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Environmental Toxicology

Validation of Nickel Bioavailability Models for Algae, Invertebrates, and Fish in Chinese Surface Waters

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Abstract: Nickel (Ni) is used primarily in the production of alloys like stainless steel and is increasingly being used in the production of batteries for the electric vehicle market. Exposure of Ni to ecosystems is of concern because Ni can be toxic to aquatic organisms. The influence of water chemistry constituents (e.g., hardness, pH, dissolved organic carbon) on the toxicity of Ni has prompted the development and use of bioavailability models, such as biotic ligand models (BLMs), which have been demonstrated to accurately predict Ni toxicity in broadly different ecosystems, including Europe, North America, and Australia. China, a leading producer of Ni, is considering bioavailability-based approaches for regulating Ni emissions. Adoption of bioavailability-based approaches in China requires information to demonstrate the validity of bioavailability models for the local water chemistry conditions. The present study investigates the toxicity of Ni to three standard test species (*Daphnia magna*, *Pseudokirchneriella subcapitata*, and *Danio rerio*) in field-collected natural waters that are broadly representative of the range of water chemistries and bioavailabilities encountered in Chinese lakes and rivers. All experimental data are within a factor of 3 of the BLM predicted values for all tests with all species. For *D. magna*, six of seven waters were predicted within a factor of 2 of the experimental result. Comparison of experimental data against BLM predictions shows that the existing Ni bioavailability models are able to explain the differences in toxicity that result from water chemistry conditions in China. Validation of bioavailability models to water chemistries and bioavailability ranges within China provides technical support for the derivation of site-specific Ni water quality criteria in China. *Environ Toxicol Chem* 2023;42:1257–1265. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Bioavailability; Biotic ligand model; Nickel; Water quality criteria

INTRODUCTION

Nickel (Ni) is a widely used metal, with the main end use being stainless steel. Increasing demand for electric vehicles as a possible mitigation against climate change has suggested that the production of Ni, an important cathode for some battery formulations, may increase substantially over the next decade. Currently, China is among the leading users of Ni for both stainless-steel and battery manufacturing.

Although Ni is an essential element for plants and microbes, exposure to excess concentrations of Ni has numerous human health and environmental consequences (Buxton et al., 2019). In terms of environmental effects, Ni is toxic at elevated concentrations to terrestrial plants and aquatic organisms. Nickel's classification as a Priority Substance in the European Union has triggered extensive research into consequences of Ni exposure to aquatic ecosystems, ultimately leading to a revision of the Ni Environmental Quality Standard (EQS) under the Water Framework Directive (WFD) in 2013. Currently, Ni has an extensive aquatic toxicity database for both temperate and tropical ecosystems. For example, Stauber et al. (2021) recently proposed a Ni Water Quality Guideline for Australia that included chronic ecotoxicity data for 44 species of aquatic organisms from nine taxonomic groups. More recently, Peters et al. (2023) published an updated database of chronic Ni toxicity that contained data for 53 freshwater species that meet

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reliability and relevance criteria for European risk assessment application.

The effects of Ni on aquatic organisms are influenced by naturally occurring water chemistry constituents including pH, water hardness (i.e., $Ca^{2+}$and Mg^{2+}), and dissolved organic carbon (DOC). The consequence of this influence is that Ni toxicity can vary substantially among surface waters. For example, Deleebeeck et al. (2008) showed that Ni toxicity to *Daphnia magna* varied by up to 30-fold among European surface waters. Nickel is complexed by DOC, whereas Ca and Mg compete with Ni for uptake. Some organisms, notably the invertebrate *Ceriodaphnia dubia*, show increasing toxicity with increasing pH (Santore et al., 2021). The outcome of these processes is that the most sensitive waters for Ni toxicity tend to have low DOC and hardness, and high pH.

A number of models have been developed to predict the influence of water chemistry on metal toxicity to achieve ecological protection goals without being unnecessarily stringent (Merrington et al., 2016). For Ni, these include mechanistic biotic ligand models (BLMs) and empirical multiple linear regression (MLR) models (Croteau et al., 2021; Peters et al., 2021). Increasingly, these models are being used to determine protective values for aquatic life. The current Ni EQS under the European Union WFD is derived using BLMs (European Commission, 2013), a proposed revision to Australia/New Zealand Water Quality Guideline utilizes MLR models (Stauber et al., 2021), and the US Environmental Protection Agency (USEPA) is currently considering both modeling approaches to revise the Ni Water Quality Criterion (WQC) for the United States (https://www.epa.gov/sites/default/files/2018-10/documents/metals-crada-workplan-2018.pdf). Previous studies have demonstrated that existing Ni bioavailability models are capable of explaining intraspecies variation due to the influence of toxicity modifying factors for a wide range of species in different regions of the world, including the United States and Australia (Besser et al., 2021; Peters et al., 2018; Schlekat et al., 2010).

In China, the current EQSs for protection of surface waters, drinking water quality, and fisheries consider water hardness and yield maximum permissible standard values ranging from 20 to 50 μg Ni/L (Ministry of Ecology and Environment of the People's Republic of China, 1989, 2002; National Health Commission of the People's Republic of China, 2006). China is updating water EQSs to protect surface water environmental quality to reflect the current state of the science. To evaluate if existing Ni bioavailability models can be considered for use in WQC derivation, similar validation exercises are needed to demonstrate the appropriateness of the models using Chinese aquatic toxicity test organisms in waters that reflect the range of water chemistries and bioavailability ranges within China.

