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Matrix in River Water, Sediments, and Biofilms Mitigates Mercury Toxicity to Medaka (*Oryzias Latipes*)

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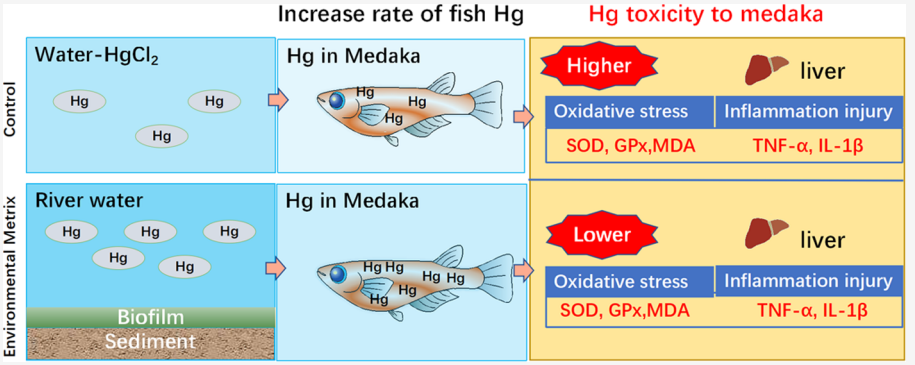

ABSTRACT: Impacts of an environmental matrix on mercury (Hg) bioavailability and toxicity to medaka (*Oryzias latipes*) were investigated in matrix-free controls and treatments with a stepwise increased environmental matrix of river water, sediments, and biofilms. Generally, river water enhanced but the presence of sediments and biofilms reduced Hg bioavailability to medaka up to 10^5 times, so that Hg_{total} concentrations/amounts among different environmental media cannot mirror Hg availability and toxicity to medaka. On average, 12.9 and 12.4% of Hg in medaka was, respectively, methylated to methylmercury (MeHg) in matrix-free and -containing treatments, indicating no influence of the environmental matrix on Hg methylation in medaka. All oxidative stress, inflammatory injury, and malformation parameters correlated strongly and significantly with Hg_{total} and MeHg concentrations in medaka, notably with steeper slopes in matrix-free controls than in matrix-containing treatments, highlighting that the environmental matrix mitigated Hg and MeHg toxicity to medaka. Moreover, oxidative stress was more strongly mitigated than inflammatory injury according to the stronger decreases of the regression line slopes from matrix-free to -containing treatments. Here, we have newly identified that the potential of the environmental matrix to decrease Hg bioavailability and mitigate Hg toxicity to fish together could buffer Hg ecotoxicity in the aquatic environment.

KEYWORDS: *mercury, methylmercury, environmental matrix, mitigating effect, oxidative stress, inflammatory injury, medaka, sediment and biofilm*

INTRODUCTION

Mercury (Hg) is a highly toxic trace metal that is widespread and persistent in freshwater and marine ecosystems.[1] It is frequently used in chlorine, caustic soda, nuclear reactors, dental offices, gold mining, or pharmaceutical antifungal products.[2] Once released into the environment, inorganic Hg deposited into the aquatic environments can be converted to methylmercury (MeHg), which is much more toxic than inorganic Hg and may undergo strong bioaccumulation and biomagnification, especially in aquatic food chains.[3] Mercury therefore poses a general health risk to fish at the top of the food chain and to the humans who consume them.[4,5]

In aquatic environments, Hg is mostly bound to the surfaces of organisms, suspended particles, sediments, and biofilms.[3] Among these environmental media, the most active Hg methylation occurs in sediments under anoxic conditions.[6] Moreover, Hg bound to sediments released into the water column can be subsequently taken up by aquatic organisms.[7] Sediments thus may serve as a sink and at the same time a long-time source of Hg.[8] In comparison, biofilm assemblies of bacteria, algae, diatoms, protozoa, and fungi are surrounded by extracellular polymeric substances that develop on and adhere to the riverbed and immersed materials.[9] Biofilms are usually the first microbial media in nature to interact with dissolved metal(loid)s in aquatic systems and can alter the bioavailability and toxicity of metal(loid)s by influencing mineral solubility,

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adsorption, and transformations between oxidized and reduced species as well as metabolism of aquatic biota.[10,11] The Hg levels in water, biofilms, and sediments have been investigated widely to assess the acute, short-term, and long-term pollution status of the aquatic environment, respectively.[12,13] However, Hg levels in environmental media cannot be taken directly to assess the toxic effects of organisms exposed to Hg-contaminated environments. Namely, there are several investigations showing that the presence of sediments or biofilms alters Hg availability to aquatic organisms.[14−16] The past studies have focused exclusively on exploring the effect of a single matrix of biofilms or sediments on the accumulation of Hg in organisms. Little is known about the combined effects of single and multiple environmental media on Hg bioaccumulation, as well as its toxicity to aquatic organisms. Usually, Hg toxicity to fish was illustrated by exposing fish such as medaka and zebrafish to environmentally relevant concentrations of Hg in aqueous solutions, e.g., 10−1000 μg L^{-1} $HgCl_2$ and 0.01−40 μg L^{-1} MeHg.[17−21] Nonetheless, toxicity tests capable of predicting the potential hazard of real Hg-contaminated aquatic environments on the basis of multiple environmental media, including biofilms and sediments, are still lacking today.

Mercury has been evidenced to induce oxidative stress and immunotoxicity in fish.[19,20,22] The oxidative stress induced by Hg toxicity is associated with the production of intracellular reactive oxygen species (ROS), which suppresses antioxidant defenses and damages macromolecules such as DNA, proteins, and lipids.[23] To combat ROS and protect cells against oxidative stress, the antioxidant defense system comprises antioxidant enzymes (such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione S-transferase) and nonenzymatic antioxidants (such as reduced glutathione and vitamin E). Besides, Hg produces notable deleterious effects on immune function in fish.[20] Cytokines, secreted from immune cells, are vital mediators of immune function, and Hg exposure could alter cytokine expression in fish.[20,22,24] Accordingly, these biochemical mechanisms of Hg toxicity in fish highlight the usefulness of antioxidant enzyme activity and cytokine levels as warning signs of Hg ecotoxicity caused by Hg exposure in real environments. Therefore, taking advantage of medaka (*Oryzias latipes*), this study aimed to assess how individual and combined presence of an environmental matrix in river water, sediments, and biofilms may influence (1) Hg bioavailability to fish, (2) Hg methylation in fish, and (3) Hg toxicity to fish, to explore the emerging significance of the matrix in river water, sediments, and biofilms on Hg ecotoxicology in the aquatic ecosystems.

