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Evidence from In Situ Bioassays and Suspect Analysis Revealed the Region-Specific Aquatic Risk across Socioeconomic Gradients in China

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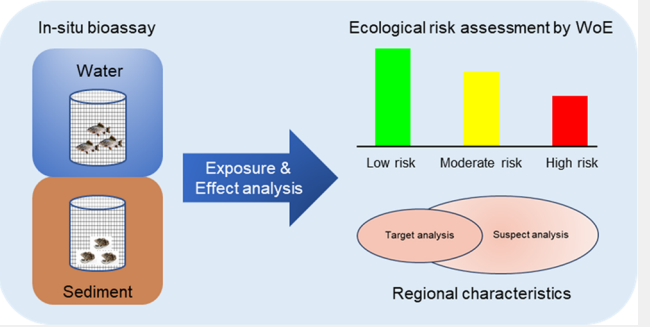
ABSTRACT: Various contaminants are present in aquatic environment and pose potential threats to pelagic and benthic organisms, calling for effective risk assessment. Traditional risk assessments based on target analysis are useful when the principal contaminants responsible for ecological risk are known; however, these approaches become challenging when dealing with chemical mixtures. In addition, the compositions of chemical mixtures often differ in regions with different levels of socioeconomic development, requiring risk assessment methods that are applicable under different pollution scenarios. Herein, *in situ* bioassays were conducted with two native species, Chinese rare minnows (*Gobiocypris rarus*) and Asian clams (*Corbicula fluminea*), in economically developed watersheds in China (Pearl River Basin (PRB) and Taihu Lake Basin (THB)) and agriculture-dominated Poyang Lake Basin (PYB). Significant lethal and sublethal effects (e.g., neurotoxicity, reproductive toxicity, and metabolic and oxidative stress) were observed in fish and clams irrespective of economic gradients. Notably, ecological effects differed significantly between water and sediment phases within the same region. Target (98 contaminants) and suspect screening (942 contaminants) revealed regional-specific characteristics. Ecological risk assessments using a weight of evidence approach demonstrated that both water and sediment in the PRB were at moderate to high risk, as was the sediment in the less developed PYB. However, the characteristics of mixture pollution varied greatly among regions. Suspect screening identified many pollutants that are not regularly monitored but are present at high environmental concentrations and are linked to local industrial production. These distinct mixture risk characteristics across different basins suggest that mitigating aquatic pollution requires region-specific management measures.



KEYWORDS: *mixture risk, in situ bioassays, weight of evidence, ecological risk assessment, suspect analysis*

1. INTRODUCTION

Aquatic ecosystems are often exposed to complex mixtures of chemical pollutants, posing potential threats to aquatic wildlife and ultimately humans. Regional disparities of economic development and population density strongly affect the compositions of chemical mixtures and subsequently aquatic risk.[1] Aquatic ecological risks are usually assessed by hazard quotients derived from chemical analysis and adverse effects measured by bioassays, either individually or in combination. While target analysis can identify tens to hundreds of chemicals, it remains challenging to elucidate adverse effects on aquatic organisms using a predetermined list of analytes.[2] Bioassays complement chemical analysis by addressing mixture effects. However, traditional laboratory bioassays may not accurately reflect actual field exposure due to alterations in the composition and bioavailability of pollutants during sample transport and storage.[3] *In situ* bioassays, characterized by their enhanced environmental authenticity and ecological relevance, exhibit tremendous promise for ecotoxicological applications.[4]

Natural environmental conditions, economic disparities, and human activity patterns significantly affect the pollution status of watersheds.[5] The inherent uncertainties in the field environment make the utilization of *in situ* bioassays with multiple native species and various end points advantageous for elucidating regional toxicological characteristics under mixture contamination scenarios.[6] Employing multiple lines of evidence for effects and exposure, a weight of evidence method can integrate *in situ* exposure and bioassay information to assess aquatic ecological risk in the study region, thereby mitigating the assessment bias associated with relying on a single line of evidence.[7−9] Concurrently, suspect analysis using

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an extended list of contaminants (ranging from hundreds to thousands) facilitates the identification of previously overlooked contaminants, enhancing our understanding of their sources, regional distribution, and toxicity contribution. Consequently, a more comprehensive elucidation of the association between mixture risk and regional characteristics, such as industrial structures, agricultural practices, and urbanization levels, can be achieved.[10] In addition, identifying additional pollutants through suspect screening can also help pinpoint potential sources within specific industries or sectors, enabling more targeted regulatory management and risk control.

The objectives of the present study were to assess the applicability of *in situ* bioassays with native species across regions with varying socioeconomic gradients, to elucidate regional characteristics of effects and exposure in water and sediment through biological and chemical analyses, to evaluate regional aquatic ecological risk using a weight of evidence approach, and to expand contaminant lists via suspect analysis to better illustrating the relationship between aquatic risk and economic development status. Three study regions were selected: the Pearl River Basin (PRB) and the Taihu Lake Basin (THB), both of which are economically developed and heavily impacted by human activities, and the Poyang Lake Basin (PYB), which is significantly influenced by surrounding agricultural activities. The aim of the present study was to enhance the understanding of ecological implications of combined pollution in different watersheds using environmentally relevant approaches, thereby providing insights for more effective pollution management strategies.

2. MATERIALS AND METHODS

2.1. Test Organisms

Two freshwater organisms, the Chinese rare minnow (*Gobiocypris rarus*) and the Asian clam (*Corbicula fluminea*), which generally inhabit different niches within aquatic environments, were employed to assess ecological risks in water and sediment, respectively. The fish, supplied by the National Aquatic Biological Resource Center (NABRC), were four-month adults with a fresh weight of 0.63 ± 0.08 g and a body length of 35.2 ± 0.6 mm. The clams were collected from a water conservation zone in Qingyuan, Guangdong, China, with a fresh weight (including shell and tissue) of 2.41 ± 0.45 g and a shell height of 11.5 ± 0.9 mm. After collection, the clams were transported to the laboratory in cooled containers. Upon arrival, both organisms were acclimatized to room temperature and subsequently transferred to 20 L glass tanks within a semistatic freshwater system containing dechlorinated tap water. The aerated freshwater used for culturing organisms was renewed every 24 h, and the system was maintained at 23 ± 1 °C under a 16:8 light−dark cycle with constant aeration. The fish were fed with commercial fish food, while the clams were fed with laboratory-cultured green algae. Both organisms were kept under laboratory conditions for 2 weeks prior to field deployment, with survival exceeding 95% during the acclimation period.