The purposes of the present study were (1) to investigate the toxicity of Ni to three standard test species in a variety of different field-collected natural waters that are broadly representative of the variety of conditions encountered in Chinese lakes and rivers, (2) to determine if the ranges of freshwater chemical parameters for which the existing BLMs were developed are similar to the ranges in China, which would indicate that the models would be applicable from the chemical perspective, and (3) to determine if existing Ni bioavailability models can explain the intraspecies variability observed in toxicity tests with standard aquatic toxicity test species (*D.magna*, *P. subcapitata*, and *Danio rerio*) performed in a range of surface waters collected throughout China. The results of our study can be used to inform the update of the EQS in China.

METHODS

Surface waters

Water collected from 20 locations was used to characterize the ranges of various water chemistry parameters in China (Figure 1, Table 2) and 11 of these locations were used in subsequent toxicity tests selected to maximize variability in pH, hardness, and DOC, while at the same time remaining in the boundaries of the BLMs. The boundaries for the BLMs are as follows: pH 6.5–8.7, Ca 0.1–88 mg $CaCO_3$/L, and DOC 0.1–30 mg/L (Nys et al., 2016; Peters et al., 2018).Water was collected in prerinsed 50-L polypropylene containers using a submersible pump and polypropylene tubing. Samples were immediately placed into coolers, transported to the testing laboratory, filtered through a 1-μm filter, and kept at 4 ± 2 °C in the dark until testing began.

Due to the longer toxicity test period compared with algae and fish, the water used to perform toxicity tests on *D. magna* was collected separately from five sites [Hai River (a), Pearl River, Yellow River (a), Fu River (Sanjiang) and Fu River (Siyuan)]. Water from 10 sites [Dian Lake, Laoyu River, Fu River (Wugan), Yellow River (b), Hai River (b), YanQi Lake, Fu River (Liangjiadu), Yinmi Lake, Yongding River, and Shahe reservoir] was used to perform toxicity tests on both *D. rerio* and *P. subcapitata*.

Toxicity tests

Daphnia magna. The toxicity study with *D. magna* was conducted following the Organisation of Economic Co-operation and Development (OECD) 211 standard test method for a 21-day exposure (OECD, 1998). *Daphnia magna* were obtained from State Key Laboratory of Environmental Criteria and Risk Assessment (SKLECRA) and cultured in incubator with a temperature of 20 ± 2 °C, and a 16:8-h light-dark ratio.The third brood (or later) daphnia used in the exposures met the acceptability criteria indicative of a healthy population (e.g., no signs of abnormal population development such as high mortality, presence of male daphnia, delayed first brood, discolored animals, etc.) Tests were considered valid if control mortality was ≤20% and the average number of neonates per surviving adult was ≥60.

Exposure treatments were prepared by adding appropriate volumes of $NiCl_2 \cdot 6H_2O$ (Macklin) to five site waters and test waters were allowed to equilibrate for at least 24 h at test temperature prior to distributing to exposure chambers for test initiation or renewal. Based on the results of range-finding experiments, nominal test concentrations were set as Hai River (a) 0 (blank control), 10, 20, 40, 80, and 160 μg Ni/L; Fu River (Sanjiang) and Fu River (Siyuan) 0 (blank control), 16, 32, 64,

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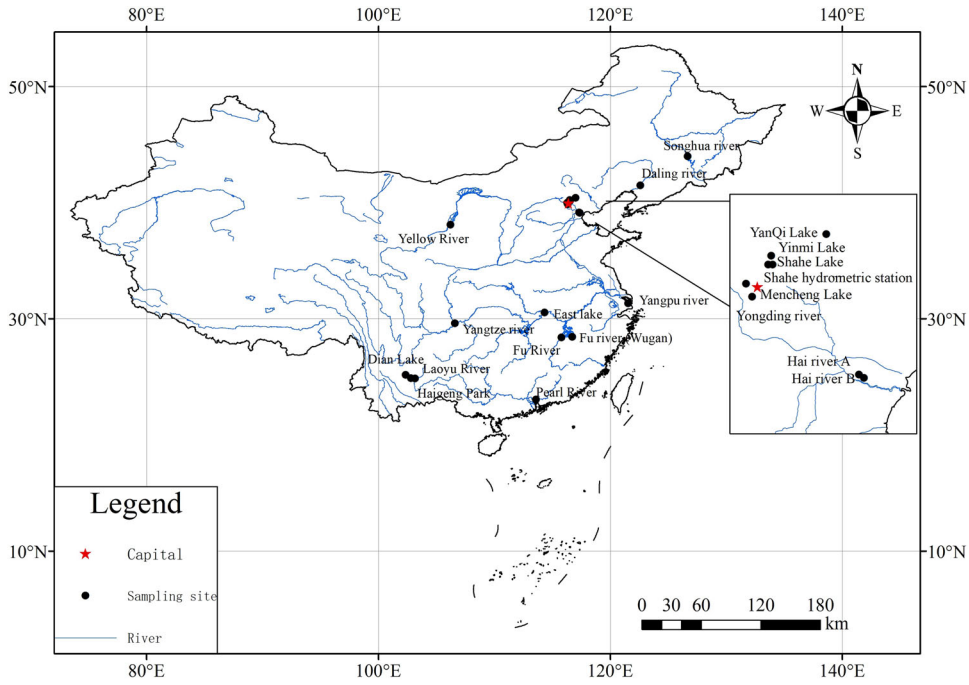