MATERIALS AND METHODS

Study Area and Water, Sediment, and Biofilm Sampling. The Dongmenqiao River (DMQ) is located downstream of the Guizhou Organic Chemical Plant in Guizhou Province, SW-China. This plant has used a large amount of Hg as the catalyst to produce acetic acid and discharged almost 100 tons of Hg into the DMQ from 1971 to 1997.[25] Since 2017, governance regulations have been implemented to reduce Hg levels in soils, atmosphere, and DMQ water. Due to the subtropical humid climate, biofilms grow generally well in DMQ. In April 2019, water (0−10 cm depths), sediment (0−10 cm depths), and biofilm samples were collected in triplicate in the upstream (near to the discharge effluent, S1), middle (S2), and downstream (S3) of DMQ for exposure experiments and Hg analysis (Figure S1). Water samples were collected following the trace Hg clean protocols.[26] For exposure experiments, surface water collected at each site was stored in a polyethylene container before use. The biofilm-attached stones collected from the shore water at each site were first gently rinsed, then stored in acid-pretreated plastic bottles, and transported back to the laboratory. The biofilm was completely detached from the stones via oscillating at 200 rpm for more than 12 h and then kept in clean plastic bottles for exposure experiments and Hg analysis. All samples were stored at 0−4 °C before use in exposure experiments.

Toxicity tests were performed with medaka (*O. latipes*). In total, 45 independent experimental units were performed in triplicate. The experimental units comprised three experimental groups: Hg-free controls, $HgCl_2$ controls (90, 120, 150, 180, and 210 ng L^{-1}, equivalent to THg levels in DMQ water), and environment matrix treatments. For $HgCl_2$ controls, stock solutions of 1 ppm $HgCl_2$ were prepared in deionized water, from which small aliquots were added to dechlorinated tap water in order to obtain desired concentrations. For matrix-containing treatments, the real environmental matrix from S1, S2, and S3 were combined as follows: (1) river water (W), (2) river water and sediment (W + S), and (3) river water, sediment, and biofilm (W + S + B; Table 2). To truly reveal the effect of the environmental matrix, the same amount of fish diet supply was purposely maintained in all experimental units. All experiments were performed in glass aquaria (60 cm × 30 cm × 40 cm) filled and renewed daily with 5 L of dechlorinated tap water in controls and with 5 L of river water in W, W + S, and W + S + B treatments. For W + S and W + S + B treatments, 1 kg of the sediment and 0.4 kg of the biofilm were placed into aquaria. First, a piece of fishing net (mesh opening of 1000 μm) was covered over the sediment and biofilm. Then, river water was gently poured in and left to stand before fish was placed, which helped to minimize fish activities disturbing the bottom substrates (Figure S2). To date, glass aquaria have been widely applied for Hg exposure experiments, mirroring that glass aquaria have been well accepted as the most optimal experimental system among all.[17−21] To minimize the loss of Hg caused by adsorption to glass aquaria, dechlorinated tap water at desired $HgCl_2$ levels and river water were renewed in glass aquaria daily for a week prior to the exposure experiment. Our time-variation monitoring of the $HgCl_2$ controls evidenced that the adsorption equilibrium of THg to the aquaria surface was achieved on the seventh day (Figure S3).

The adult medaka (*O. latipes*, 3 month old) were acquired from Zhongke water quality company in Wuxi, China (http://www.casaet.com/), and cultured in a flow-through system with continuously aerated and triple-filtered recirculating water for 2 weeks of acclimation in the laboratory. The THg and MeHg concentrations in medaka prior to exposure experiments were 5.65 ± 0.42 and 0.39 ± 0.03 ng g^{-1}, respectively. Since MeHg is notoriously slow to be excreted,[27,28] the MeHg burden in medaka prior to the experiment is clearly a carry-over from the previous diet. After acclimation to the experimental setup, uniform-sized medaka were randomly distributed into glass aquaria (six fish per aquarium) and fed twice a day with rations of hatched artemia. The hatched artemia was a clean diet with a THg concentration of 0.26 ± 0.13 ng g^{-1} and MeHg undetected (methodic detection limit: 0.002 ng g^{-1}). The supplied diets corresponded to a daily intake of 4.0 g of artemia (fresh weight) for six fishes per aquarium, which were completely eaten up within 5 min each time. Also, fish feces

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were removed every day. Therefore, the contribution of the remaining artemia and feces to Hg methylation and to adsorb Hg to prompt its bioaccumulation in each treatment could be excluded. Accordingly, the contribution of fish diet to the increase of THg (5.27 ± 0.17 ng g^{-1}, Table S1) and MeHg concentrations (negligible) in medaka varied little among all controls and treatments.

After 21 days of exposure, medaka in each aquarium were collected and placed on ice (without using an anesthetic), and their livers were dissected and preserved in different Eppendorf tubes for biochemical assays within 1 day. A part of the fish body was stored at −80 °C for THg and MeHg quantification. All experimental protocols were approved by the Chinese Legislation and Animal Committee of the Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences.

Quantification of Total Mercury and Methylmercury in Dongmenqiao Samples and Medaka. The THg concentration in DMQ water was determined with a chromatography/cold vapor atomic fluorescence spectrometer (GC−CV-AFS; Model III, Brooksrand) following USEPA method 1631 after 0.5% BrCl oxidation with subsequent reduction with 0.2% v/v $NH_2OH{\cdot}HCl$ and $SnCl_2$.[29] The MeHg in water was measured using GC−CV-AFS after distillation and ethylation following USEPA method 1630.[26] The sediment, biofilm, and medaka samples were freeze-dried and homogenized for THg and MeHg analysis. Total Hg in the sediment and biofilm was analyzed with a DMA-80 Total Mercury Analyzer (Milestone Srl, Italy) after USEPA method 7473.[30] Methyl Hg in the sediment and biofilm was determined using HNO_3 leaching/CH_2Cl_2 extraction, ethylation, trapping on Tenax trap, isothermal GC separation, and CV-AFS detection.[31] To quantify THg in medeka, 0.1−0.2 g of the sample was digested with 10 mL of a mixture of HNO_3 and H_2SO_4 (7:3 (v/v)) at 95 °C for 3 h before CV-AFS analysis. For MeHg speciation in medaka, 0.1−0.2 g of samples were digested in 5 mL of KOH solution at 75 °C for 3 h, diluted to 25 mL with deionized water, and then quantified using CV-AFS after GC separation (Yan et al., 2006).[32] Total Hg and MeHg in certified reference materials were all satisfactorily recovered with the aforementioned methods, including 95−97% of THg found in GSD-5a, 93−96% of MeHg in ERM-CC580, and 93−101% and 92−107% of THg and MeHg in TORT-2, respectively.

