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Assessment of Environmental Pollution of Taihu Lake by Combining Active Biomonitoring and Integrated Biomarker Response

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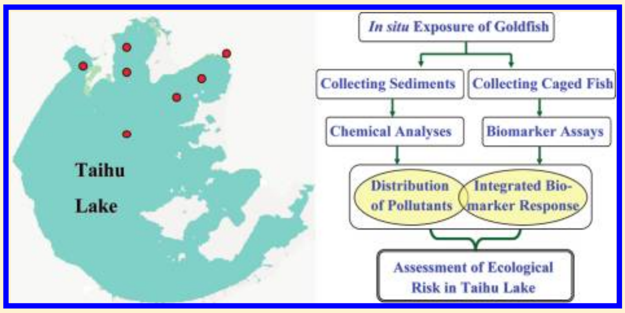
S Supporting Information

ABSTRACT: Goldfish (*Carassius auratus*) bred in clean water were transferred into different stations in Taihu Lake for active biomonitoring exposures. The biotransformation enzymes 7-ethoxyresorufin-O-deethylase (EROD) and glutathione-S-transferase (GST), the antioxidant defense enzymes catalase (CAT), reduced glutathione content (GSH), and lipoperoxidation (TBARS) and metallothionein (MT) in liver were determined as biomarkers during the field exposure period. At the same time, the contents of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and heavy metals in the surface sediments of biomonitoring sites were measured. The total concentrations of PAHs ranged 378.6−1047.8 μg kg^{-1} dry weight, PCBs ranged 0.76−3.27 μg kg^{-1}, OCPs ranged 1.12−3.08 μg kg^{-1}, and heavy metal Zn ranged 31.82−85.04 mg kg^{-1}, Cu ranged 13.04−91.02 mg kg^{-1}, Ni ranged 4.96−28.66 mg kg^{-1}, and Pb ranged 9.33−22.44 mg kg^{-1}. The results indicate that persistent organic pollutants exist mainly in Meiliang Bay, while heavy metals are present mainly in Gong Bay. The integrated biomarker response (IBR) was calculated by combining multiple biomarkers to a single value and used to evaluate the toxically induced stress level of populations in different areas. The results demonstrate that Tuoshan and Mashan in Meiliang Bay as well as Dagongshan in Gong Bay showed higher IBR values compared to the other sites. IBR values were in good agreement with OCP concentrations.



INTRODUCTION

Taihu is the third largest freshwater lake in China. The lake water has been used for agricultural and industrial purposes and as the major drinking water source for several cities, including Shanghai, Suzhou, and Wuxi. With rapid population rise, as well as industrial and agricultural developments near the lake, the water quality in Taihu Lake has been deteriorating and noxious algae blooms have been occurring with increasing frequency in the lake.[1] The rapid spread of water pollution and the speedup of eutrophication among Taihu Lake Basin in recent years is becoming the primary factor that restricts sustainable development of economy in that region. Since Taihu Lake has an access to the Yangtze River, where algae is absent and nutrient concentration is relatively low, transfer of water from the Yangtze River into Taihu Lake to reduce severe algae bloom and improve water quality was proposed and has been carried out since 2002.[2] In the water diversion, water from the Yangtze River is introduced into Gong Bay located in northeastern Taihu Lake through Wangyu River. Water in the lake is discharged through Zhihu Port on the north side and Taipu River on the southeast side. Water transfer had positive effects on decreasing the concentration of phytoplankton and total nitrogen in some subzones though pollution of persistent organic pollutants and heavy metal remains, especially in the northern end of Taihu Lake.[3,4]

Various biochemical parameters in fish have been tested for their responses to toxic substances and their potential use as biomarkers of exposure or effect.[5] The use of biomarkers as surrogate measures of biological impact of contaminants within the environment has been studied in several polluted areas. A multiple biomarker approach combined with chemical analysis could provide better evaluation of the environmental hazard.[6] Active biomonitoring (ABM) involves the transplantation of organisms that are collected from a (generally) unstressed, unpolluted population to selected polluted sites. The chemical and biological consequences of this translocation, which usually involves caging of the organisms, can then be monitored in space and time, to assess the effects of exposure on selected end points.[7] Since van der Oost et al. first used biomarker responses in caged carp (*Cyprinus carpio*) to assess water pollution,[8] ABM has been extensively used in ecosystem health assessment, and produces better results than passive biomonitoring since organisms already present in situ may have adapted to the pollutants.