2.2. *In Situ* Bioassays and Sample Collection

As illustrated in Figure S1, a total of 12 sampling sites were selected for field deployment. Four sites (PR1−PR4) were located along the Guangzhou Reach of the Pearl River, covering upstream, midstream, and downstream sections. Five sites were situated in the THB, specifically within Taihu Lake (TH1−TH3) and Gehu Lake (GH1 and GH2) in Changzhou. Additionally, three river sites (PY1−PY3) were selected from PYB in Nanchang. Detailed descriptions of these sites are summarized in Table S1. Meanwhile, a reference site (Rf), located in a drinking water reservoir near Guangzhou, was also assessed and considered to be a good-quality water site due to the absence of obvious contamination sources. Water quality parameters, including dissolved oxygen, pH, temperature, conductivity, and ammonia, were measured on-site for all sites used in the *in situ* bioassays. These parameters fell within ranges that ensured the survival of both organisms (Tables S2 and S3).

The homemade *in situ* bioassay device is depicted in Figure S1. In brief, four series-connected stainless-steel chambers were placed in both water and sediment. Following previously reported procedures,[6] 10 fish were placed in each chamber suspended in the water, while 15 clams were placed in chambers embedded in the sediment. The entire device was secured in place with nylon ropes and heavy stones to prevent movement during the 10 day exposure period, after which the device was retrieved. Preliminary results indicated no significant difference in the survival and body weights of fish and clams during the *in situ* exposure, regardless of feeding status (Table S4). Therefore, no additional food was provided during the field bioassays. Surface water (4 L) was collected and stored in acid-treated brown glass bottles with 1 mL of 10% sodium azide at 4 °C in the dark for subsequent analysis within a week. Surface sediments (up to the top 20 cm) were collected and passed through a 0.5 mm sieve to remove rocks and coarse debris. Both water and sediment samples were transported to the laboratory and stored at −4 and −20 °C, respectively, until further analysis. Water samples were analyzed within 7 days of the sampling. The surviving organisms were sieved from water and sediments, respectively, rinsed with reconstituted water, and stored in liquid nitrogen before transport to the laboratory.

2.3. Chemical Analysis

Target contaminants were analyzed in sediment and water following our previous study.[6] A total of 98 substances were analyzed, including 16 polycyclic aromatic hydrocarbons (PAHs), 3 polycyclic musks (PCMs), 21 polychlorinated biphenyls (PCBs), 8 polybrominated diphenyl ethers (PBDEs), 19 organochlorine pesticides (OCPs), 9 organophosphate pesticides (OPs), 11 pyrethroids (PYRs), fipronil and its 2 metabolites (FIPs), 7 metals, and a metalloid (As). These compounds were selected because they have been previously detected in study regions and are a potential risk to aquatic species.[11,12] In addition to target analysis, a suspect analysis was conducted to semiquantify 942 organic contaminants using the Compound Composer software on a gas chromatograph−mass spectrometer/mass spectrometer (GC−MS/MS) in full scan mode. Any duplicates of compounds found between the target and suspect lists were retained in the target list and deleted from the suspect list. The concentrations derived from target analysis were used for further analysis. Detailed information regarding chemicals and reagents, samples extraction and cleanup, instrumental analysis, and ions of the target compounds is provided in the Supporting Information (SI) and Tables S5−S7.

2.4. Biomarker Analysis

Chinese rare minnows were dissected to obtain liver, brain, and muscle tissues, while Asian clams were dissected to obtain digestive glands, gills, and viscera after an 8 h purge in aerated tap water. Tissue samples were then thawed on ice and homogenized in ice-cold phosphate buffer saline (PBS, 100 mmol/L, pH = 7.4, w:v = 1:9) using a Bullet Blender Blue-24 homogenizer (Next Advance Inc., Averill Park, NY, USA). The homogenates were centrifuged at 10000 rpm for 10 min at 4 °C. The supernatant was collected and used for subsequent enzymatic analyses, including those related to metabolism [ethoxyresorufin-*O*-deethylase (EROD), ethoxycoumarin *O*-deethylase (ECOD), glutathione *S*-transferase (GST)), antioxidation (superoxide dismutase (SOD), calatase (CAT), glutathione peroxidase (GPX)), lipid peroxidation (malondialdehyde, MDA)], neurotoxicity (acetylcholinesterase, AChE), and reproductive toxicity (vitellogenin, Vtg). The Vtg was measured only in the liver of male fish. All enzyme assays were conducted in triplicate at 25 °C. Finally, the enzymatic data were summarized using an enhanced integrated biomarker response index (EIBR).[13,14] More details of enzymatic assays and the EIBR calculation are provided in the SI.