FIGURE 1: The distribution of sampling sites in the Chinese freshwater system.

and 128 µg Ni/L; Pearl River 0 (blank control), 28, 56, 112, and 224 µg Ni/L; and Yellow River (a) 0 (blank control), 30, 60, 120, and 240 µg Ni/L, respectively. Exposures were initiated by randomly distributing organisms directly into the test beakers. Ten parallel samples were prepared for each concentration, and 30 mL of solution was poured into a 100-mL beaker then one daphnia (6–24 h post hatch) was added to each beaker. The daphnia were fed algae (*P. subcapitata*) every 24 h. Test water was renewed every 48 h. The time of first neonate, numbers of neonates, and time between broods were recorded daily. The temperature, pH, DOC, and hardness of the experimental solution were measured and recorded before and after changing the experimental solution at 0, 7, 14, and 21 days. The dissolved concentration of Ni ions of each parallel sample was measured daily using Inductively coupled plasma mass spectrometry (ICP-MS).

Danio rerio. The toxicity study with *D. rerio* was based on the USEPA standard method "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" for a 7-day exposure (USEPA, 2002). Based on range-finding experiments, the nominal concentrations for evaluating the chronic toxicity exposure to fish were set as a geometric progression: 0 (blank control), 5, 10, 20, 28,

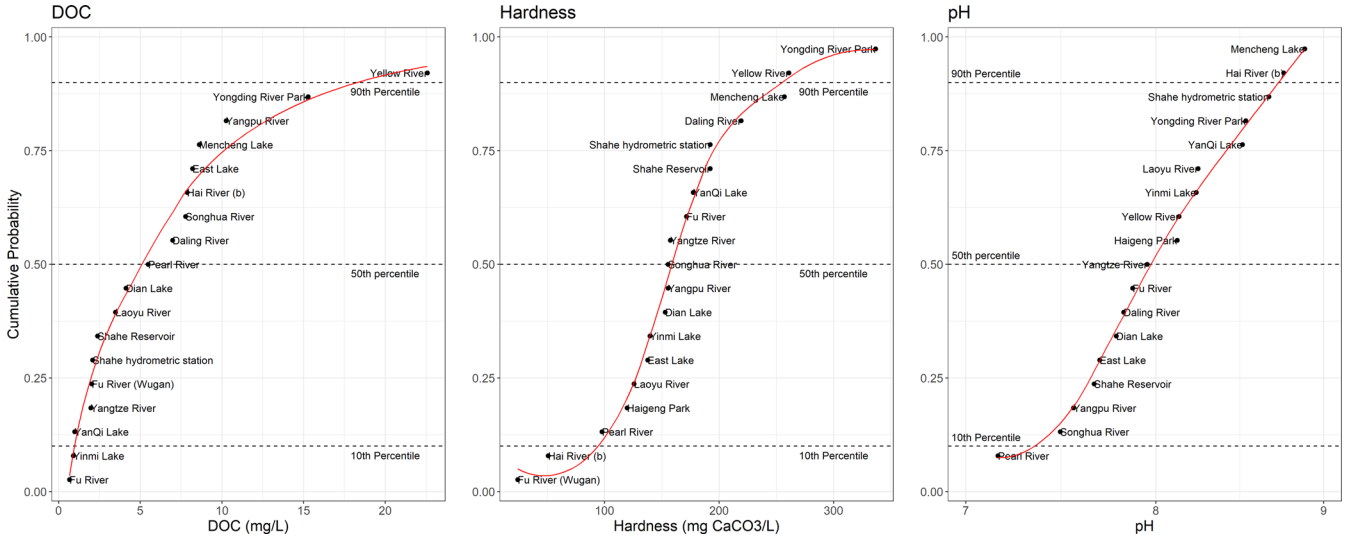


FIGURE 2: The probability distribution of DOC, hardness, and pH values in 20 Chinese freshwater sites. DOC, dissolved organic carbon.

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56, 80, 113, and 160 μg Ni/L. *Danio rerio* have been maintained in a circulating aquaculture system for at least 5 years in the SKLECRA laboratory. Adult zebrafish are raised in treated tap water and fed with shrimp twice daily. Zebrafish embryos were exposed at 25 ± 1 °C, 1000 lux light intensity, and 16:8-h light:dark cycle. The exposure solution was natural water, filtered by a 0.45-μm polypropylene fiber membrane, and stored in the dark at 4 °C. Zebrafish embryos with a time interval of less than 6 h from the same batch were selected, and the embryos were exposed within 36 h. At least five exposure levels and one control were set in each experiment, with three parallel replicates conducted at each exposure concentration. Ten zebrafish embryos were placed in each replicate. The semistatic experiment method was adopted to change the exposure solution every 24 h, and dissolved Ni concentration was measured daily using ICP-MS. Incubation was recorded in each vessel every day starting at 48 h. Embryo death, body segment loss, no separation of caudal segment and yolk sac, and cardiac arrest were recorded every 24 h. The number of hatchings, malformation, and survival were observed under a microscope (M165C; Leika).

Pseudokirchnerialla subcapitata. The toxicity study with *P. subcapitata* was based on the OECD 201 (OECD, 2011) standard method for a 96-h exposure. Based on range-finding experiments, the nominal concentrations for evaluating the chronic toxicity of algae were 0 (blank control), 20, 40, 80, 160, 320, 640, and 1280 μg Ni/L. Four nutrient stock solutions were prepared in pure water, according to the compositions given below (according to OECD 201). Supporting Information, Table S1 lists the different group contents of nutrient media, such as macro- and trace nutrients.