Biotransformation phase I enzyme 7-ethoxyresorufin-O-deethylase (EROD) and phase II enzymes glutathione S-transferase

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(GST) activities have been used as biomarkers for exposure to polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs).[9,10] The antioxidant defense enzymes such as catalase (CAT), reduced glutathione content (GSH), and lipoperoxidation (TBARS) have been proposed as biomarkers of contaminant mediated oxidative stress in a variety of marine and freshwater organisms and their change reflects a specific response to variable pollutants including pesticides and heavy metals.[11−13] Metallothionein (MT) measurements have also been performed in transplanted fish since they are useful indicators of heavy metal exposure.[14] The purposes of our study were to investigate the occurrence, level, and spatial variation of persistant organic pollutants and heavy metals and to determine multiple biomarker responses of fish in different districts of Taihu Lake influenced by the water diversion project. The integrated biomarker response (IBR) was computed with biomarker measurements obtained in transplanted fish to assess the ecological risk of the polluted area of Taihu Lake.

MATERIALS AND METHODS

Animals. The goldfish (*Carassius auratus*) can be found in freshwaters throughout China and was demonstrated to be a very sensitive species in the study of biotransformation responses.[15] Immature goldfish of both sexes weighing 154.2 ± 25.6 g were obtained from the Nanjing Institute of Fishery Science. The fishes were acclimatized for two weeks in dechlorinated municipal water prior to the test. Fish were fed every day with commercial fish food. Feces and uneaten food were removed every day by suction.

Study Area. According to the flow characteristics of Taihu Lake and the water transfer route of the diversion project, the study was conducted in several districts (Figure 1), including the conveying water district (S1, the outlet of Wangyu River), Gong Bay (S2, Xiaogongshan and S3, Dagongshan), Meiliang Bay (S4, Tuoshan and S5, Mashan), Zhushan Bay (S6), and the diluting area of transferred water (S7 near lake center). The control fish were cultured in dechlorinated municipal water in a laboratory in Hohai University because various pollutants are present in the whole Taihu Lake and a field negative control site for comparison was not available. Water temperatures ranged from 8 to 10 °C, with pH 7.0 ± 0.2, and dissolved oxygen (DO) of 8.5 ± 0.5 mg L^{-1} for the control during the test period.

Active Biomonitoring Techniques. The goldfish were transferred from the laboratory and deployed into Taihu Lake at the seven monitoring sites (S1−7 in Figure 1) in December 2009. The bioindicator organisms were deployed in nontoxic columnar polyethylene cages (70 cm diameter and 1.5 m high) in groups of six and suspended in the water at about 20 cm below the water surface. Each cage was fixed by three wooden pegs. Three fish were collected and delivered to the laboratory at each station after 7 and 14 d of exposure, respectively. At the same time, two surface sediment samples were collected at each ABM station with a stainless steel grab sampler and used for chemical analysis. In the laboratory fish were killed by cervical transection and livers were collected. Liver tissues were carefully dissected, washed in 0.15 M of cold KCl, weighed, immediately frozen in liquid nitrogen, and stored at −80 °C. The following physicochemical water quality properties were determined in situ at all seven sites: pH, temperature, DO, and conductivity using portable meters.