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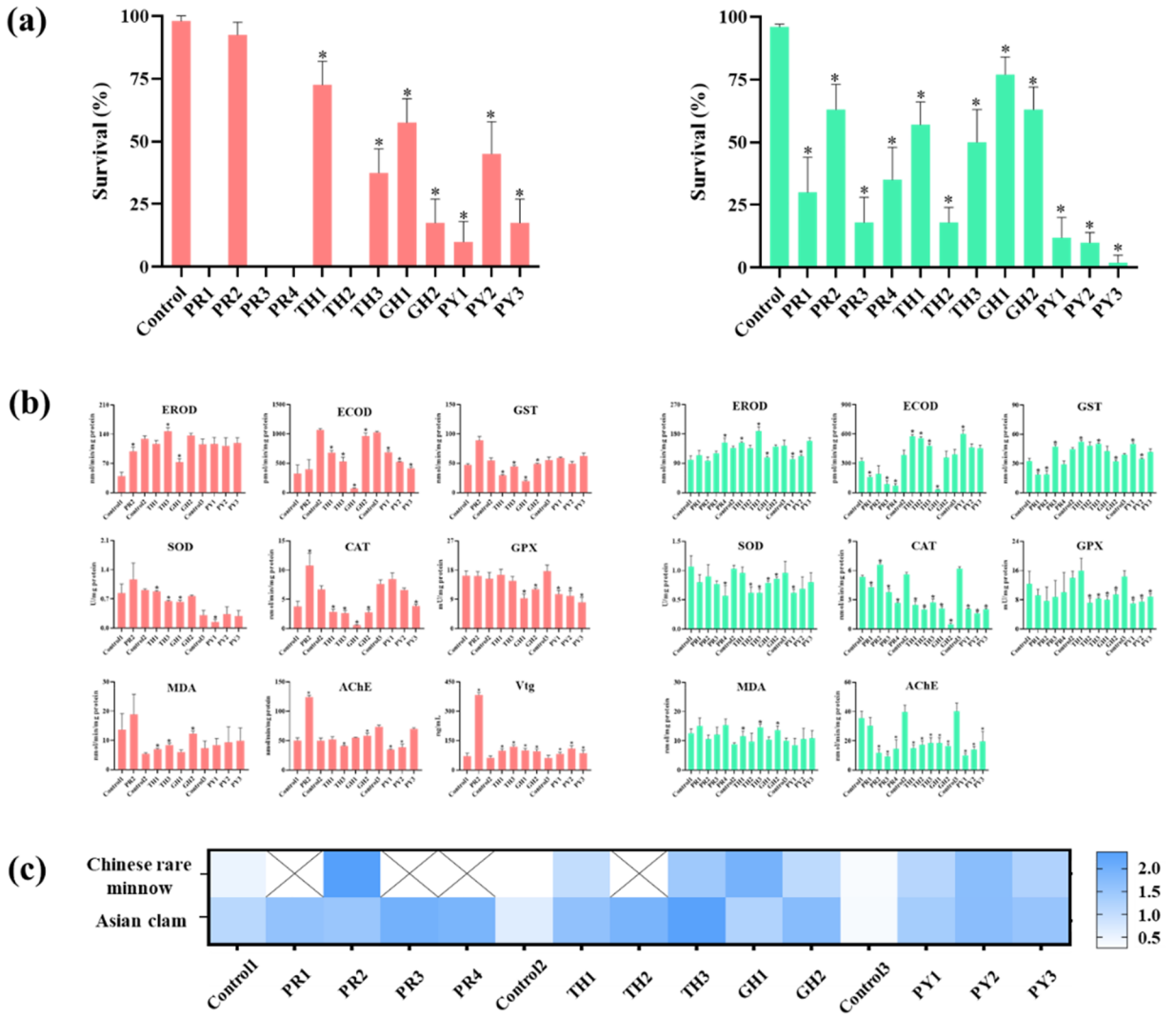

Figure 1. Biological effects of Chinese rare minnows (red) and Asian clams (green) after 10 days of *in situ* bioassays. (a) Survival; (b) enzyme activities; and (c) enhanced integrated biomarker response (EIBR). Asterisks indicate significant differences ($p < 0.05$) between the field sites and laboratory control. X's indicate that there was no EIBR value because no test organisms survived at this site.

2.5. Weight of Evidence Assessments

Risk assessment was conducted using a weight of evidence (WoE) approach integrating three lines of evidence: survival, EIBR, and hazard quotient (HQ). While survival and EIBR were directly measured *in situ* effects, HQs were estimated from the measured chemical concentrations in sediment or water. A method for order preference by similarity to ideal solution (TOPSIS) combined with gray relational analysis (GRA) was used to prioritize the ecological risk of the study sites. The distance between the evaluation index and the ideal solution is inversely related to the correlation, and a smaller distance indicates a higher correlation and a better quality of sediment and water. By establishing two virtual comparison points representing the best and worst sites, all study sites were evaluated simultaneously, and samples were ranked in descending order based on the calculated relative closeness to the ideal solution. The ranking of each site determined its risk priority, with categorization into high, medium, and low risk. The calculation process was executed using an Excel VBA (Visual Basic for Application) program developed by Jiang et al.,[15] with loose strength weights applied. The weight values of individual lines of evidence were optimized and determined through an intuitionistic fuzzy analytic hierarchy process approach (IFAHP).[6] Given that the observed effects directly reflect mixture toxicity and that the presence of pollutants does not necessarily imply adverse effects, preference ratings of levels 1 and 2 were assigned for effects and exposure assessments, respectively (Table S8). In this context, effects were considered to be more significant than exposure, with survival being a much more critical measure than EIBR. Consequently, the weight of biological survival, EIBR, and concentration of target pollutants were calculated as 0.694, 0.251, and 0.055, respectively, using our self-designed Excel application (Table S9). Additionally, the potential ecological risk index (RI) was utilized to investigate potential hazards of metals and a metalloids in sediment. Detailed information on HQ and RI calculations is shown in the SI.

2.6. Data Analysis

Data analysis was performed using R (version 4.2.2). A one-way analysis of variance (ANOVA), followed by the Tukey's Honest Significant Difference (HSD) multiple comparison, was conducted to assess statistically significant differences between the treatment and the control groups. Prior to analysis, data were checked to ensure that they met the ANOVA assumptions of normality and homogeneity of variances. Statistical significance was determined at $p < 0.05$.