Algae were cultured in growth medium for 5–7 days in appropriate light incubator parameters and the temperature was controlled at 22 ± 1.5 °C. Light intensity was controlled at 6600 lux, 16:8-h light:dark cycle. Humidity was controlled at 40%–50%, with stirring every 2 h. When algae cell concentrations reached appropriate levels (5×10^3–10^4 cells/mL), the inoculum solution was centrifuged (<5000 r/min), the supernatant removed, and the precipitate rinsed with deionized water. The precipitate was then centrifuged a second time to reduce the nutrient stock solutions in the next-stage growth medium. After the second centrifugation, the upper layer of algal liquid was decanted and the algal precipitate retained.

For toxicity exposures in natural waters, the waters were amended with appropriate nutrient stock solution. Three parallel samples were prepared for each concentration level and the stock algae culture was added to the exposure waters with an initial density of 5–20 × 10^4 cells/mL. Exposure chambers were stirred six times a day and the position of each experimental group in the incubator was randomly changed at these intervals. Throughout the exposure, a portion of the algal was removed every 24 h to measure the biomass using a UV-spectrophotometer with the wavelength established at 680 nm. The dissolved Ni concentration was measured at the beginning and end of the experiment using ICP-MS.

Chemical analysis

A full suite of water chemistry parameters was measured in the toxicity tests to facilitate characterization and bioavailability modeling efforts, with variables including temperature, pH, hardness, DOC, total and dissolved Ni^{2+}, Ca^{2+}, Mg^{2+}, sodium (Na^+), potassium (K^+), chloride (Cl^-), and sulfate (SO_4^{2-}). All variables were measured at test initiation. Measurements of pH and temperature were made daily (PHS-3C; Inesa). Dissolved Ni measurements were taken from freshly prepared test solutions prior to each renewal and at test termination or complete mortality, whichever occurred first. In addition, within the *D. magna* test, dissolved Ni concentrations were measured daily.

Samples collected for DOC analysis were passed through a 0.45-μm nylon mesh filter following a 5-mL rinse with water sample, acidified with concentrated sulfuric acid, and stored in amber glass bottles. DOC concentrations were determined via total organic carbon analysis meter (Shimadzu). Other analytes were analyzed at the initiation of the test using ICP-MS (Ca^{2+}, Mg^{2+}, Na^+, and K^+) or ion chromatography (SO_4^{2-} and Cl^-).

Toxicity data analysis

Chronic effect concentrations that represented 10%, 20%, or 50% reduced performance relative to controls (EC10, EC20, or EC50, respectively) and 95% confidence limits were calculated using R (DRC package; Ritz et al., 2015). Effect concentrations for algal growth (control-normalized) and *D. rerio* survival were measured using a two-parameter log-logistic regression (LL.2) with the upper and lower limits prescribed at 1 and 0, respectively. Meanwhile, the effect concentrations for *D. magna* reproduction were measured using a three-parameter log-logistic regression (LL.3) with the lower limit set at 0. The toxicant concentrations used in these calculations were averages of all dissolved measurements for each treatment within each toxicity test.

Bioavailability model validation

The accuracy of bioavailability model predictions was evaluated by comparing the observed toxicity for each species with the predicted toxicity from the Ni BLMs (Nys et al., 2016) and MLR models (Peters et al., 2021), respectively. The full water chemistry used for bioavailability modeling predictions is provided in Table 1. Multiple linear regression predictions were generated following the procedure described by Peters et al. (2021) for Australian freshwaters. For the BLM predictions, the invertebrate species were made using the chronic Ni BLM calibrated for *D. magna* (Deleebeeck et al., 2008), *P. subcapitata* BLM predictions were performed using the chronic Ni BLM for *P. subcapitata* (Deleebeeck et al., 2009), and *D. rerio* predictions were generated using the chronic Ni BLM for *Oncorhynchus mykiss* (Deleebeeck et al., 2007). All BLMs used the Windermere Humic Aqueous Model VI (WHAM-Model VI; Tipping, 1998) to calculate metal speciation, with stability constants for inorganic metal complexes taken from the

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TABLE 1: Locations and average water quality of surface water systems where water was collected for nickel toxicity determinations

				mg/L as $CaCO_3$			mg/L						
No.	Sample sites	Location	Test species	Hardness	Alkalinity	pH	Ca	Mg	Na	K	SO_4	Cl	DOC
1	Hai River (a)	Tianjing	Daphnia	142.6	98.8	8.2	20.6	8.7	7.8	2.3	28.1	7.6	8.8
2	Pearl River	Guangzhou	Daphnia	89.6	124.3	7.6	26.6	10.3	79.5	9.9	101.0	67.3	3.8
3	Yellow River (a)	Yinchuan	Daphnia	280.3	173.4	8.6	38.5	26.1	47.8	2.6	39.9	91.3	14.9
4	Fu River (Sanjiang)	Nanchang	Daphnia	32.8	36.2	7.7	6.8	2.8	15.9	3.6	17.0	16.3	2.7
5	Fu River (Siyuan)	Nanchang	Daphnia	28.9	37.4	7.8	7.0	3.0	12.6	3.2	14.2	16.4	4.3
6	Yellow River (b)	Yinchuan	Algae, Fish	258.9	127.9	7.5	68.6	28.8	66.3	4.0	110.0	54.5	9.3
7	Hai River (b)	Tianjing	Algae, Fish	109.1	69.4	8.2	34.8	8.6	9.0	2.3	4.0	25.0	4.2
8	YanQi Lake	Beijing	Algae, Fish	146.9	76.4	8.0	38.7	16.6	9.0	3.4	44.7	10.9	6.6
9	Fu River (Liangjiadu)	Nanchang	Algae, Fish	43.8	31.8	7.8	14.6	3.4	8.4	2.8	7.5	12.8	3.9
10	Yinmi Lake	Beijing	Algae, Fish	98.6	60.7	7.5	30.0	7.9	5.3	1.9	30.4	8.4	3.9
11	Yongding River	Beijing	Algae, Fish	232.5	44.8	9.0	21.4	49.0	130.0	12.3	230.0	182.0	12.7
12	Shahe Reservoir	Beijing	Algae, Fish	165.3	83.6	7.8	42.8	18.4	57.9	14.0	74.7	100.0	7.7