Chemical Analyses. Sediment samples were dried-frozen, spiked with surrogate standards, and extracted in a Soxhlet

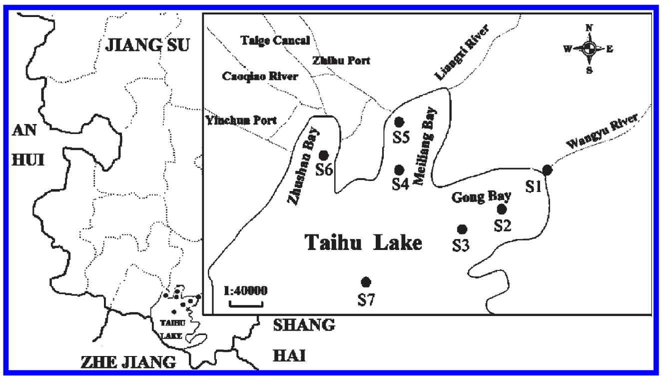

Figure 1. Location of the study area and active biomonitoring sites in Taihu Lake.

apparatus. The concentrations of 16 PAHs, identified as priority pollutants by the U.S. Environmental Protection Agency (EPA), were determined using a gas chromatograph (Thermo Fisher) equipped with a splitless injector and coupled to a flame ionization detector (FID) according to method described in EPA 8100. Ten PCB congeners (PCB 28, 52, 101, 112, 118, 138, 153, 155, 180, 198) and OCPs (hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethanes (DDTs)) were determined using a gas chromatograph (Thermo Fisher) equipped with a splitless injector and coupled to a ^{63}Ni electrical capture detector (ECD), as described in EPA 8082 and EPA 8081A, respectively. The concentrations of Zn, Cu, Ni, and Pb elements in sediments were measured using an ICP-MS (Leeman Laboratories) after digestion. Further details are provided in the Supporting Information.

Biomarker Assays EROD, GSH, GST, TBARS, CAT, and MT. Liver samples were homogenized in nine volumes of cold buffer (0.15 M KCl, 0.1 M Tris−HCl, pH 7.4) and centrifuged for 25 min (9000*g*) at 4 °C. The supernatants were used as the extract for enzymatic activity determination. The use of S9 fractions is convenient and relatively cheap compared to using cytosol and microsomes. EROD activity was quantified at 572 nm using a microplate reader.[15] GSH content was determined at 405 nm following the method of Redegeld et al.[16] GST activity was determined at 340 nm using 1-chloro-2,4-dinitrobenzene as a substrate.[17] TBARS was determined to reflect the state of lipid peroxidation of cell membranes.[18] CAT activity was determined by the method of ammonium molybdate.[19] The concentration of MT was determined by Cd-hemoglobin saturation method.[20] Protein concentrations were determined with the Coomassie Protein Assay Kit,[21] with bovine serum albumin as the standard. The measurements were done on a microplate reader at 595 nm. Further details of biomarker assays are provided in the Supporting Information.

Calculation of the IBR. A method for integrating all the measured biomarker responses into one general "stress index", termed "Integrated Biomarker Response" (IBR),[22] was applied to evaluate an integrated impact of toxicants from different monitoring sites. Details of IBR calculation can be found in the Supporting Information.

Statistical Analysis. For each biomarker, the data were expressed as mean ± SD. All data from different treatments were checked for normality. Data from different stations were compared by a one-way analysis of variance (ANOVA) and statistically different treatments were identified by Dunnett's *t*