Biochemical analysis. The liver tissue of the adult medaka was dissociated and homogenized in ice-cold physiological saline (1:10, W/V) and then centrifuged at 2,500*g* at 4 °C for 10 min. Then, the supernatants were immediately removed for the soluble protein, antioxidant enzymes, and inflammatory factors analysis. The soluble protein was quantified with the BCA protein detection kit (cat. no. A045−3−2, Nanjing Jiancheng Bioengineering Institute, China), whereas the SOD and GPx activities as well as malondialdehyde (MDA) concentrations were determined using commercially available kits (for SOD, cat. no. A001−3−2; for GPx, cat. no. A005−1−2; for MDA, cat. no. A003−1−2, Nanjing Jiancheng Bioengineering Institute, China). Finally, the interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) levels in the supernatants were measured using the ELISA method (for IL-1β, cat.no.H002; for TNF-α, cat.no.H052−1, Nanjing Jiancheng Bioengineering Institute, China).

RESULTS AND DISCUSSION

Mercury and Methylmercury in Dongmenqiao River Water, Sediment, and Biofilm. The THg concentrations in DMQ water and biofilms ranged from 93.4 to 202 ng L^{-1} and 0.7 to 4.9 mg kg^{-1}, respectively (Table 1), higher than the environmental quality standards for China's third-class surface water (100 ng Hg L^{-1})[33] and most values found in the uncontaminated biofilm (0.01−6.3 μg kg^{-1}).[11,34−36] In comparison, the historical THg pollution in DMQ was better reflected by THg in sediments with concentrations (4.6−51.3 mg kg^{-1}) almost 1000 times higher than the background values (6.39−56.22 μg kg^{-1}).[37] The concentrations of THg and MeHg in the sediment were up to 10 times higher than in those in the biofilm, suggesting a higher ability of the sediment to retain Hg than the biofilm. Based on Table 1, we calculated the partition coefficients (K_d) at the sediment− and biofilm−water interfaces, which describe the Hg equilibrium between the solid and dissolved phases. For THg, the K_d values ranged from 22,800 to 458,000 L kg^{-1} and from 3470 to 48200 L kg^{-1} for the sediment and biofilm, respectively. Apparently, Hg has higher mobility in biofilms than in sediments in DMQ. Interestingly, MeHg had smaller (13,100−20,600 L kg^{-1}) for the sediment but larger K_d values (7710−58,200 L kg^{-1}) for the biofilm than THg, similar to literature values ($10^{3.4}$−$10^{6.2}$ and $10^{3.8}$−$10^{6.6}$ for biofilm Hg and MeHg[38] as well as 10^4−10^6 and 10^3−10^5 for sediment Hg and MeHg, respectively).[39] While biofilms and sediments consist mainly of organic compounds and minerals, respectively, they retain Hg and MeHg from water generally in very different ways. In sediments, inorganic Hg seems to be more strongly sorbed by humic substances than MeHg,[40,41] while in biofilms, inorganic Hg and MeHg accumulation can be influenced by the binding functional groups in exopolysaccharide-embedding microbes.

Table 1. Physicochemical Parameters of Water, Total Mercury (THg), and Methylmercury (MeHg) Concentrations in the Water, Sediment, and Biofilm of the Dongmenqiao River[a]

	S1	S2	S3
physicochemical parameters of water			
pH	8.42 ± 1.01	8.54 ± 1.10	8.56 ± 0.92
temperature (°C)	24.5 ± 2.23	25.4 ± 2.10	25.5 ± 1.90
dissolved oxygen (mg L^{-1})	6.60 ± 1.00	6.30 ± 0.70	6.20 ± 1.10
salinity (g L^{-1})	0.39 ± 0.02	0.40 ± 0.07	0.39 ± 0.04
conductivity (ms cm^{-1})	8.93 ± 2.10	9.22 ± 1.70	8.98 ± 1.50
total suspended solids (mg L^{-1})	23.1 ± 2.00	22.6 ± 2.32	20.2 ± 4.25
Hg concentrations			
THg in water (ng L^{-1})	202 ± 4.84	112 ± 2.46	93.4 ± 1.70
MeHg in water (ng L^{-1})	0.17 ± 0.02	0.30 ± 0.17	1.45 ± 0.01
THg in sediment (mg kg^{-1})	4.63 ± 0.55	51.3 ± 2.65	13.7 ± 1.03
MeHg in sediment (μg kg^{-1})	35.1 ± 2.47	44.9 ± 3.67	18.3 ± 0.59
THg in biofilm (mg kg^{-1})	0.69 ± 0.01	4.93 ± 0.87	4.52 ± 0.79
MeHg in biofilm (μg kg^{-1})	9.92 ± 0.69	10.7 ± 1.32	10.8 ± 0.81

[a]S1, S2, and S3 represent sampling sites in the upstream, middle, and downstream of the Dongmenqiao River. Values, represented as mean ± SEM of three replicate

Effect of the Environmental Matrix on Mercury Bioaccumulation in Medaka. The THg and MeHg