CI = confidence interval; DOC = dissolved organic carbon.

National Institute of Standards and Technology (USA) database. Following the recommendations of Van Laer et al. (2006), who calibrated WHAM-Model VI with measured Ni speciation data in natural waters, we set the input of the fulvic acid concentration (mg/L) for the modeling at 0.8 times the measured DOC concentration, used a Ni-fulvic acid binding constant of log $K_{MA} = 1.75$, and we assumed that $Fe^{3+}$and Al^{3+}activities were controlled by their colloidal $Fe(OH)_3$ and $Al(OH)_3$ precipitates, respectively. All these assumptions were consistent with the assumptions made in the development of the BLMs in the original papers as cited above and Nys et al. (2016). Under the assumption that the biotic ligand parameters, which describe competitive binding of Ca^{2+}, Mg^{2+}, H^+, and Ni^{2+} for the biotic ligand, are constant across organisms, we fitted the intrinsic sensitivity of each species in each individual test and calculated the average sensitivity coefficient based on all tests for the same species. An analysis of the performance of bioavailability models was conducted following the recommendations of Garman et al. (2020) and Besser et al. (2021). An overall model performance score (MPS) was calculated for both species-specific and pooled versions of both bioavailability models (i.e., BLM and MLR) based on an average of three criteria: (1) an r^2value which accounts for the accuracy, precision, and overall fit of model predictions against the observed values, (2) the fraction of predicted toxicity values that are within a factor of 2 from the observed toxicity (factor of agreement, FA), and (3) an analysis of model residuals corresponding to major toxicity modifying factors. Specific equations for the r^2 and residual score metrics are shown in Besser et al. (2021), Equations 2 and 3, respectively. Within our analysis, the overall residual score comprised residuals for pH, hardness, alkalinity, and DOC.

RESULTS AND DISCUSSION

Distribution of water chemistry

To identify representative natural waters with different water chemistry constituents for toxicity testing, the pH, hardness, and DOC were measured in 20 water samples collected from different Chinese watersheds (Figure 1). Water chemistry varied considerably among the waters (Table 2). In general, Ni toxicity in freshwater is influenced by DOC, hardness, and pH (Buxton et al., 2019; Merrington et al., 2016). pH values ranged from 6.8 to 8.9, with 10th, 50th, and 90th percentiles of 7.3, 8.0, and 8.7, respectively (Figure 2). Dissolved organic carbon values ranged from 0.7 to 22.6 mg/L, with 10th, 50th, and 90th percentiles of 1.0, 4.8, and 15.0 mg/L, respectively. Water hardness ranged from 23.9 to 336.6 mg $CaCO_3$/L, with 10th, 50th, and 90th percentiles of 75.0, 155.0, and 258.0, respectively. Qualitative analysis of combinations of these parameters suggested that YanQi Lake, Yinmi Lake, Shahe hydrometric station, Haigen Park, Laoyu River, Fu River would be classified as high

TABLE 2: Locations and average water quality of surface water systems where water was collected for identified water chemistry and bioavailability

No.	Sample sites	DOC (mg/L)	pH	Hardness (mg/L as $CaCO_3$)	Relative bioavailability
S1	YanQi	1.0	8.5	177.3	High
S2	Yinmi Lake	0.9	8.2	139.4	High
S3	Shahe Lake	2.4	7.7	192.2	Medium
S4	Shahe hydrometric station	2.1	8.7	192.2	High
S5	Dian Lake	4.1	7.8	152.9	Medium
S6	Haigeng Park	/	8.1	119.5	High
S7	Laoyu River	3.5	8.2	125.5	High
S8	Fu River	0.7	7.9	171.3	High
S10	Hai River (b)	7.9	8.8	50.5	Low
S11	Yellow River	22.6	8.1	260.9	Low
S12	Yangtze River	2.0	8.0	157.4	High
S13	Yangpu River	10.3	7.6	155.4	Low
S14	East Lake	8.2	7.7	137.4	Low
S15	Pearl River	5.5	7.2	97.6	Medium
S16	Daling River	7.0	7.8	219.1	Medium
S17	Songhua River	7.8	7.5	155.4	Low
S18	Mencheng Lake	8.6	8.9	256.9	Low
S19	Yongding River Park	15.3	8.5	336.6	Low
S20	Fu River (Wugan)	2.0	6.8	23.9	High

DOC = dissolved organic carbon.

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bioavailability scenarios whereas Hai River A was classified as a site with low bioavailability. Other sites were classified as medium bioavailability.