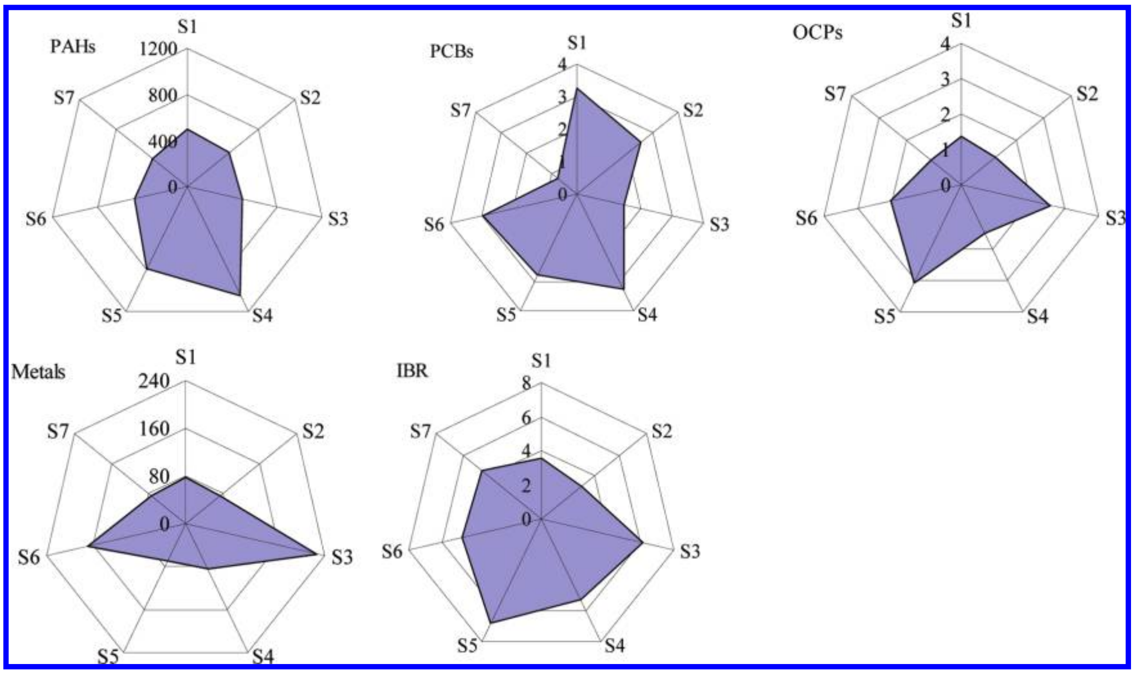

Figure 2. Star plots of ΣPAHs (μg kg^{-1}), ΣPCBs (μg kg^{-1}), ΣOCPs (μg kg^{-1}), total heavy metals (mg kg^{-1}), and IBR after 14 d exposure from seven monitoring sites.

test. All differences were considered significant at $p < 0.05$. Statistical analyses were performed using SPSS 12.0.

RESULTS AND DISCUSSION

Spatial Distribution of Pollutants. The physicochemical parameters of water during the period of exposure were obtained as follows: water temperature 7.9 ± 0.62 °C, pH 7.63 ± 0.16, DO 9.2 ± 0.85 mg L^{-1}, and conductivity 51.3 ± 3.83 ms m^{-1}. The concentrations of PAHs, PCBs, OCPs, and heavy metals in surface sediments at each site are shown in Tables S1−S4 of the Supporting Information. The spatial variation of pollutant concentrations is presented in Figure 2. The radius coordinates in these star plots are the total concentrations of PAH, PCB, OCP, or heavy metal in surface sediments at different sites, respectively. The highest total PAH concentrations were found in the sediments from S4 and S5 in Meiliang Bay, the total concentrations were 1047.8 and 800.0 μg kg^{-1} dry weight, respectively, and the lowest were detected in those from S7 in the lake center (378.6 μg kg^{-1}); the other sites (S1, S2, S3, and S6) showed a moderate PAH concentration (from 462.0 to 503.8 μg kg^{-1}). The predominant PAHs were three ring (such as fluorine, phenanthrene, and anthracene) and five ring (such as benzo(b)fluoranthene and benzo(g,h,i)perylene) aromatic hydrocarbons. The predominant PCBs were PCB 28 and PCB 118, the sum of which was more than 70% of total PCBs at all the sites except site 7. The sediments from the outlet of Wangyu River and Meiliang Bay presented higher total PCB concentration (3.27 and 3.25 μg kg^{-1} dry weight, respectively) compared to those from Gong Bay and Zhushan bay, while the lowest value was still found in the lake center. The highest OCP concentration was found in the sediments from site 5 in Meiliang Bay (ΣOCPs = 3.08 μg kg^{-1} dry weight), followed by site 3 in Gong Bay and site 6 in Zhushan Bay, whereas the lowest was from sites 1, 2, and 7 (1.12−1.36 μg kg^{-1}). The concentrations of Zn ranged from 31.82 (S5) to 85.04 (S3) mg kg^{-1}, Cu from 13.04 (S5) to 91.02 mg kg^{-1} (S3), Ni from 4.96 (S5) to 28.66 (S3) mg kg^{-1} and Pb from 9.33 (S1) to 22.44 (S3) mg kg^{-1}. The concentrations of the four metals were all highest in the sediments from S3 in Gong Bay.