2.7. Quality Assurance and Quality Control

Instrumental performance was monitored by analyzing a calibration standard every 10 samples, with variations in individual target analytes remaining within 20%. A method blank (solvent), a matrix blank (Milli-Q water and control sediment), and a matrix spike and its

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duplicate (clean sediment or water spiked with target compounds) were analyzed every 20 samples. Except for naphthalene that was found with background concentration, no target analytes were detected above their reporting limits (RLs) in the blanks. Naphthalene concentrations in the blank samples were below 10% of the lowest levels detected in all field samples, thus no background subtraction was performed. Both the fish and clams had trace levels of PAHs and PCMs, but these levels were at least an order of magnitude lower than those found in organisms exposed *in situ* in the field, indicating negligible pollutant exposure stress in the control group.[4]

The recoveries for matrix spike samples were acceptable for PAHs (62−106%), PCMs (78−101%), PCBs (74−104%), PBDEs (72−93%), OCPs (65−99%), OPs (67−106%), PYRs (61−103%), FIPs (77−102%), and metals and As (81−107%). The recoveries (mean ± standard deviation) of the surrogate standards were acceptable for naphthalene-d_8 (44 ± 8%), acenaphthylene-d_{10} (64 ± 13%), phenathrene-d_{10} (73 ± 9%), chrysene-d_{12} (75 ± 10%), perylene-d_{12} (89 ± 9%), DBOFB (73 ± 11%), PCB-67 (88 ± 12%), PCB-169 (91 ± 8%), BDE-77 (76 ± 5%), BDE-181 (81 ± 6%), and BDE-205 (77 ± 8%). To validate the semiquantitative results in suspect analysis, chemical standards containing 22 OCPs, 5 OPs, and 9 PYRs (Table S10) at 200 ng/mL each were analyzed using the Compound Composer software. The standard compounds were well-separated and accurately identified, with relative errors in retention time and quantitative concentrations ranging from −0.100 to 0.186 min and −101 to 47%, respectively.

To minimize uncertainty due to transportation stress on test organisms, laboratory and travel controls were implemented. The survival of laboratory control (98 ± 2% and 96 ± 1%, respectively) was not significantly different from that of the travel control (88 ± 13% and 93 ± 19%, respectively) for Chinese rare minnows and Asian clams, respectively, indicating that the field *in situ* bioassays are feasible.

3. RESULTS AND DISCUSSION

3.1. *In Situ* Adverse Effects across Regions

3.1.1. Lethal Effect. At the end of the 10-d exposure, the survival of test organisms exhibited significant variations across the three regions and between the two species, underscoring region-specific toxicity characteristics (Figure 1a and Table S11). Both species showed high survival in the controls, confirming the reliability of the *in situ* bioassay results. Comparatively, substantial lethality was noted for both species across all three regions. The highest survival for Chinese rare minnows was 93 ± 5% at site PR2, while survival for both fish and clams at all other sites was significantly lower than those in the controls. In the PRB, Chinese rare minnows exhibited 100% mortality at all sites except PR2, where survival was comparatively higher. Similarly, Asian clams in the PRB showed low survival, except for site PR2 (65 ± 10%). The high mortality observed in both fish and clams in the PRB was consistent with the documented degradation of the aquatic environment in this highly urbanized region.[16,17] In the THB, fish survival ranged from 18% (GH2) to 73% (TH1), while there were no survivors in the TH2. The mortality of fish in THB may be partially attributed to algal toxins from intense cyanobacterial activity during the bioassays in June.[18,19] Across the three regions, THB exhibited the highest average survival for Asian clams (37 ± 29%). The survival of sediment-dwelling Asian clams under *in situ* exposure may be influenced by factors such as temperature variations, oxidation in micro-habitats, and episodic pollutant inputs, in addition to long-term sediment contamination.[20] Despite Poyang Lake being a nature reserve with a relatively low population density and anthropogenic impacts compared to the PRB and THB, the survival of organisms in the PYB was surprisingly low. This could be due to residual pesticides from agricultural practices, which are highly toxic to nontarget aquatic organisms.[21,22] Overall, the lowest survival rates for Chinese rare minnows and Asian clams were observed in the PRB and the PYB, respectively. The inconsistent lethal effects between the two species across different regions can be attributed to their distinct living habitat (fish in water and clams in sediment) and the different composition of pollutants in environmental compartments.[6]

3.1.2. Sublethal Effects. The enzymatic activities of the surviving Chinese rare minnows and Asian clams were assessed, with results shown in Figure 1b and Tables S12 and S13. Due to the absence of surviving fish at most sites in the PRB and TH2, no enzymatic data are available for those sites. The biomarkers examined included metabolic enzymes (EROD, ECOD, and GST), oxidative enzymes (SOD, CAT, and GPX), and specific enzymes (AChE and Vtg).[6] As shown in Figure 1b, both organisms exhibited a significant induction or inhibition of metabolic enzymes compared to the controls. Both species exhibited a significant inhibition of ECOD activity at site GH1. Fish often show similar EROD and ECOD responses to environmental pollutants such as PAHs.[23] The GST activities in Chinese rare minnows were inhibited at sites within the THB, suggesting a reduced capacity to eliminate contaminants, which may have contributed to the lethality. Oxidative stress was prevalent across the sites, with CAT activities being inhibited at all sites except for PR2, where significant induction occurred. In THB, both species demonstrated a significant inhibition of SOD and GPX, along with elevated MDA levels, indicating increased oxidative damage and potential irreversible harm.

In addition to the above nonspecific bioactivities, both organisms exhibited notable changes in AChE activity. It is widely recognized that exposure to OPs and carbamates can lead to the inhibition of AChE activity in aquatic organisms. Recent studies have also revealed that additional organic contaminants, such as PAHs, DDTs, and plasticizers, may disrupt AChE.[24] Although AChE inhibition in Chinese rare minnows was observed only at the PYB sites, a significant reduction in AChE activity was noted at all study sites compared to their respective controls (Figure 1b). A positive correlation was found between OP concentrations and AChE activities in fish ($r^2 = 0.49$, $p < 0.0026$) and clams ($r^2 = 0.56$, $p < 0.0001$) (Figure S2). Acute fish toxicity tests demonstrated that a high level of AChE inhibition was required before detrimental effects became apparent.[25] Furthermore, aquaculture and shipping activities in the PRB and THB may contribute to the resuspension of sediment-bound neurotoxic pollutants to surface water.[26] The Vtg was exclusively measured in surviving male Chinese rare minnows, and Vtg levels at all sites were significantly induced compared to the respective control (Table S12), implying a widespread distribution of estrogens and their analogues (endocrine disruptors) in the three study regions. Contaminants in wastewater are considered to be significant sources of estrogenic compounds, and the induction of Vtg in male rainbow trout downstream from wastewater treatment plant effluent receiving water was previously reported.[27] Variability in Vtg concentrations among male fish may be influenced by population size and river flow.[28] The notably elevated Vtg level at the urban river site PR2 (383 ± 13 ng/mL) appeared reasonable, given the substantial annual wastewater discharge