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Table 2. Absolute Amounts of Total Mercury (THg) and Methylmercury (MeHg), THg and MeHg Concentrations in Medaka, Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx) Activities, Malondialdehyde (MDA), Interleukin-1β (IL-1β), and Tumor Necrosis Factor-α (TNF-α) Levels in Medaka Liver, and Malformation Rates from Different Experimental Treatments[a]

		absolute Hg amount in the treatments		Hg in medaka		oxidative stress in medaka liver			inflammation in medaka liver		
controls	THg (ng L^{-1})	THg (μg)	MeHg (ng)	THg (ng g^{-1})	MeHg (ng g^{-1})	SOD (U mg^{-1})	GPx (U mg^{-1})	MDA (nmol mg^{-1})	TNF-α (ng L^{-1})	IL-1β (ng L^{-1})	malformation rate (%)
$HgCl_2$	0	0.014	–	11.1 ± 0.20^{a}	0.79 ± 0.07^{a}	330 ± 15.9^{a}	512 ± 10.0^{a}	5.10 ± 1.20^{a}	154 ± 13.3^{a}	35.3 ± 2.83^{a}	0
$HgCl_2$	90	0.46	–	14.7 ± 0.50^{a}	1.07 ± 0.09^{a}	334 ± 16.7^{a}	461 ± 23.1^{a}	6.14 ± 0.31^{a}	150 ± 12.1^{a}	37.1 ± 2.97^{a}	3.52 ± 1.07^{a}
$HgCl_2$	120	0.61	–	17.3 ± 0.71^{a}	1.25 ± 0.11^{a}	252 ± 12.6^{b}	353 ± 17.6^{b}	8.30 ± 0.42^{b}	163 ± 12.7^{a}	36.2 ± 2.89^{a}	6.33 ± 1.21^{b}
$HgCl_2$	150	0.76	–	19.3 ± 0.20^{a}	1.48 ± 0.10^{a}	231 ± 10.1^{b}	281 ± 12.0^{c}	9.76 ± 0.89^{b}	182 ± 10.2^{ab}	40.1 ± 1.98^{a}	8.05 ± 1.23^{b}
$HgCl_2$	180	0.91	–	18.7 ± 1.10^{a}	2.11 ± 0.16^{a}	178 ± 0.35^{c}	246 ± 10.3^{c}	10.9 ± 0.20^{bc}	192 ± 6.32^{b}	44.2 ± 1.52^{b}	11.4 ± 1.35^{c}
$HgCl_2$	210	1.06	–	23.8 ± 1.00^{b}	2.40 ± 0.21^{b}	172 ± 8.58^{c}	166 ± 8.28^{d}	12.1 ± 0.60^{c}	203 ± 15.0^{b}	46.5 ± 3.73^{b}	13.1 ± 2.17^{c}
treatments	site										
W	S1	1.00 ± 0.02	0.85 ± 0.01	65.8 ± 3.30^{c}	9.58 ± 0.81^{c}	379 ± 18.9^{a}	463 ± 23.1^{a}	9.30 ± 0.47^{b}	158 ± 12.6^{a}	38.1 ± 3.05^{a}	39.3 ± 8.14^{d}
W	S2	0.56 ± 0.10	1.50 ± 0.01	66.4 ± 3.40^{c}	10.0 ± 0.82^{c}	342 ± 17.1^{a}	449 ± 22.5^{a}	8.18 ± 0.41^{b}	163 ± 13.0^{a}	41.0 ± 3.28^{a}	36.1 ± 5.91^{d}
W	S3	0.47 ± 0.01	7.00 ± 0.01	75.1 ± 3.70^{c}	11.6 ± 0.99^{c}	264 ± 13.2^{b}	427 ± 21.3^{a}	6.53 ± 0.33^{a}	178 ± 14.3^{ab}	45.4 ± 3.64^{b}	27.5 ± 5.92^{d}
W + S	S1	(4.60 ± 0.60) × 10^3	(3.52 ± 0.20) × 10^4	135 ± 5.90^{d}	18.7 ± 1.59^{d}	288 ± 14.4^{b}	284 ± 14.2^{c}	14.9 ± 0.75^{c}	182 ± 14.6^{ab}	49.1 ± 3.93^{b}	56.2 ± 9.21^{e}
W + S	S2	(5.13 ± 2.60) × 10^4	(4.49 ± 0.20) × 10^4	128 ± 5.00^{d}	17.7 ± 1.50^{d}	268 ± 13.4^{b}	193 ± 9.63^{d}	13.8 ± 0.69^{c}	199 ± 15.9^{b}	66.2 ± 5.30^{c}	49.0 ± 7.12^{e}
W + S	S3	(1.37 ± 0.10) × 10^4	(1.83 ± 0.40) × 10^4	127 ± 4.90^{d}	16.7 ± 1.42^{d}	161 ± 8.03^{c}	172 ± 8.62^{d}	14.2 ± 0.71^{c}	192 ± 15.4^{b}	74.4 ± 5.96^{c}	53.9 ± 12.2^{e}
W + S + B	S1	(4.88 ± 0.40) × 10^3	(4.00 ± 0.50) × 10^4	165 ± 13.2^{e}	20.5 ± 1.74^{d}	224 ± 11.2^{b}	157 ± 7.87^{d}	18.4 ± 0.92^{d}	272 ± 21.8^{c}	84.0 ± 6.72^{d}	65.4 ± 10.0^{e}
W + S + B	S2	(5.33 ± 0.80) × 10^4	(5.03 ± 0.30) × 10^4	171 ± 12.5^{e}	22.1 ± 1.88^{d}	241 ± 12.1^{b}	127 ± 6.37^{d}	17.4 ± 0.87^{d}	294 ± 23.5^{c}	86.7 ± 6.93^{d}	57.0 ± 4.50^{e}
W + S + B	S3	(1.55 ± 0.10) × 10^4	(2.37 ± 0.50) × 10^4	167 ± 14.9^{e}	25.3 ± 2.15^{e}	188 ± 9.42^{c}	166 ± 8.27^{d}	16.2 ± 0.81^{cd}	282 ± 22.6^{c}	95.5 ± 7.64^{d}	55.2 ± 7.93^{e}

[a]All values are shown as mean ± SE (n = 3 per treatment). S1, S2, and S3 represent sampling sites in the upstream, middle, and downstream of the DMQ river. W, W + S, and W + S + B represent DMQ water, DMQ water and sediment, DMQ water, sediment, and biofilm exposure, respectively. The same column with different letters (a−e) indicates statistical difference by one-way ANOVA followed by the LSD test ($p < 0.05$).