The routine physicochemical parameters of the water in Taihu Lake show insignificant differences at various stations. However, the profiles of organic pollutants and heavy metals vary remarkably at different stations, as shown in Figure 2. Meiliang Bay has the most serious organic pollution compared with the other areas, while S3 in Gong Bay showed the highest contents of heavy metals. It is noticed that the concentrations of all selected contaminants at S1 were higher that those at S2. Many toxic organic pollutants including HCHs, DDTs, and PCBs were detected in the Yangtze River in a previous study, and the OCP level in sediments at Nanjing section of the Yangtze River was higher than that in Taihu Lake.[23] These contaminants could enter Taihu Lake by water diversion. Thus, PCBs at S2 and OCPs at S3 still sustained higher level. In contrast, Meiliang Bay is near the Wuxi Industrial Park and receives a large amount of effluent from wastewater treatment plants as well as untreated domestic sewage, and the pollution of persistent organic pollutants in the sediments was still serious. The water quality in Zhushan Bay was less affected by the diverting water. Instead, the decrease of organic pollutants and heavy metal concentrations was mainly attributed to the implementing the ecological dredging project in this bay. The concentration of PCBs was the highest at the outlet of Wangyu River (S1), while the other pollutants were at medium level in this area. In general, the levels of persistent organic pollutants were lowest at S7, although the concentrations of PAHs and OCPs as well as metal contents at S7 were comparative with S1 and S2.

Biomarker Responses during ABM. No mortality occurred during the caging experiment. Liver GSH and MT contents, EROD, GST, and CAT activities, and TBARS level in caged