Based on the distributions of DOC, pH, and hardness, additional samples were collected from specific sites for toxicity testing. The chemistry of these waters and the tests the waters were used for are shown in Table 1. The natural waters chosen for toxicity testing capitalized on the range of bioavailability parameters, as exemplified by the Fu River sites, where the hardness concentrations were below the 10th percentile of the hardness observed across 20 Chinese waters (74.9 mg/L $CaCO_3$), and the hardness concentrations for the Yellow River sites exceeded the 90th percentile of the hardness observed across 20 Chinese surface waters (257.6 mg/L $CaCO_3$). The Fu River (Sanjiang and Siyuan; water hardness of 32.8 and 28.9 mg/L as $CaCO_3$, pH of 7.7 and 7.8, DOC of 2.7 and 4.3 mg/L) was representative of a system with high Ni bioavailability and was expected to result in the greatest Ni toxicity to daphnia. A similar situation was also expected in Fu River (Wugan), with high Ni bioavailability resulting in substantial Ni toxicity to fish and algae. In contrast, the Yellow River sites (water hardness of 280.2 and 258.9 mg/L as $CaCO_3$, pH 8.6 and 7.5, DOC 14.9 and 9.3 mg/L, respectively) were representative of a system with low Ni bioavailability and were expected to exhibit the lowest Ni toxicity. The remaining waters were expected to be in the midrange in terms of Ni toxicity.

Toxicity results and species sensitivity comparison

Measured toxicity thresholds (EC10, EC20, and EC50s listed in Table 3) of *D. magna*, *P. subcapitata*, and *D. rerio* are plotted as a function of location in Figure 3. The toxicity thresholds for *D. magna* (triangles) all aligned very closely with one another regardless of the geographic location and effect level. Meanwhile the EC10, EC20, and EC50 values for the algae (circles) span much greater ranges in concentrations and do not overlap one another because the dose responses for algae were shallower that those for *D. magna*. In general, *P. subcapitata* was the most sensitive, followed by *D. magna*, with *D. rerio* being substantially less sensitive than either the algae or the invertebrate.

Performance of BLM and MLR model predictions

Two bioavailability models, the European Ni BLM (Nys et al., 2016) and the Ni MLR model (Peters et al., 2021), were used to generate EC50 toxicity predictions based on water chemistry parameters for the present study (Figure 4, Supporting Information, Table S2). The models were applied on both a species-specific basis (i.e., invertebrate data were applied to the *D. magna* model, algae data applied to an algae model, and *D. rerio* data were applied to a fish model) and a pooled basis (i.e., all data were grouped independent of species and applied to a "pooled" version of each model).

To assess the comparative performance of each model, a series of analyses were performed following recommendations made by Garman et al. (2020) and Besser et al. (2021) which assessed accuracy, precision, and bias within the models. The "pooled-all" analysis evaluated each of the following metrics based on all individual datapoints being pooled across species, rather than taking an average of the performance of each species prior to pooling the data set. By retaining the individual datapoints prior to pooling across species, the performance of the pooled model is representative of the underlying hypothesis that the pooled model is capable of generating predictions independent of the species tested.

The first metric considered in the evaluation of model performance is r^2, which is calculated as a ratio of the difference between the predicted and observed toxicity over the distance between the observed toxicity and the mean observed toxicity (Equation 2, Besser et al., 2021). In this manner, r^2 quantifies the accuracy of the prediction over the precision of the observed toxicity. The species-specific BLM and MLR model performed comparably for both the algae and invertebrate data ($r^2 = 1.00$), however the MLR model performed marginally

TABLE 3: Chronic effect concentration (μg Ni/L) for *Daphnia magna*, *Pseudokirchnerialla subcapitata*, and *Danio rerio*

		Daphnia magna			*Pseudokirchneriella subcapitata*			*Danio rerio*		
		μg/L			μg/L			mg/L		
No.	Sample site	EC10	EC20	EC50	EC10	EC20	EC50	EC10	EC20	EC50
1	Hai River (a)	98.8	109.7	131.3	ND	ND	ND	ND	ND	ND
2	Pearl River	142.3	147.7	157.3	ND	ND	ND	ND	ND	ND
3	Yellow River (a)	75.1	95.6	144.5	ND	ND	ND	ND	ND	ND
4	Fu River (Sanjiang)	48.8	51.2	55.8	ND	ND	ND	ND	ND	ND
5	Fu River (Siyuan)	57.0	61.2	69.1	ND	ND	ND	ND	ND	ND
6	Yellow River (b)	ND	ND	ND	15.8	67.5	805.0	2.0	4.5	19.0
7	Hai River (b)	ND	ND	ND	17.4	60.3	505.2	4.7	6.6	11.7
8	YanQi Lake	ND	ND	ND	12.9	49.1	486.3	6.6	12.5	36.6
9	Fu River (Liangjiadu)	ND	ND	ND	8.3	26.4	189.5	11.6	14.9	22.6
10	Yinmi Lake	ND	ND	ND	6.9	29.0	338.7	7.0	10.6	21.8
11	Yongding River	ND	ND	ND	7.6	28.4	269.8	9.6	10.8	13.2
12	Shahe Reservoir	ND	ND	ND	36.9	99.2	537.1	12.0	16.0	25.9

ND = not determined.