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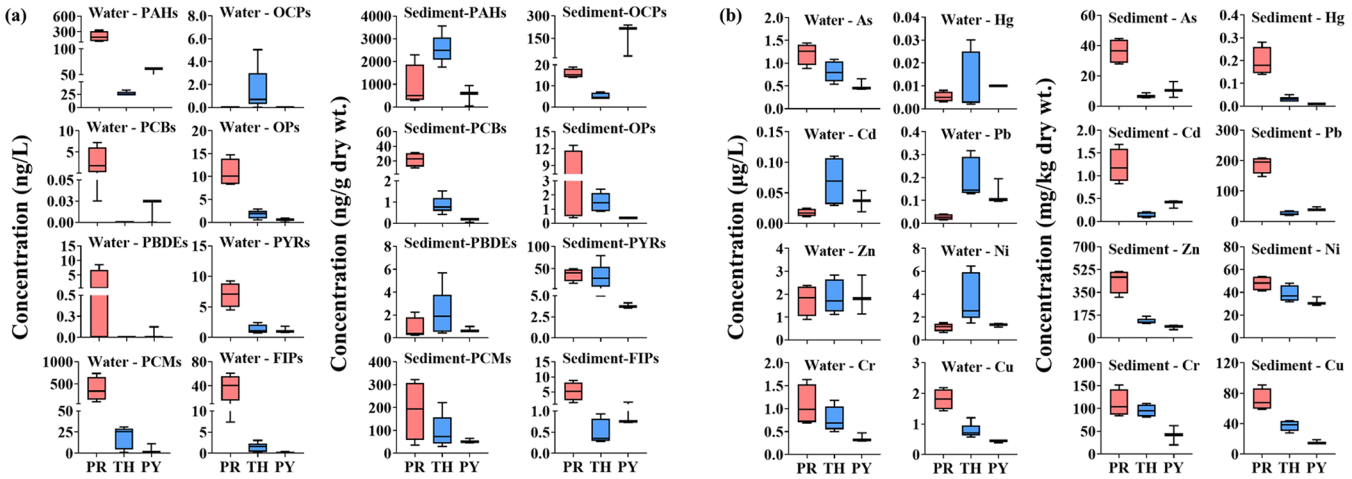

Figure 2. Concentrations of organic pollutants (a) and metals/metalloid (b) in water and sediment across the Pearl River Basin (PRB), the Taihu Lake Basin (THB), and the Poyang Lake Basin (PYB), China.

in Guangdong and the high population density in China, despite fish survival being the highest at this site among all sites.

To integrate the enzymatic effects, EIBR values were calculated for individual sites, consistently exceeding control levels at all sites (Figure 1c). The highest EIBR values were 2.37 at PR2 for fish and 2.31 at TH3 for clams, indicating significant enzymatic abnormalities that ultimately impact organism survival.[29] A linear correlation between survival and EIBR was observed in Asian clams across sites (r^2 = 0.37, p = 0.0129) (Figure S3), demonstrating consistent *in situ* lethal and sublethal effects. Due to the extremely high mortality of fish at several sites, no significant relationship between lethality and EIBR was found for Chinese rare minnows. At PR2, where fish exhibited the highest EIBR but the lowest mortality, an association with elevated Vtg was observed. This suggests that while acute lethality was not detected at some sites, long-term sublethal effects caused by endocrine disruptors should not be overlooked. The discrepancies between fish and clam responses may be attributed to species-specific differences, hydrological conditions, and contaminant variations in water and sediment.[30] Overall, *in situ* bioassays effectively revealed various stresses on field organisms, underscoring the importance of using multiple test species and toxicity end points. Regional contamination characteristics significantly influenced the survival and enzymatic activity of test organisms in different niches, highlighting the need to identify responsible toxicants in aquatic environments.

3.2. Concentrations and Potential Risk of Target Pollutants across Regions

Most target pollutants were detected in water and sediment across the three regions, exhibiting notable concentration variations (Figure 2 and Tables S14–S17). Among target organic pollutants, PAHs and PCMs occurred at relatively high concentrations and detection frequencies in both water and sediment, whereas PCBs and PBDEs were the lowest in water while their sediment concentrations were comparable to those of some pesticides. The occurrence of pesticides showed distinct regional disparities, with current-use pesticides (CUPs) such as PYRs, OPs, and FIPs dominating in PRB and THB, while legacy OCPs were prevalent in sediments of PYB.

In water samples, PAHs and PCMs accounted for over 90% of the total concentrations, with significantly higher average levels in the highly developed PRB than those in the THB and PYB ($p < 0.05$). The elevated PAH levels in the PRB may be attributed to industrial and domestic wastewater discharge and surface runoff related to vehicular emissions and atmospheric deposition.[31] High population density and increased wastewater discharge likely accounted for the elevated PCM levels in the PRB.[32] Low detection frequencies of legacy PCBs and PBDEs are reasonable due to their high hydrophobicity.[33] Legacy pesticides (OCPs) were detected above the RL only in the THB (1.45 ± 2.03 ng/L), while CUPs were frequently detected but at low concentrations, with notably high concentrations observed in the PRB. Among the CUPs, FIPs were present in water with higher levels and detection frequencies (above 90% in all regions), reaching the highest concentrations in the PRB (37.2 ± 22.1 ng/L), consistent with previous reports (14.8 ± 12.9 ng/L).[34] Compared to FIPs, OPs are more readily metabolized, while PYRs are more hydrophobic and tend to accumulate in sediment. The higher CUP concentrations in the PRB are primarily attributed to severe pest infestations and heavy rainfall, resulting in increased usage and continuous inputs in this region.[35] As for metals, they were found at low concentrations in water at all sites, except for mercury, which was not detected in the PYB (Figure 2b and Table S16). Compared to the "Standards for Drinking Water Quality" (GB5749-2006) and the "Environmental Quality Standards for Surface Water" (GB3838-2002) from China, metal concentrations in water across all regions were below the class III water standard, indicating relatively low metal risk.