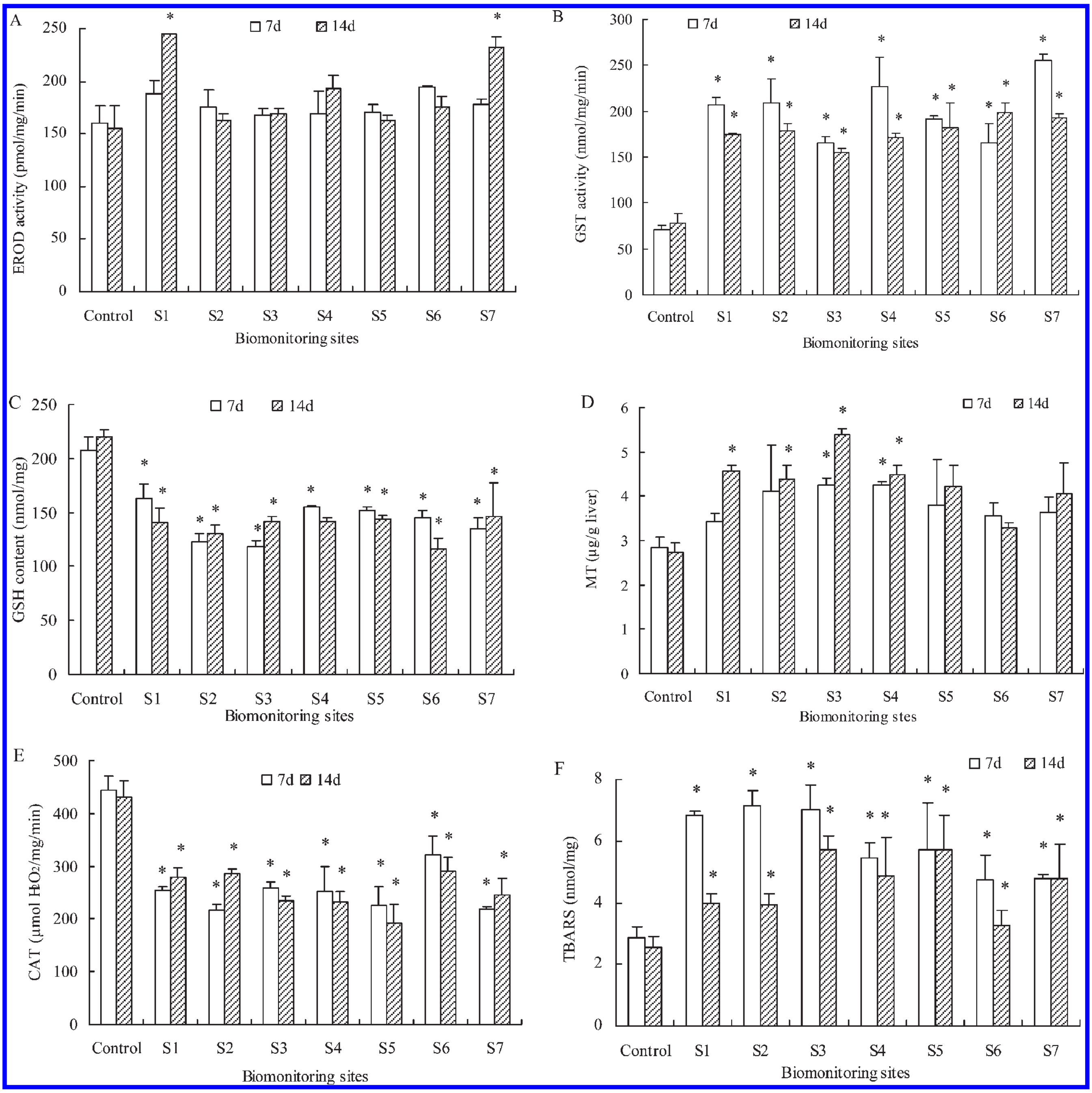

Figure 3. Biomarker responses measured in the liver of goldfish encaged at seven monitoring sites. Asterisks indicate values that are significantly higher than control values ($p < 0.05$).

goldfish are presented in Figure 3. The enzymatic activities in the control fish did not alter significantly during the experimental period. EROD activity was induced only at S1 and S7 after 14 d of exposure, while EROD activity did not differ significantly from the control at all other cases (Figure 3A). The cytochrome P4501A (CYP1A) is of critical importance in the metabolism of many xenobiotics. Induction of hepatic mixed-function oxidase enzymes of phase I, especially CYP1A and associated EROD activity, is considered a common indicator of exposure of fish to environmental pollutants, such as PCBs[10] and PAHs.[24] The injection dosages more than 1 mg kg^{-1} of benzo(b)fluoranthene, benzo(g,h,i)perylene, and indeno(1,2,3-cd)-pyrene were found to induce dose-dependent increases in EROD and GST activities in *Carassius auratus*.[15] However, EROD activity was increased about 50% only at S1 and S7. Possible reasons for the lack of EROD responses at the higher PAH and PCB polluted sites S4−6 are the short exposure times, less accuracy of S9 analyses compared to microsomal fractions due to substrate inhibition, and the presence of CYP1A inhibitor there, such as estrogenic substances.[25] Apparent lack of CYP1A response in fish from a PCB contaminated site was reported in a previous study.[26]

GST activity was substantially induced at all the sites during the exposure period, and the induction rate was higher at 7 d than that at 14 d for most sites (see Figure 3B). GST may play an important role in detoxifying strong electrophiles with toxic, mutagenic, and carcinogenic properties. It can catalyze the conjugation of the tripeptide glutathione with the xenobiotic in phase II of the biotransformation process and promote its elimination from the organism.[27] Elevated expression of GST has a protective effect against environmental carcinogens. Increased GST activity in fish liver has been demonstrated in various fish species as the result of exposure to PCBs,[28] PAHs,[15] and pesticides.[29] GST activity showed 1.3- to 2.3-fold increases at 7 d. However, there was not significant difference among all sites after 14 d of exposure. EROD and GST are considered related biomarkers, and the metabolites formed by phase I biotransformation are conjugated via phase II enzymes (e.g., GST) before excretion.