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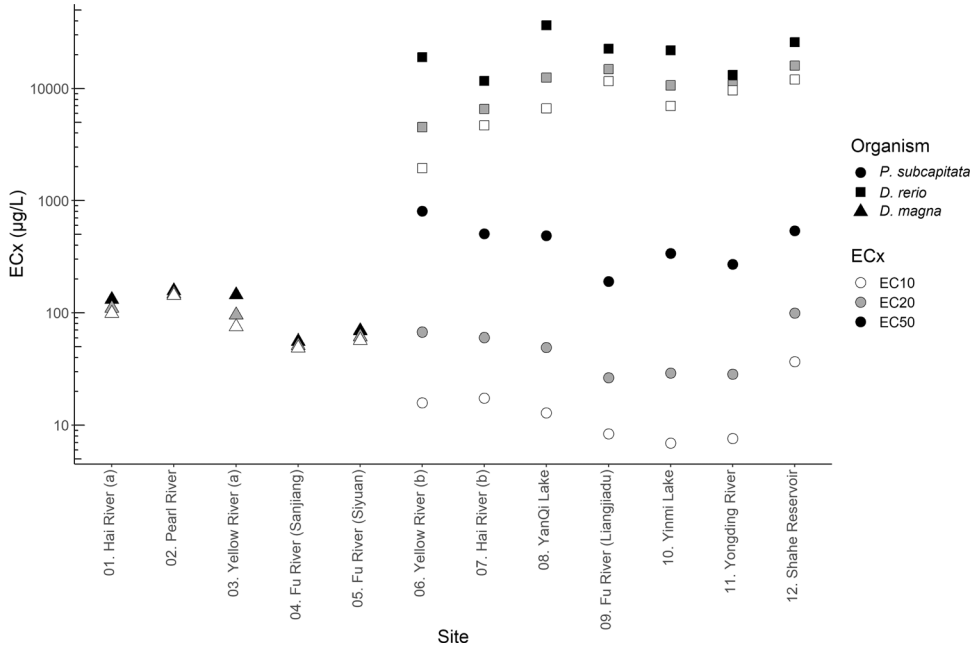


FIGURE 3: Comparison of effect concentration (ECx) EC10, EC20, and EC50 values of three trophic levels with 15 specific sites.

better for the fish and "pooled all" data sets (Table 4). The BLM r^2 for *D. rerio* resulted in a negative value, indicating that the accuracy of the model prediction was greater than the precision of the observed data.

The second parameter is an agreement factor (FA), which quantifies the fraction of predicted toxicity values that occur within a factor of 2 of the observed toxicity. The factor of 2 agreement is often cited as a measure of validity in model performance (Meyer et al., 2018) although analyses employing a factor of 3 have also been employed in recent studies (Peters et al., 2018, Price et al., 2022). In all cases, the MLR models resulted in an equivalent or higher FA than the BLMs, but all BLM predictions across all species are within a factor of 3. Considering that the intraspecies variability was as high as 50-fold (e.g., the difference between the highest and lowest EC10 values for *P. subcapitata*), this represents a substantial feat in model performance. For *D. rerio*, only four out of seven results were within a factor of 2 of the species-specific BLM-predictions, but six out of seven predictions were within a factor of 2 for the species-specific MLR model. Interestingly, results with *D. rerio* showed that water chemistry had less of an impact on Ni toxicity than for the algae and invertebrate species. Intraspecies variability based on EC50 values was 4.0 for *D. rerio*, compared with 6.3 and 12.9 for *D. magna* and *P. subcapitata*, respectively. Previous studies have shown that when species are less intrinsically sensitive to Ni toxicity, as is the case with *D. rerio*, the influence of water chemistry is less pronounced and the BLM predictions may suffer (Schlekat et al., 2010).

TABLE 4: Model performance comparison

	r^2		FA		RS		MPS	
Species	BLM	MLR	BLM	MLR	BLM	MLR	BLM	MLR
Algae	1.00	1.00	0.71	0.86	0.89	1.19	0.87	1.02
Invertebrate	1.00	1.00	1.00	1.00	1.03	0.91	1.01	0.97
Fish	−0.13	0.25	0.57	0.86	1.56	1.34	0.67	0.82
Pooled all	0.21	0.48	0.74	0.89	1.18	1.13	0.71	0.83

BLM = biotic ligand model; FA = fraction of modeled values that occur within a factor of 2 of observed values; MLR = multiple linear regression model; MPS = model performance score, calculated as average of r^2, FA, and RS = r^2 = measure of goodness of fit (Equation 2, Besser et al., 2021); RS = overall residual score (Equation 3, Besser et al., 2021).

The third metric of the MPS is the overall residual score, which quantifies the relationship between the toxicity residual (i.e., ratio of predicted toxicity to observed toxicity) because it is influenced by toxicity modifying factors. In practice, the residual score is determined by integrating a combination of the slope and *p* value of this relationship (Equation 3, Besser et al., 2021) to assess any bias in the predictions as a result of these factors. For this analysis, the toxicity modifying factors pH, alkalinity, hardness, and DOC were evaluated to contribute to the overall residual score shown in Table 4. Although the species-specific MLR model received a higher residual score for the algae data, the residual score for the BLMs better performed for the invertebrate, fish, and "pooled all" data sets. Ultimately, the overall MPS was determined by taking the average of the r^2, FA, and overall residual-score values. As shown in Table 4, the MLR MPS exceeds that of the BLM for the algae data, fish data, and "all species" data. Conversely, the BLM performs slightly better for the invertebrate data (MPS = 1.01 BLM vs. 0.97 MLR). Notably, the overall MPS values were close for both models, with the difference in MPSs never exceeding 0.15. This largely indicates that either model would be sufficient for providing accurate toxicity predictions in natural Chinese waters. Indeed, this point is further supported by the overall agreement in toxicity predictions generated by each model.