Occurrences of sediment-bound pollutants showed different patterns from those in water. The highest average PCM concentrations were observed in the PRB (187 ± 129 ng/g dry wt); however, THB sediments contained significantly higher PAH concentrations (2551 ± 648 ng/g dry wt). The increasing use of PCMs has resulted in their consistently increasing presence in aquatic environment over the years (Figure S4), with concentrations comparable to those in developed countries over the past decade (Table S18). Despite their low acute toxicity, PCMs are suspected of inducing estrogenic activity, liver damage, and skin irritation, and they are potentially bioaccumulative, warranting further attention.[36]

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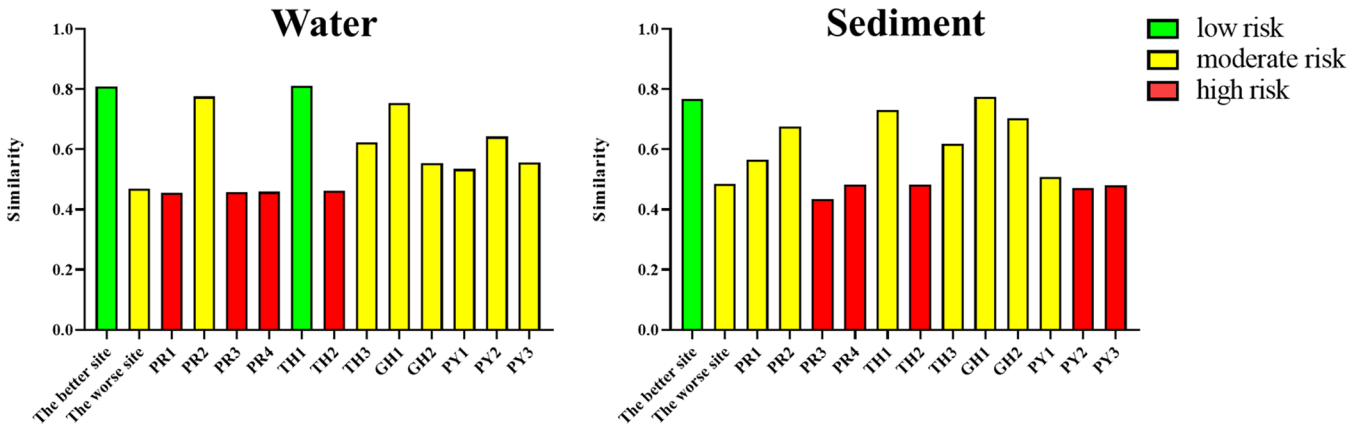

Figure 3. Ecological risk at individual samples sites. The assessment was conducted using a weight of evidence (WoE) approach including three lines of evidence (survival, enhanced integrated biomarker response (EIBR), and hazard quotient (HQ)). Different colors indicate the risk ranking.

It should be noted that versalide, which was banned due to its neurotoxicity and estrogen receptor activity, was detected in the PRB (2.25 ± 2.56 ng/g dry wt), as a result of earlier use and persistence.[11] Although BDE-209 was detected in all sediment samples, the sum concentrations of PCBs and PBDEs accounted for less than 2% of the total concentrations of target organic contaminants. Regarding pesticides, the PYB showed extremely high levels of legacy OCPs (162 ± 113 ng/g dry wt), reflecting severe historical residue issues. The detection of persistent OCPs in PYB sediment was reasonable given the long history of agricultural practices in this region.[21] Conversely, CUPs were frequently detected in sediments at all locations, but OP and pyrethroid concentrations in the PYB were significantly lower than those in the other two regions. Due to their widespread use in residential areas and urban landscape maintenance,[34] sediment concentrations of FIPs in the PRB were significantly higher than those in the THB and PYB, which were comparable. Among CUPs, PYRs had the highest concentrations in all three regions, consistent with previous studies on the composition of sediment-bound CUPs in the PRB.[37] Unlike limited regional variations in metal concentrations in water, the levels of metals in sediment exhibited significant differences among the three regions (Figure 2b and Table S17). Sediment concentrations of As, Cd, Pb, Zn, and Cu in the PRB were significantly higher compared with those in the THB and PYB ($p < 0.05$), which may relate to the high industrialization levels in this region. Ecological risk from sediment-bound metals was assessed using the risk index (RI) with the values of 452 ± 79.6, 141 ± 29.4, and 66.3 ± 13.0 in the PRB, PYB, and THB, respectively (Tables S19 and S20), indicating high potential metal-related risks in the PRB. Specifically, Cd and Hg contributed 57 and 21%, respectively, to the total RI in the PRB, which is consistent with previous observations of significant metal risks in sediments within Guangzhou urban waterways.[38]

Spatial distribution patterns of sediment-bound pollutants were assessed using redundancy analysis, with pollutants accounting for 70.7% of the cumulative variance among sampling sites (Figure S5). In the PRB, a variety of pollutants, including PCMs, OPs, PCBs, metals, and FIPs, dominated the contamination profile. The complex mixtures in the PRB likely imposed significant stress on aquatic organisms, as evidenced by low organism survival and excessive contaminants in both water and sediments. The main contaminants in the other two regions were less diverse. In the THB, PAHs and PBDEs were the primary pollutants, while the persistently high levels of banned OCPs were the important contaminants in PYB.

The ecological risk of the target pollutants was estimated by dividing their concentrations in water and sediment by their respective toxicity thresholds (risk quotient), with rankings presented in Figure S6 and Table S21. Risk quotients for these pollutants in water ranged from 0.0001 to 10, with most pollutants at all study sites below 1, indicating low risk. For pollutants in sediment, risk quotients ranged from 0.0001 to 100, with most between 1 to 10 in PRB, indicating moderate risk, while most pollutants in the THB and PYB were below 1, indicating low risk. The rankings of the total risk quotient for water were PRB (7.28 ± 2.27) > THB (2.87 ± 1.21) > PYB (1.76 ± 0.22), and for sediments, it was PRB (85.5 ± 26.1) > THB (47.5 ± 24.8) > PYB (16.6 ± 7.92). Using a threshold of <10 for low risk and >100 for high risk, water samples from all sites demonstrated low risk, whereas most sediments indicated moderate risk. However, risk quotient rankings were inconsistent with survival and EIBR at some sites, highlighting the need for a comprehensive risk evaluation that integrates multiple lines of evidence.

