $R^2 = 0.45$, $P < 0.001$; BLM-Cu(II) vs. DCu (b): $y = 0.26x + 1.44$, $P = 0.11$; BLM-Zn(II) vs. DZn (b): $y = 0.26x + 1.44$, $R^2 = 0.57$, $P < 0.001$

BLM-predicted Metal Speciation

The BLM-predicted concentrations of free metal ions, inorganic metal complexes, and metal-DOC complexes were presented in Table 2. For each sampling site, the Cu-DOC concentration ($13.2 \pm 2.0 \mu\text{g/L}$) was highest among all Cu species ($P < 0.001$). The Zn-DOC concentrations ($15.2 \pm 10.8 \mu\text{g/L}$) were higher than other Zn species in the influent ($P < 0.001$). However, the values of Zn-DOC and Zn^{2+} were similar in the treatment cells, effluent, and stream ($P = 0.16$). The percentage of each BLM-predicted metal species in dissolved metals was shown in Fig. 3b, d. The percentage of Cu-DOC in DCu was as high as $99.4 \pm 1.1\%$, while BLM-Cu(II) only accounted for $0.6 \pm 1.1\%$ of DCu (Fig. 3b). The percentage of BLM-predicted Zn speciation in DZn followed the trend of Zn-DOC $>$ Zn_{inorg} $>$ Zn²⁺ in the influent ($P < 0.001$, Fig. 3d). The percentage of Zn_{inorg} obviously decreased from the influent to the treatment cells, and an increase was observed in the percentage of Zn²⁺ in the treatment cells. Except for the influent, both Zn-DOC and Zn²⁺ were the major species with an obviously higher percentage than that of Zn_{inorg} ($P < 0.001$). There was no relationship between BLM-Cu(II) and DCu ($P = 0.11$); however, the BLM-Zn(II) indicated a strong and positive correlation with DZn ($y = 0.26x + 1.44$, $R^2 = 0.57$, $P < 0.001$, Fig. 2).

Linear regressions between DGT-indicated metal concentration and BLM-predicted metal concentration were shown in Fig. 4. Though BLM-Cu(II) were not related to DGT-Cu ($P = 0.47$), while a significant linear relationship was found between BLM-Zn(II) and DGT-Zn ($R^2 = 0.35$, $P < 0.001$).

Discussions

The Wetland Function

The function of the FWS wetland is determined by the major chemical, physical, and biological processes, mostly depending on the pH, temperature, DOC, ORP, and sulfur dynamics in the water. Generally, vegetation plays a fundamental role in the wetland treatment system (Batool and Saleh 2020). Giant bulrush (*Schoenoplectus californicus*) was planted in the H-02 wetland cells to serve as the primary carbon source for the ecosystem because of its low metal accumulation (Bach et al. 2008; Murray-Gulde et al. 2005).

Wetland treatment cells 1 and 2 were the main constructions of the H-02 wetland system, where heavy metals were removed from the surface water. During the sampling period, total metal concentrations in the wastewater decreased after being processed by the treatment cells (Fig. 1), both total and dissolved metal concentrations in the wetland cells were significantly lower than those in the influent. Metal concentrations in the effluent and stream were lower than the National Pollutant Discharge Elimination System limit ($12.3 \mu\text{g/L}$ for Cu and $110 \mu\text{g/L}$ for Zn) (Kadlec and Wallace 2008). Metal removal efficiencies of H-02 wetland were relatively high with the averages of $73.8 \pm 10.2\%$ for Cu and $75.2 \pm 16.0\%$ for Zn (Fig. S3), keeping a consistent level from 2018 to 2019 and were close to the results from 2008 to 2017 (63.8% for Cu and 70.5% for Zn) (Xu et al. 2019). Our results were in the range of removal efficiencies of other constructed wetlands, which were 48–80% of Cu and 55–88% of Zn (Ayaz et al. 2020; Crites et al. 1997; Hadad et al. 2006; Khan et al. 2009).