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concentrations in medaka exposed to $HgCl_2$ solution ranged from 14.7 to 23.8 and from 1.07 to 2.40 ng g^{-1}, respectively (Table 2). However, a significant increase in THg and MeHg accumulation in medaka was only observed at 210 ng L^{-1} $HgCl_2$ as compared with the Hg-free control ($p < 0.05$). In comparison, DMQ water from all sites increased remarkably THg and MeHg accumulation in medaka. The THg and MeHg concentrations in medaka exposed to DMQ water (65.8−75.1 and 9.58−11.6 ng g^{-1}, respectively) were significantly higher than those exposed to $HgCl_2$ solution ($p < 0.05$). This finding suggests that river water exposure increased Hg availability to medaka. In nature, fish accumulate Hg either directly from the aqueous phase or from dietary food sources (i.e., trophic transfer).[42] The aqueous uptake usually represents 18−68% inorganic Hg accumulation in fish, whereas MeHg accumulation in fish occurs mainly via ingesting MeHg-containing food.[43] Generally, there are plenty amounts of suspended matter in natural river water (20.2−23.1 mg L^{-1}, Table 1), which plays an important role in the transport of inorganic Hg and MeHg in aquatic environments, for a large proportion of Hg in the aqueous phase is attached to suspended particles.[44] Suspended matter consists of inorganic particles and particulate organic matter as well as biogenic particles such as bacteria, algae, and phytoplankton. Inorganic Hg tends to bind more strongly to mineral particles and detrital organic matter, whereas MeHg is more strongly associated with biogenic particles.[45,46] Although the presence of suspended particles and dissolved organic substances should limit the biotic dissolve uptake of Hg via gills and surface adsorption,[47] 3 times higher Hg accumulation in medaka was still observed in W treatments than in the controls with equivalent Hg doses. In parallel to dissolved uptake,[47] suspended particles could be ingested, and part of Hg associated could be desorbed in the digestive tract and absorbed by medaka. Similar ingestion of suspended particles associated with polycyclic aromatic hydrocarbons has been evidenced in the case of zebrafish.[48] Among suspended particles, the nanoparticle has a particularly high specific surface area to enrich aqueous Hg^{2+}.[49,50] The nanosized Hg particle has been widely detected in the muscles of fishes (e.g., tuna, swordfish, salmon, and trout).[51] In the case of the very small nanosized Hg particle (e.g., 3−4 nm), its bioavailability could be even higher than dissolved Hg.[50] These factors, together with dietary exposure, explain the greater importance of ingestion than aqueous uptake for the overall Hg and MeHg accumulation in fish.[42]

Sediment and biofilm remarkably decreased Hg bioavailability to medaka. Although the average THg and MeHg amounts of sediment and biofilm in each treatment were 3−4 orders of magnitude higher than those of DMQ water, the accumulation of THg and MeHg in medaka increased less than 3 times (Table 2). Taking the difference in THg and MeHg concentrations in medaka among treatments of different medium combinations, we calculated the net Hg accumulation in medaka taken up from Hg in each environmental medium. The results indicated each μg of Hg appearing in different environmental media increased THg concentrations in medaka in order of fish diet (241 kg^{-1}) > river water (48.8−125 kg^{-1}) > $HgCl_2$ in water (7.50−12.1 kg^{-1}) > biofilm (0.022−0.107 kg^{-1}) > sediment (0.001−0.015 kg^{-1}; Table S1), reflecting that the availability of Hg to medaka followed the same trend. Such order agreed well with Hg mobility in the order of water ≫ biofilm > sediment Hg based on the K_d values (previous section) and sediment and biofilm decreased about 10^5 and 10^3 times Hg availability to medaka, respectively. Apparently, the uptake of Hg from water is the decisive pathway for Hg bioaccumulation in medaka next to dietary exposure. Similar to medaka, low bioavailability of sediment Hg was also reported by Olsvik et al., in which Atlantic cod (*Gadus morhua*) larvae were exposed to Hg-contaminated sediments (16.0−19.5 mg Hg kg^{-1}), but THg and MeHg concentrations in fish only slightly increased by 30 ng g^{-1}.[15] de Carvalho et al. also observed that the Hg present in contaminated sediments was scarcely available to the *Danio rerio* and *Oreochromis niloticus*, for there was no difference in Hg concentrations in these two fishes before and after the sediment exposure experiment.[14] Moreover, Issa et al. showed that the presence of a biofilm could significantly reduce 60−70% accumulation of inorganic Hg in *Daphnia* by decreasing bioavailable Hg in the aqueous phase.[16] Such conclusion was based on the fact that *Daphnia* accumulated Hg(II) mainly through Hg absorption from the aqueous phase rather than from ingesting Hg-containing solid phases, e.g., biofilms and sediments.[16]

It was also found that the accumulation pattern of Hg in medaka differed remarkably among matrix-containing and -free treatments (Table 2). Namely, there were strong and significant correlations between THg ($r = 0.960$, $p < 0.01$) and MeHg concentrations ($r = 0.930$, $p < 0.01$) in medaka and THg amounts in matrix-free treatments (Figure S4), revealing that Hg accumulation in medaka in matrix-free treatments was dose-dependent. On the other hand, among the treatments with the same environmental media, THg and MeHg concentrations in medaka varied little with THg amounts in treatments ($p > 0.05$), so that Hg accumulation in medaka exposed to the environmental matrix was much less dose-dependent. Therefore, we may conclude that Hg accumulation in medaka might not be a suitable indicator to reflect Hg pollution in real environmental media.