GSH content in the liver decreased significantly as compared with the control in all the cases. However, the inhibition rate did not change much during the exposure period (Figure 3C). GSH is involved in processes essential for synthesis and degradation of proteins, formation of deoxyribonucleotides, regulation of enzymes, and protection of cells against reactive oxygen species.[30] GSH content was reduced from 22% at S1 to 43% at S3 on day 7, and from 33% at S7 to 47% at S6 on day 14, respectively. The decrease in GSH content at the period of in situ exposure suggests a depletion of nonenzymatic antioxidant reserves. Several enzymes are related to the cellular GSH content, which regulates the synthesis, conjugation, and oxidation rates of GSH.[31]

MT content increased significantly at S3 and S4 on day 7 and at S1, S2, S3, and S4 on day 14. The most significant rise of MT content was observed at S3 after 14 d of exposure (Figure 3D). MTs are a family of ubiquitously occurring low-molecular-weight cysteine- and metal-rich proteins that function in metal detoxification (Cd and Hg) and essential trace metal (Cu and Zn) homeostatic mechanisms.[14] MTs have the capability to bind a large amount of metals. It can be induced by many metals, and they have been proposed as sensitive biomarkers of metal exposure in aquatic organisms.[32] MT concentrations elevated in all the sites during in situ exposure. The most significant MT induction was found at S3, where the concentration of heavy metals was highest. However, MT concentrations were observed to increase only 20−25% at S6 with higher metal levels. The reason of lacking response was probably that only four heavy metals were considered in this work, and other heavy metals such as Hg and Ag could exert important influence. In addition, MT synthesis may be reduced in the presence of high levels of organic contaminants due to an increased demand for cysteine residues for GSH synthesis. Estradiol and estrogenic PCBs appeared to inhibit cadmium-mediated MT induction in Arctic char.[33]

CAT is a major primary antioxidant defense component responsible for catalyzing the decomposition of H_2O_2 to H_2O and sharing this function with glutathione peroxidase (GSH-Px). CAT activity was inhibited significantly at all sites. The inhibition rate of CAT did not change significantly within the exposure duration (Figure 3E). In the presence of low H_2O_2 levels, organic peroxides are the preferred substrate for GSH-Px, but at high H_2O_2 concentrations, they were metabolized by CAT.[34] The higher inhibition rate of CAT was found at S3, S4, and S5 after 14 d of exposure, where the higher concentration of OCPs was detected compared to most other sites. It seems that the marked lowering of CAT activity could be the consequence of the increased O_2^- production caused by the accumulation of OCPs in fish.[35]

TBARS level reflects the state of lipid peroxidation of the membranes. TBARS level was significantly elevated at all sites after 7 d exposure, and the induction rate decreased at 14 d for most sites (Figure 3F). The increase of lipid peroxidation levels indicates an oxidative damage of hepatic cell membranes caused by pollutants. Damiens et al.[36] observed an especial elevation of TBARS in transplanted mussels in the Bay of Cannes and thought this oxidative stress may be due to copper or PCBs. TBARS levels in caged fish were increased 0.3- to 1.3-fold at 14 d, but not related to a single pollutant group in this study.