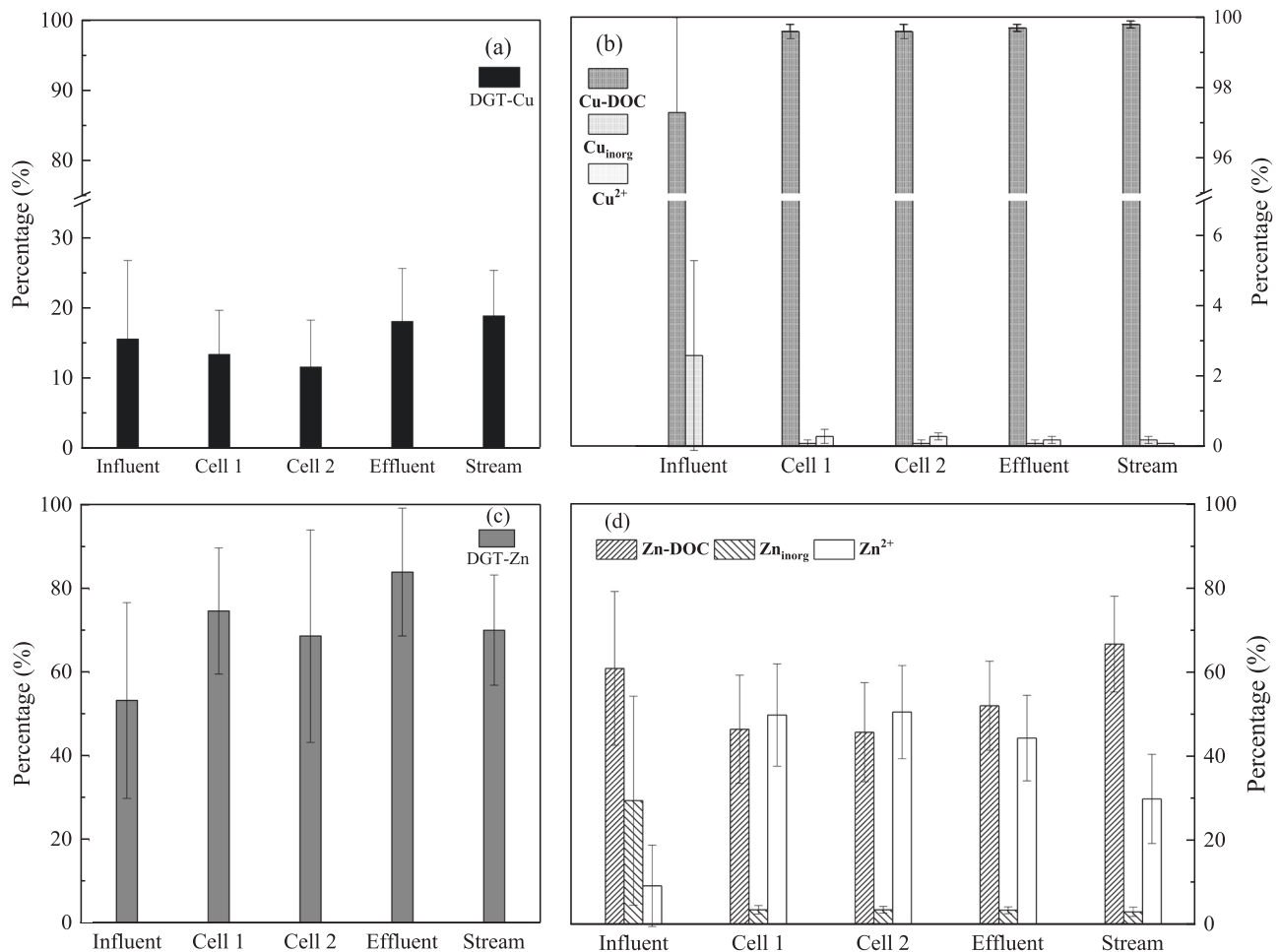


Fig. 3 Percentages of DGT-indicated (a and c) and BLM-predicted (b and d) metals in dissolved metals in the water of each sampling site. Each value was presented with mean and SD ($n = 12$)

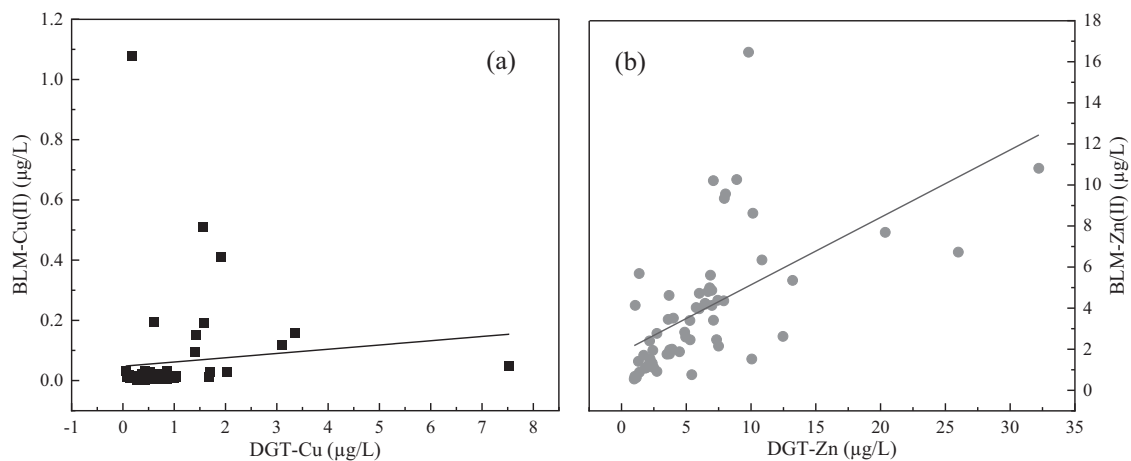


Fig. 4 Linear regressions between DGT-indicated metal concentrations (DGT-Cu and DGT-Zn) and BLM-predicted inorganic metal concentrations (BLM-Cu(II) and BLM-Zn(II)) in all sampling sites.