Mercury Methylation in Medaka. Since our fish diet contained negligible MeHg, inorganic Hg was the only form of Hg presented in the $HgCl_2$-free and -containing controls. Together with the almost constant quantity of fish diet given in each control and treatment, 2−6 times higher MeHg concentrations in medaka in all $HgCl_2$ controls than those prior to exposure experiments ($p < 0.05$; Table S1) may mirror the ability of medaka to in vivo methylate Hg. In fact, Hg methylation in fish has been addressed in prior studies. For instance, Rudd et al. found that intestinal contents of freshwater fish were capable of converting Hg^{2+} to MeHg at 0.5−4% day^{-1}.[52] Wang et al. reported that 0.67−1.6% of the ingested Hg^{2+} can be converted into MeHg in tilapia muscle.[53]

Generally, MeHg accumulation in medaka increased with increasing THg concentrations in medaka (Figure 1a). Interestingly, MeHg-to-THg ratios observed in medaka from $HgCl_2$ controls (12.9%) and matrix-containing treatments (12.4%) were almost identical, suggesting that the environmental matrix influenced in vivo Hg methylation by medaka limitedly. In matrix-containing treatments, MeHg concentrations in medaka correlated additionally with total MeHg amounts in all treatments ($r = 0.775$, $p < 0.05$; Figure 1b). Nevertheless, such a correlation was much weaker and less significant than that with THg concentrations in medaka ($r = 0.973$, $p < 0.01$). According to the regression slopes, MeHg in environmental media increased the MeHg concentrations (0.02%) in medaka far less than in vivo methylation by medaka (~12%), pinpointing that MeHg in medaka originated

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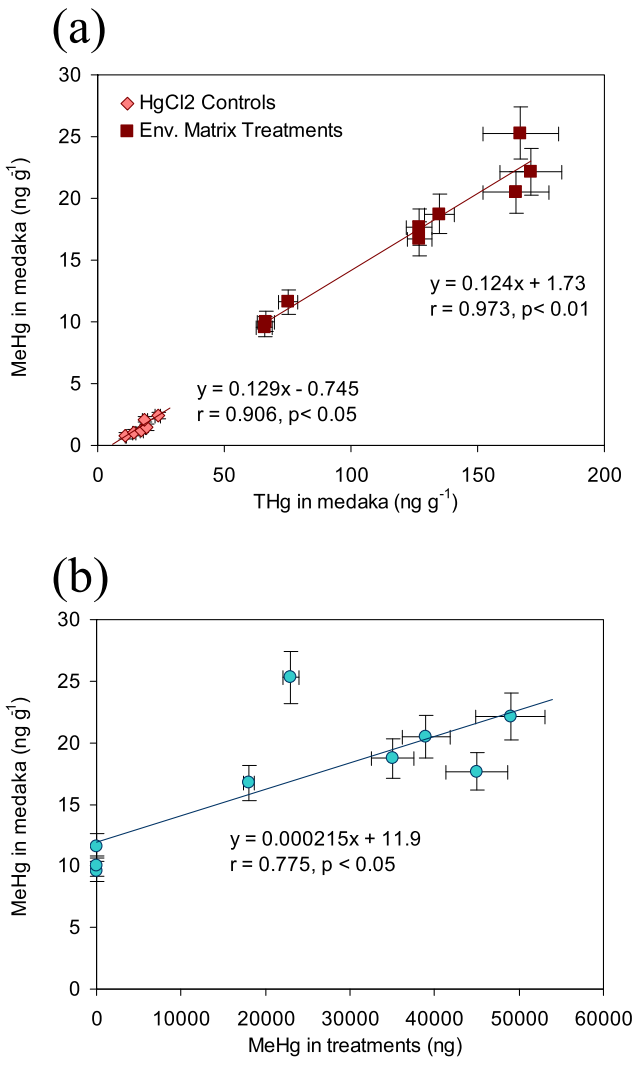

Figure 1. Correlations between (a) total mercury (THg) and methylmercury (MeHg) concentrations in medaka in the $HgCl_2$ controls and treatments containing environmental matrices and (b) MeHg concentrations in medaka and the total MeHg amount in the treatments containing environmental matrices. Mean values and standard deviations of three replicates are shown.

prevalently via in vivo Hg methylation rather than uptake from environmental media.

Effects of $HgCl_2$ Exposure on Mercury Toxicity in Medaka Liver. Detectable oxidative stress and inflammatory injury were generally observed in all $HgCl_2$ controls by decreasing SOD and GPx activities and increasing MDA, IL-1β, and TNF-α levels in medaka liver relative to the $HgCl_2$-free controls (Table 2). In parallel, the toxicity of Hg was also reflected by the malformations in medaka exposed to $HgCl_2$, i.e., spinal curvature and internal area bleeding (Table 2). In the $HgCl_2$-free control, there was no significant malformation in medaka. However, medaka malformations were observed in all $HgCl_2$ controls, with rates increased from 3.52% in 90 ng L^{-1} to 13.1% in 210 ng L^{-1} $HgCl_2$ controls. Surprisingly, significantly elevated IL-1β and TNF-α levels in medaka liver were first observable at 180 and 210 ng L^{-1} $HgCl_2$, respectively, which increased by 25−32% for TNF-α and 104−153% for IL-1β. Such an observation revealed that Hg^{2+} concentrations lower than 180 ng L^{-1} would not cause any marked inflammatory injury in medaka liver. In comparison, significant changes in SOD and GPx activities as well as MDA levels as compared to the $HgCl_2$-free control have already been found at 120 ng L^{-1} (Table 2), decreased by 25−104% for SOD and 31−68% for GPx and increased by 63−138% for MDA, reflecting that oxidative stress is more sensitive to Hg toxicity than inflammatory injury in medaka liver.

Oxidative stress is a common mechanism underlying Hg-induced hepatotoxicity.[54] This mechanism mainly occurs due to the accumulation of ROS and the impairment of enzymes and antioxidants that serve to detoxify ROS. Superoxide dismutase catalyzes the superoxide anion radical into H_2O_2, while GPx is responsible for catalyzing the decomposition of H_2O_2 and lipid hydroperoxides to prevent the production of ROS.[55] In addition, the MDA level is often used to monitor lipid peroxidation, which has been known as a major contributor to the impairment of cell function under oxidative stress.[56] Thus, the significant changes in SOD and GPx activities as well as MDA levels in the medaka liver in this study indicated an active response to counteract oxidative stress. Inflammation is also a mechanism of Hg-induced toxicity.[57] Oxidative stress can alter immune competence and therefore has been considered as a mechanism for Hg-induced immunotoxicity.[20] Excessive ROS generation has been evidenced to trigger proinflammatory cytokine (e.g., IL-1β and TNF-α) production in immune cells,[24] which may explain why oxidative stress is more sensitive to medaka than inflammation. Here, administration of $HgCl_2$ significantly increased TNF-α and IL-1β levels, reflecting inflammatory responses.