Integrated Biomarker Response. The IBR was calculated by combining different biomarkers to a single value, which can be used to describe the toxically induced stress level of populations in different areas. IBR index after 14 d of exposure in situ was evaluated and are represented as a star plot (see Figure 2). In general, the IBR values indicate a large range of variation at different monitoring sites. IBR values for stations S3, S4, S5, and S6 located in the northern bays of Taihu Lake are higher than those obtained at the other stations. The S5 located in Meiliang Bay shows the highest IBR value, whereas the S2 exhibits the lowest IBR value, and the difference between the two sites was approximately 2 times. The result at S7 with the least levels of organic pollutants exhibited a relatively higher IBR than S1 and S2 showed that other contaminants, not measured, might be present.

No single biomarker can unequivocally measure environmental degradation. The ability to differentiate between clean and polluted sites would be at best incomplete using a single biomarker approach.[37] A pool of available biomarkers, by allowing information to be summarized in the form of a multivariate data set, can provide a more valid basis for interpretation of ecotoxicological surveys.[22] Given that the IBR is an indicator of environmental stress, sites 3, 4, and 5 are most stressful places for fish, followed by sites 6 and 7. The least negative biological effects were found at sites 1 and 2, probably attributed to the pollutant dilution by water diversion.

Heavy metal star plot, as well as PAH, PCB, and OCP star plots, are also shown in Figure 2 for the purpose of comparison with IBR results. A visual agreement can be observed between the OCP gradient in the sediments and the IBR variation. However, the concentrations of other pollutants were not in accordance with the IBR variation (see Figure 2). Even so, high PAH and PCB levels observed at S4 seem to have influenced integrated biomarker response. The S3 shows a high MT score relative to the other stations, and the content of heavy metals was highest there. However, the very high heavy metal contents found at S6 do not seem to have influenced biomarker responses, especially MT and the IBR value. The biomarker responses at S5 and S6 could be caused by a mixture of organic contaminants including PAHs, PCBs, and OCPs.

IBR has been previously used as a possible tool for environmental risk assessment. Broeg and Lehtonen[38] calculated IBR using a biomarker set consisting of 4−6 biomarkers in flounder (*Platichthys flesus*), eelpout (*Zoarces viviparous*), and blue mussel (*Mytilus sp.*) populations of the Baltic Sea, and found the IBR was able to distinguish inter- and intraregional as well as seasonal differences of responses in all the three species studied. Damiens et al.[36] found that IBR values calculated from AChE, GST, and CAT activities and TBARS concentrations in the three successive experiments were in good agreement with copper and PCB concentrations in transplanted mussels but not with PAH concentrations. Pereira et al.[39] investigated metals accumulation and oxidative stress responses in gills of *L. aurata* in a eutrophic coastal system with a moderate contamination in winter and summer. It was found that intersite differences on the basis of IBR were more accentuated in winter, and that IBR represents a potential measure of *L. aurata* health at the óbidos lagoon to provide stronger evidence of the existing impact.

The present study demonstrates that the aquatic environmental quality seemed to be improved by transferring water from the Yangtze River at the outlet of Wangyu River (S1) and Xiaogongshan in Gong Bay (S2). Nevertheless, Tuoshan (S4) and Mashan (S5) in Meiliang Bay as well as Dagongshan (S3) showed higher IBR values compared to the other sites. When the water transfers were carried out, water in Wangyu River and Gong Bay was first replaced by the water from the Yangtze River. Therefore, the water transfers might have active effects on the ecosystem health in these districts since the nutrient concentration is relatively low in the Yangtze River.[40] Site 3 is located at the common boundary of Gong Bay and the center of lake, and some persistent organic pollutants and heavy metals were readily deposited there due to a decrease in flow velocity. Meiliang Bay is nearly an enclosed area of Taihu Lake and water in this zone was difficult to exchange with water from Gong Bay or the center of lake because of hydrodynamic conditions, and so the water transfers could not exert distinct ecological influences.[40]

■ ASSOCIATED CONTENT

S Supporting Information. Detailed description of chemical analyses, biomarker assays, and IBR calculation; Tables S1−S4: concentrations of PAHs, PCBs, OCPs, and heavy metals in surface sediments at seven biomonitoring sites. This information is available free of charge via the Internet at http://pubs.acs.org.

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