For DGT-Cu vs. BLM-Cu(II) (a): $y = 0.01x + 0.05$, $P = 0.47$; DGT-Zn vs. BLM-Zn(II) (b): $y = 0.33x + 1.85$, $R^2 = 0.35$, $P < 0.001$

Totally, the H-02 constructed wetland still functions well and maintains high metal removal efficiency. A three-stage performance pattern of the FWS metal-removing wetland

was hypothesized by Xu et al. (2019), which includes the plateau stage, the trough stage, and the recovery stage. The removal efficiency derived from this study indicated

that the current performance of the H-02 wetland was in the second plateau stage (Xu et al. 2019).

Xu and Mills (2018) found that the metal removal processes were primarily related to sulfur cycling in the treatment cells of H-02 wetland. Sulfate reduction dominated the sulfur cycling during the warm months of a year (February to August), and the produced sulfide caused precipitation of metals from the surface water; while during the cold months (September to next February), sulfide oxidation dominated the sulfur cycling, and the primary removal process of metals shifted to the adsorption of organic matter (Xu and Mills 2018). The sulfur cycle in this study showed a similar dynamic: (1) sulfate concentrations in the treatment cells were generally higher than the influent during the cold months, indicating the production of sulfate compounds; (2) sulfate concentrations in the cells were generally lower than the influent during the warm months, indicating the consumption/reduction of sulfate compounds (Fig. S3). However, the noticeable drop of sulfate concentrations in all sampling sites in July 2018 should be caused by the low concentrations in the influent, which might be due to the biogeochemical changes in the retention basin but not just the sulfur dynamics in the treatment cells. The carbon cycle in the wetland also influences metal removal mechanisms. DOC, as one of the bacterial energies, facilitates and accelerates microbial processes, such as the bacterial sulfate reduction in the warm season (Xu and Mills, 2018). Meanwhile, DOC is a strong binding agent that combines metals and hydrophobic organics in the water (Pinney et al., 2000), and its labile fractions, e.g., fluvic acid, can combine with metals and form bioavailable metal-fulvic acid complexes (Xu and Mills, 2018).

Metal Speciation Predicted by BLM

The speciation of dissolved metals was modeled by BLM. Copper and Zn indicated distinct speciation in the water of the H-02 wetland. There was no relationship between BLM-Cu(II) and DCu ($P = 0.11$); however, the BLM-Zn (II) indicated a strong and positive correlation with DZn ($P < 0.001$, Fig. 2b). The most possibility might be because the major fraction of DCu was Cu-DOC complexes ($99.4 \pm 1.1\%$), and only a minor fraction was Cu^{2+} and Cu_{inorg} ($0.6 \pm 1.1\%$, Fig. 3b). Unlike DCu, DZn species, including Zn-DOC complexes ($55.2 \pm 14.6\%$), Zn^{2+} ($45.8 \pm 14.4\%$), and Zn_{inorg} ($3.6 \pm 1.0\%$), distributed relatively even in all sites (except the influent) with a surprisingly high percentage of Zn^{2+} (Fig. 3d). Generally, the percentage of Cu-DOC was higher than Zn-DOC and the percentage of Cu^{2+} was lower than Zn^{2+} , demonstrating their dissimilar capability of complexing organic ligands. This was consistent with previous conclusions that Cu was a much stronger binding element than Zn when competing

for metal sorption sites on organic matter (Machemer and Wildeman, 1992).

A large variation of the percentage of Cu_{inorg} was observed in the influent (Fig. 3b). Considering the water quality parameters input in the BLM, the only parameter showing the obviously different pattern was the pH whose fluctuation during the sampling period was significantly higher than the others (Fig. S3). The large treatment cells provide a neutralization condition for the wastewater. The wastewater discharged from the source pipes was slightly alkaline (8.8 ± 0.8). In contrast, the water in the effluent and stream after being treated by the cells was almost close to neutral (6.8 and 7.3, Table 1 and Fig. S2), which was similar to the results observed from the last ten years (from 2007 to 2018) (Xu and Mills 2018; Xu et al. 2019). Thus we assumed the change of pH values influenced Cu speciation by affecting the binding strength between BIM-Cu(II) and organic matter, and the conversion between Cu^{2+} and Cu_{inorg} (Morel and Hering 1993). Likewise, the different percentages of Zn^{2+} and of Zn_{inorg} between the influent and the other sites can be explained with the pH values. After water being treated by the wetland cells, the percentage of Zn_{inorg} decreased and of Zn^{2+} increased (Fig. 3d). The lowered pH (Fig. S2) and increased H^+ ions would compete against metal cations to form inorganic complexes with bicarbonate, carbonate, and hydroxide (Morel and Hering 1993), resulting a release of Zn^{2+} .