Besides medaka, similar toxic effects caused by $HgCl_2$ and CH_3HgCl exposure have been evidenced in, e.g., zebrafish, *Ictalurus melas*, and Korean rockfish, but at much higher Hg levels in waters (1−30 μg L^{-1}).[20,22,57,58] For example, the SOD activity of male zebrafish increased by ∼20% after 30 day of exposure to 15 μg L^{-1} $HgCl_2$ treatment.[57] Also, there were decreased GPx activities in zebrafish larvae and significantly upregulated expressions of IL-1β and TNF-α levels in the liver of zebrafish larvae when exposed to 4 and 16 μg L^{-1} $HgCl_2$ for 7 days.[20] Based on the aforementioned results, it could be concluded that inorganic Hg-induced oxidative stress and immune responses were variable for different fish species, and medaka could react more sensitively to Hg^{2+} than zebrafish and Japanese flounder.

The changes of all toxicological parameters investigated show a linear response not only to the Hg concentrations in medaka ($r \geq 0.89$, $p < 0.05$, Figure 2) but also to $HgCl_2$ concentrations in the $HgCl_2$ controls ($r \geq 0.91$, $p < 0.05$, Figures S5 and S6). Accordingly, in the matrix-free systems, the Hg toxic effect on medaka was governed by the bioavailability and bioaccumulation of Hg and could be predicted by Hg concentrations in not only medaka but also water.

Impact of the Real Environmental Matrix on Hg Toxicity in Medaka Liver. Surprisingly, although 3−4 times higher Hg concentrations in medaka were found in DMQ water than in $HgCl_2$ controls, there was almost no increase in oxidative stress and inflammatory injury (Table 2). Accordingly, the matrix presented in river water such as dissolved organic matter (DOM) has effectively mitigated Hg toxicity in fish. Natural DOM, even at low concentrations, e.g., ∼3 mg L^{-1}, strongly complexes with Hg^{2+} and CH_3Hg^+.[59] The DOM, especially with aromatic thiols, could bind directly with Hg to prevent the binding of Hg with L-glutathione in the

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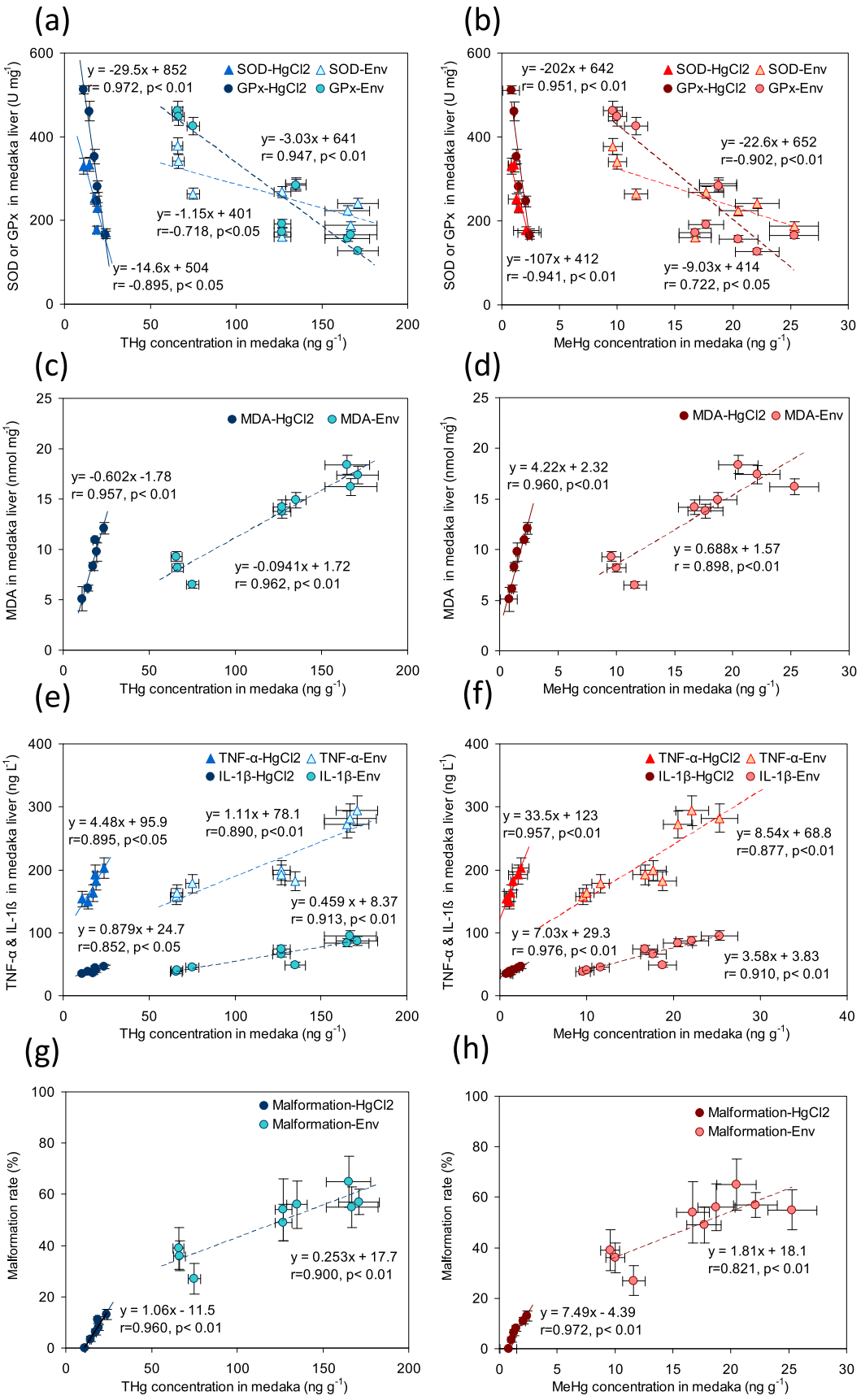

Figure 2. Correlation of superoxide dismutase (SOD), glutathione peroxidase (GPx) activities (a, b), malondialdehyde (MDA) levels (c, d), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) levels (e, f) in medaka liver, and malformation rates (g, h) with total mercury (THg) and methylmercury (MeHg) concentrations in medaka in $HgCl_2$ controls and treatments containing the environmental matrix. Mean values and standard deviations of three replicates are shown.