3.3. Ecological Risk Assessment Using a Weight of Evidence Approach

To improve the accuracy of assessing the ecological risk of water and sediment across different regions, three lines of evidence, including survival, EIBR, and contaminant concentrations, were integrated using a weight of evidence approach following the evaluation criteria established in our previous study.[6] Risk levels were categorized as low, moderate, or high. Both water (PR1, PR3, PR4, and TH2) and sediments (PR3, PR4, TH2, and PY2) at four sites were classified as high risk, with the remaining sites showing moderate risk, except for a low-risk site (TH1) (Figure 3). By integrating exposure and effect data, we identified significant ecological risks from both water and sediments in the PRB as well as sediments in the PYB. The contribution of individual lines of evidence to the overall risk varied among regions, which differed significantly from the risk ranking based solely on the environmental concentrations of target contaminants (Figure S7). This discrepancy highlighted the high uncertainty in assessments that overlook effect-related information. The present study underscored the importance of integrating biological effects and environmental concentrations through a weight-of-evidence analysis to improve the accuracy of ecological risk

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Figure 4. Detected frequencies (A) and the concentrations of suspect organic pollutants in water (B) and sediments (C) within individual chemical classes (shown in different colors) across the Pearl River Basin (PRB), the Taihu Lake Basin (THB), and the Poyang Lake Basin (PYB), China. The number in the cycle is the number of compounds detected.

assessments for environmental mixtures.[4,39] One limitation of relying solely on exposure data was the limited number of target pollutants analyzed; thus, further suspect analysis was conducted to expand the list of pollutants.

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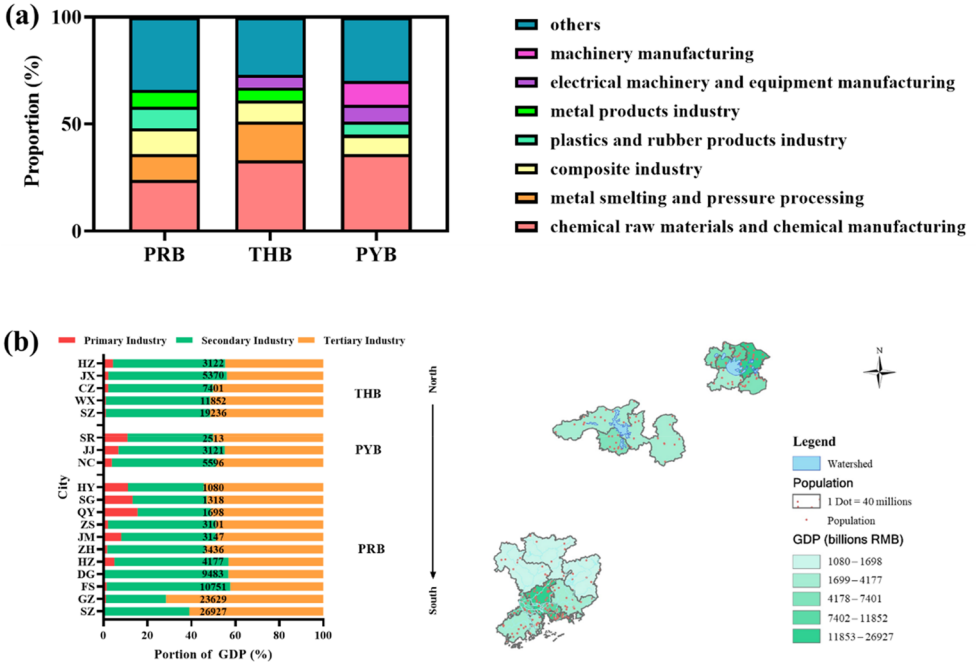

Figure 5. Socioeconomic gradient of study regions. Classification and proportion of contamination sites by types of industries in the Pearl River Basin (PRB), the Taihu Lake Basin (THB), and the Poyang Lake Basin (PYB), China (a). Gross domestic product (GDP), primary industrial production (PIP), secondary industrial production (SIP), tertiary industrial production (TIP), and population (POP) for the major cities in the PRB, THB, and PYB, China (b).

3.4. Suspect Analysis of Water and Sediment Samples

Nearly a thousand suspect pollutants were semiquantified (Figure 4 and Tables S22–S24). These substances were classified into seven categories according to their application purposes or sources, including chemical raw materials (such as plastics-, cosmetics-, and dyeing-related materials), pesticides (such as insecticides, herbicides, and fungicides), plasticizers, natural products, *n*-alkanes, aromatics and derivatives, and pharmaceuticals and personal care products (PPCPs).[40] Although the pollutants in water and sediments varied, the frequently detected suspect pollutants in the three regions exhibited distinct regional characteristics, underscoring the influence of economic disparities on the presence of pollutants. As shown in Figure 4, the THB had the most diverse range of pollutants (101 and 93 compounds in water and sediment, respectively), while the highest total detected concentrations of pollutants were found in the PRB, with plasticizers, *n*-alkanes, and pesticides as the predominant constituents.