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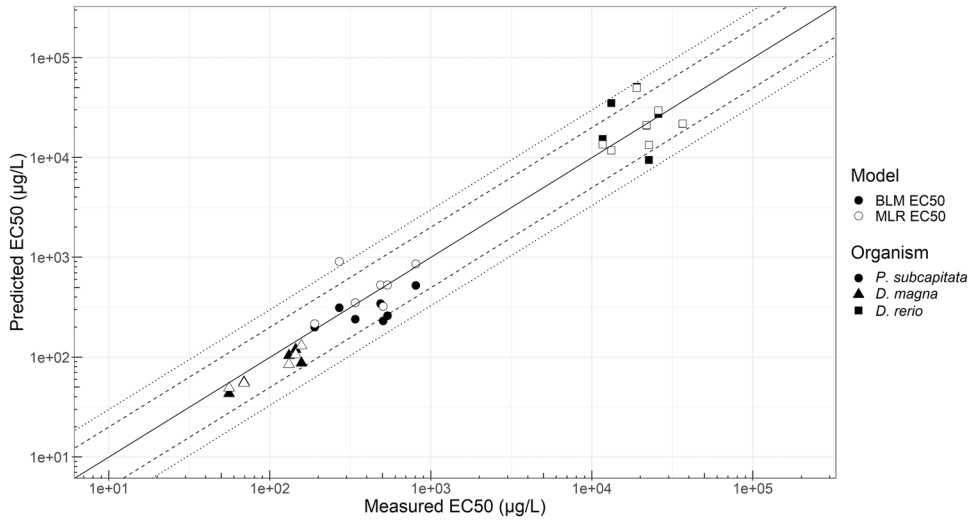


FIGURE 4: Comparison of species-specific Ni bioavailability model predictions against observed results of three different trophic levels. Solid line is a 1:1 ratio, dashed line represents a twofold divergence from 1:1, and the fine dotted line represents a threefold divergence. EC50, median effect concentration.

Five algal tests were performed in culture medium and these tests provide evidence of the variability that is inherent in the tests, rather than the variability that results from differences in the water chemistry of the different waters tested. EC_{50} values have a mean value of 119 µg/L with relative standard deviation (RSD) of 13%, whereas the BLM predictions for these tests have a mean value of 132 µg/L with an RSD of 3%. The variability in BLM predictions reflects minor differences in the test water chemistry between tests, most likely due to small differences in the average pH. The RSD of the experimental results reflects variability due to differences in water chemistry and the inherent variability of the test system.

High-quality ecotoxicity data were generated for three species of aquatic organisms in natural water that varied in different water chemistry factors, resulting in high (e.g., 10-fold difference) intraspecies variability (e.g., for *D. magna* EC10 was a 10-fold difference between Pearl River (a) site and the Fu River [Sanjiang] site). The greatest difference occurred in the EC10 of algae with nearly 50-fold difference between Fu River (Wugan) with low hardness, DOC, and pH compared with water chemical factors in Dian Lake. This high intraspecies variability will have a substantial influence on the toxicity assessment of Ni to aquatic organisms and will influence the derivation of site-specific Ni water quality criteria in China. Because both Ni bioavailability models were able to successfully account for the variability, the present study supports the use of bioavailability normalization of water chemical conditions in China.

The present study once again demonstrates that the existing Ni bioavailability models are robust and yield consistent outcomes across aquatic species, ranges of water chemistry, and geography. Schlekat et al. (2010) showed that the same European BLMs as used in the present study explained intraspecies variability for a range of invertebrates, including insects, as well as a vascular plant that were tested in natural waters from North America. Peters et al. (2018) demonstrated that the European models were able to explain intraspecies variability for five Australian aquatic species (including an alga, a vascular plant, two invertebrates, and a fish) that were tested in natural Australian waters with water hardness as low as 1 mg $CaCO_3$/L. The present study adds Chinese water chemistry to the groups of waters for which these models have been validated, suggesting that the models are extremely robust in determining the influence of water chemistry constituents on the toxicity of dissolved Ni to those aquatic organisms that are commonly considered in the determination of protective values for aquatic life.

CONCLUSIONS

Bioavailability models for Ni toxicity have been validated for three standard test species in field-collected natural water samples from a range of different Chinese surface waters. Comparison of experimental data against both BLM and MLR model predictions showed that the existing Ni bioavailability models can explain the differences in toxicity that result from water chemistry conditions in China. The present study demonstrates that BLM and MLR modeling approaches can be developed and validated to predict Ni toxicity to freshwater aquatic organisms from existing data sets. Empirical toxicity prediction approaches may provide a viable alternative for taking account of the bioavailability of Ni in freshwaters for regulatory purposes.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5595.

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Author Contributions Statement—**Jia He**: Data curation; Investigation; Writing–original draft; **Chen Wang**: Investigation; Methodology. **Christian Schlekat**: Project administration; Validation; Writing–original draft; Writing–review & editing. **Fengchang Wu**: Conceptualization; Writing–review & editing. **Elizabeth Middleton**: Software; Validation; Writing–original draft; Writing–review & editing. **Emily Rogevich Garman**: Writing–review & editing. **Adam J. Peters**: Methodology; Software; Validation; Writing–review & editing.

Data Availability Statement—Data for the present study is found in the Supporting Information.

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