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intercellular and thus reduce Hg-induced toxicity.[60] A similar result has been found by Li et al. that DOM can effectively mitigate the MeHg toxicity in embryonic zebrafish.[61]

Compared with W treatments, the toxic effect caused by Hg in W + S and W + S + B treatments became more remarkable and all significantly different from the $HgCl_2$ control ($p < 0.05$; Table 2). The values of MDA, TNF-α, and IL-1ß were about 3, 1.5, and 2 times higher in W + S treatments and 4, 2, and 3 times higher in W + S + B treatments than the $HgCl_2$ control, respectively. Similarly, the decreased extent of SOD and GPx activities were more notable in W + S + B treatments (decreased by 34 and 71%, respectively) than in W + S treatments (decreased by 28 and 58%, respectively). Thus, among all matrix-containing treatments, the toxic effect of Hg to medaka was in the general order of W + S + B > W + S > W treatments. It is noteworthy that the toxicological parameters of medaka correlated significantly with THg and MeHg concentrations in medaka, independent of the environmental matrix ($p < 0.05$; Figure 2) but not with THg amounts in the treatments ($p > 0.05$). Such findings highlight the following: (1) Both oxidative stress and inflammation induced by Hg in medaka were more likely governed by Hg accumulation in medaka. In the presence of a real environmental matrix, the concentration or amounts of THg in treatments cannot reflect Hg toxicity. (2) The toxic effect caused by Hg to medaka was less dependent on the environmental matrix. After Hg intake, the toxicity of Hg to medaka seems to be similar regardless of its environmental origin. Apparently, different Hg species that occurred in the presence of the environmental matrix, e.g., organic matter-complexed Hg and particle-associated Hg, underwent similar transformation and metabolism processes in fish, which should be addressed in the follow-up research to support such a hypothesis.

Based on this, we presumed that the influence of the environmental matrix on Hg toxicity in medaka liver can be evaluated by comparing regression line slopes in matrix-containing and -free treatments. Here, we observed all steeper regression slopes for the matrix-free treatments than the DMQ matrix-containing treatments for not only all toxicological parameters but also malformation rates (Figure 2), reflecting again the mitigating effect of the environmental matrix on Hg toxicity. Notably, such a mitigating effect varied among different toxicological parameters. More concretely, the slopes of SOD, GPx, and MDA levels on THg concentrations in medaka of the matrix-free treatments were 12.7, 9.75, and 6.38 times steeper than those of matrix-containing treatments, respectively (Figure 2). These indicated the mitigative effect of the environmental matrix on oxidative stress in medaka liver in the order of SOD > GPx > MDA, which might be related to their role in the antioxidant process. The SOD−CAT system was the first defense line against ROS.[62] Superoxide dismutase catalyzes the dismutation of superoxide into H_2O_2, which is in turn broken by GPx,[55] and MDA is the major product of lipid peroxidation caused by the decrease of antioxidant enzyme activity.[56]

As to the inflammatory factor levels, the slopes for TNF-α and IL-1β levels on THg concentrations in medaka of matrix-free treatments were 4.04 and 1.92 times steeper than those of matrix-free treatments, respectively (Figure 2), suggesting a higher mitigating effect of the environmental matrix on TNF-α than the IL-1β level in medaka liver. Moreover, the difference in the slope for cytokines was on average smaller than that for antioxidant enzyme activities, indicating that the environmental matrix had stronger mitigating effects on the oxidative stress than on immunotoxicity (Figure 2). Namely, the inflammatory impairment often follows oxidative stress induced by Hg hepatotoxicity.

It is also noted that regression line slopes of toxicological parameters with MeHg contents in medaka were remarkably steeper than those of THg (Figure 2). This could reflect the general higher toxicity of MeHg than inorganic Hg.[22] Similarly, dietary CH_3HgCl uptake has been evidenced to have higher oxidative stress than $HgCl_2$ in Korean rockfish.[22] These altogether reflected the dependence of the Hg toxicity on its chemical form. Methyl Hg can bind more efficiently to cysteine in fluids mimicking methionine membranes by amino acid transporters, which facilitates MeHg transport to tissues.[63] However, due to the higher THg concentrations than MeHg and Hg methylation in medaka, we are not able to truly differentiate whether the environmental matrix impacts MeHg toxicity differently from inorganic Hg to medaka. Further toxicity testing with only MeHg is essential to address these hypotheses.

■ ENVIRONMENTAL IMPLICATIONS

In summary, we have identified a new significance of the environmental matrix in aquatic Hg biogeochemistry, namely, mitigating Hg toxicity after its uptake in fish. Our results demonstrated the presence of sediments and biofilms capable of reducing Hg bioavailability to fish, buffering the ecotoxicological impact of Hg in the aquatic ecosystem. On the other hand, the matrix in river water may increase the availability of THg and MeHg to fish. The environmental matrix taken up together with Hg by fish can substantially mitigate Hg toxicity, e.g., in the order of SOD > GPx > MDA > TNF-α > IL-1β to medaka. Such an effect was independent of the environmental media. Accordingly, it should be noted that toxicity tests based on matrix-free designs cannot reflect the true Hg toxicity to fish. Moreover, future designs for Hg toxicity tests to fish should consider more carefully the impact caused by the environmental matrix, e.g., by not only including the environmental matrix but also considering the changing physicochemical conditions under river flowing conditions. Also, this study raises an open question of whether such a mitigating effect could also be so significant for other aquatic organisms. Furthermore, the underlying mechanisms of the mitigating effects caused by the environmental matrix during digestion and metabolism in fish need to be explored in greater depth in future studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c05408.

Further information on statistical analysis; figures (the sampling sites along the Dongmenqiao River (DMQ), schematic diagram of the experimental unit, explanation of Hg adsorption to aquaria, correlation between Hg concentrations in medaka and THg amounts in $HgCl_2$ controls, correlation between SOD and GPx activities and MDA levels in medaka liver and $HgCl_2$ amounts in matrix-free treatments, and correlations of TNF-α and IL-1β levels in medaka liver and $HgCl_2$ amounts in matrix-free treatments); and table (increasing rate (kg^{-1}) of THg and MeHg concentrations in the medaka

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muscle caused by Hg amounts in fish diet, spiked $HgCl_2$, and environmental matrix). (PDF)

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Notes

The authors declare no competing financial interest.

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