In the water phase, the compound with the highest concentrations in both the PRB and PYB was 4-methyl-2,6-di-t-butylphenol (BHT) (Tables S22 and S24), which is widely used in polymer materials, petroleum products, and food processing industries. This chemical was previously detected at high concentrations in surface water in Jiangsu[41] and wastewater from Guangzhou, China.[40] Additionally, many raw materials used in the pharmaceutical and plastic industries were often found in the THB, including 30 compounds with relatively high concentrations. Squalane, another industrial chemical, was also detected at high concentrations in all samples, likely due to its extensive use in PPCPs.[42] Besides the above-mentioned chemicals, other industrial chemicals such as antioxidants, intermediates, and degradation products related to the pharmaceutical, plastics, and pesticide synthesis industries were also detected, but at relatively low concentrations and detection frequencies. Beyond the target pesticides, additional pesticides were frequently detected with varying concentrations across regions. Notably, chloroneb and spirodiclofen were found at high concentrations and detection frequencies. Plasticizers exhibited the highest concentrations at most sampling sites, particularly in the densely populated PRB. Five natural products, primarily steroid compounds, were also identified. The concentrations of *n*-alkanes in PRB were substantially higher than those at other sites, while elevated concentrations of aromatics and their derivatives were found in both the PRB and THB. Given the extensive aquaculture industries in the THB, it is reasonable to detect high levels of sterols (cholestane, cholesterol, cholestanol, and coprostanol).[41] Caffeine was the most frequently detected PPCP.

Compared to water samples, fewer suspect pollutants were detected in sediments, particularly in the PRB. This finding aligned with the higher lethality observed in fish than in clams in this region. Pesticides were most frequently detected in sediments from the THB and PYB, whereas *n*-alkanes were the dominant sediment-bound contaminants in the PRB, predominantly with more carbon ($C_n > 24$), accounting for 55–66% of the total. The carbon preference index (CPI) values of *n*-alkanes in the PRB, THB, and PYB were determined to be 0.62, 2.31, and 2.36, respectively, indicating that anthropogenic input was the primary source of *n*-alkanes in sediments, particularly in the PRB. Elevated levels of aromatic hydrocarbons and their derivatives were found in the PRB and THB sediments, consistent with the results of target analysis.

Collectively, the pollutants detected in both target and suspect analyses showed that intense human activities have caused complex mixture pollution in the PRB and THB. Specifically, the PRB exhibited a dominance of pollutants originating from the automobile, food, electronic equipment, rubber, and plastic products industries, while the THB showed a prevalence of pollutants derived from spice products, pharmaceutical manufacturing, and textile industries. In

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contrast, the less developed PYB was mainly impacted by pollutants from the agro-food processing industry and pesticide synthesis intermediates.

3.5. Regional Pollution Characteristics and Socioeconomic Gradients

Contaminated construction sites and industrial land in urban areas not only reflect local developmental history but also release pollutants into the surrounding aquatic environment, thus closely tying them to regional pollution characteristics.[43] To evaluate the effectiveness of the identified contaminants in revealing these regional pollution characteristics, we collected data from 119 contaminated sites in three regions (Tables S25−S27). These data were obtained from the publicly available list of contaminated construction sites released by the Department of Ecology and Environment (PRB: https://gdee.gd.gov.cn/; THB: http://sthjt.jiangsu.gov.cn/; and PYB: http://sthjt.jiangxi.gov.cn/). The number of contaminated sites in the PRB, THB, and PYB were 66, 39, and 14, respectively, reflecting their urbanization and industrialization gradients. The classification of these sites by industry type revealed distinct industry characteristics for each region (Figure 5a). In all three regions, secondary industry was the predominant economic activity, with chemical raw materials and chemical product manufacturing industries representing the highest proportion, averaging 32% (Figure 5b).

In the PRB, a notable presence of metal smelting and plastic manufacturing industries (23%) is observed, reflecting the region's emphasis on export-related sectors such as electronics, garments, and toys. This industrial profile aligned with the elevated concentrations and detection frequencies of metals and contaminants associated with plastic materials in the PRB compared to those in the THB and PYB. In particular, certain contaminated sites in PRB, not classified under major industries, encompass activities such as wood processing, ink production, cement manufacturing, and petroleum processing. Correspondingly, pollutants related to these sites exhibit higher detection frequencies and concentrations of chemical raw materials and *n*-alkanes as revealed through suspect screening (Figure 4). Contaminated sites in THB are composed of a higher proportion of metal smelting and composite material industries (29%), alongside sectors related to textile printing and dyeing (10%). This industrial landscape explains the prevalence of cosmetics, dye materials, and PPCPs detected in the THB (Figure 4). Despite its comparatively lower level of industrialization, the PYB demonstrates a relatively higher concentration of machinery and electrical machinery manufacturing industries (20%). Consequently, chemical raw materials associated with automobile manufacturing, electronic information, aerospace, and food processing are identified in the PYB (Figure 4). The industry characteristics across the studied regions closely correlate with findings from target and suspect screening, underscoring the potential utility of an extended list of suspect contaminants in ecological risk assessment and source identification. Overall, the contamination pattern revealed through target and suspect screening aligns with regional industry profiles, validating the need for broadening suspect screening strategies for comprehensive contaminant inventories. The distinct mixture risk profiles observed in different basins emphasize the necessity of implementing region-specific management measures to mitigate aquatic pollution effectively.

4. CONCLUSIONS

In situ bioassays were employed to examine aquatic ecological risks across three regions exhibiting varying socioeconomic gradients. By integrating evidence of exposure and *in situ* effects, distinct regional profiles concerning mixture pollution and risk were unveiled. While chemical analysis alone indicated low risk in the PYB, sediment risk was discerned upon the incorporation of *in situ* bioassays. Target analysis results provided limited insights into the toxicants within the high-risk regions. Further suspect screening findings demonstrated the presence of a diversity of pollutants that are not routinely monitored, which were characterized by elevated environmental concentrations and potential risk linked to local industrial activities. Different mixture risk characteristics among the study basins suggest that mitigating aquatic pollution requires region-specific management measures.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00083.

Detailed information on chemicals and reagents, sample preparation and instrumental analysis, enzymatic analysis and data analysis; figures showing the sampling areas and sites and risk quotients; tables showing the coordinates of sampling sites, physiochemical properties, concentrations of the detected contaminants in individual samples, correlations between concentrations and environmental parameters, lists of target contaminants and instrumental parameters, weight values for each line of evidence, survival and individual biomarkers of test organisms after 10 days of *in situ* exposure, concentrations of targeted contaminants, and suspected screening results and contaminated sites from the PRB, THB, and PYB (PDF)

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Notes

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