Metal Bioavailability Indicated by DGT

DGT technique has been used as a metal speciation tool in the seawater, river water, and wastewater (Buzier et al. 2006; Dunn et al. 2003; Meylan et al. 2004; Munksgaard and Parry 2003). Research indicated that the DGT accumulated not only free and inorganic metal species but also labile metal-organic complexes (Ferreira et al. 2008; Philipps et al. 2018). Our results of Cu demonstrated the similar conclusion. Linear regression indicated that DGT-metal and dissolved metal showed a positive relationship ($P < 0.001$, Fig. 2a). The percentage of DGT-Cu were much higher than the percentage of BLM-Cu(II) in the wetland cells, effluent, and stream ($P < 0.01$, Fig. 3a, b), indicating DGT in the water also combined a large amount of labile Cu-DOC complexes. However, the %DGT-Cu ($15.5 \pm 8.1\%$) was much lower compared to the result of natural freshwater ($66 \pm 17\%$) (Meylan et al. 2004). The most possibility of the low %DGT-Cu might be Cu^{2+} and Cu_{inorg} in H-02 constructed wetland only accounted $0.6 \pm 1.1\%$ in all sites. Dissimilarly of DGT-Cu, the close percentages of DGT-Zn and BLM-Zn(II) in all sampling sites (Fig. 3c, d) illustrated the DGT targeted Zn species overlapped with Zn^{2+} and Zn_{inorg} predicted by BLM, and the presence of DOC did not influence the performance of

DGT. Zn, as a much weaker binding element than Cu (Kerndorff and Schnitzer 1980; Machemer and Wildeman 1992), was not greatly complexed with DOC in the water, so its diffusion coefficient was not lowered. The extremely high Zn^{2+} concentrations in Fig. 3d also corresponded to this conclusion.

Comparing Techniques

In the output of BLM, we usually assume the combination of predicted free metal ions and inorganic metals (BLM-Cu(II) and BLM-Zn(II)) are the bioavailable and toxic metal species. Under this assumption, the DGT-measured Zn overlapped with BLM-Zn(II), but the DGT-Cu was different from the BLM-Cu(II). There was no relationship between DGT-Cu and BLM-Cu(II) ($P = 0.47$, Fig. 4a) but a strong correlation between DGT-Zn and BLM-Zn(II) ($R^2 = 0.35$, $P < 0.001$, Fig. 4b), suggesting Zn lability and/or bioavailability can be predicted by both DGT-Zn and BLM-Zn(II) in the water of H-02 wetland. However, care should be taken relative to Cu. Considering BLM-Cu(II) was not the primary species of DGT-Cu, BLM is not appropriate to explore Cu lability and/or bioavailability in the H-02 wetland or in the water with moderately high DOC concentrations. Balistrieri and Blank (2008) discovered the similar inconsistent performances of both DGT and geochemical modeling relative to different elements. For instance, the dynamic Zn concentrations predicted by models WHAM VI, NICA-Donnan, and SHM were all related to the DGT labile concentrations; but only the WHAM VI successfully predicted the DGT-Cu (Balistrieri and Blank 2008). These findings demonstrated the appropriate use of passive sampling techniques and geological modeling. In most cases, a combination of techniques, which could improve the accuracy of prediction, should be a much-optimized approach for a monitoring project when biomonitoring can be substituted; while in some situations, biomonitoring is irreplaceable due to the limitations of passive sampling techniques (Xu et al. 2020).

Conclusion

In this study, we explored the speciation and bioavailability of Cu and Zn in the water of the H-02 wetland with passive sampling device DGT and geochemical modeling BLM. Our results indicated the influence of water chemistry, such as pH and DOC, on metal speciation, which thus affected the performance of DGT, especially with the presence of metal mixtures. The conclusion that DGT and BLM were both appropriate tools to evaluate the lability and/or bioavailability for Zn while only DGT was good for Cu brings up the complexity of predicting speciation and

bioavailability of metal mixtures with DGT. Though techniques like passive sampling and geochemical modeling are easy, convenient, and cost-effective, it is critical to understanding the water chemistry in the environmental media, realize the heterogeneity of diffusive capability among different elements, and cautiously interpret data when geochemical modeling and/or passive samplers such as the DGT are applied. Biomonitoring often acts as the “early warning signal”, plays significant role in ecological risk assessment. DGT, BLM, and biomonitoring coupling should be the future strategy of management and ecological risk assessment.

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Compliance with Ethical Standards

Conflict of interest The authors declare no competing interests.

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