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Intergrading water quality, benthic fauna and toxicity test for risk assessment on an urban-rural river

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No problem statement and objectives provided in Abstract

Abstract: Climate change, river pollution and biodiversity loss are increasing and becoming global ~~environment~~ concerns. As the Yellow River is China's Mother River, providing daily water to 114 million people. The climate change and human activity intensity lead to water shortage in cities even nearby the main stream in the lower reaches of the Yellow River. Hence, many cities and towns become water-scarce cities. In this study, water and sediment samples from Juancheng, a typical city in the lower reaches of the Yellow River, were collected and evaluated by integrating traditional physico-chemical indicators, benthic Index of Biological Integrity (B-IBI) and Zebrafish embryotoxicity test (ZET). The results showed that water dissolved oxygen, pH, total nitrogen and total phosphorus were closely correlated with teratogenic rate (TR), lethal rate, richness index and as well as Shannon Winner index. A total of 21 species of benthic organisms were collected, including molluscs, arthropods and linkages, with upper reaches of the river having the highest biomass, and B-IBI values indicated that urban reaches was larger than rural reaches. The TR of zebrafish embryos in raw water was greatest at rural reaches and showed a significant difference from the negative control ($p < 0.05$). When exposed to sediment samples under 100 mg/mL, the hatching rate showed an inverse ratio to the TR, and the lethal effects at rural reaches all reached more than 96%. The results suggest that farm wastewater may be the main sources of pollution in this river containing urban-rural reaches, special focus on protection of the watershed in the rural area is highly recommend.

No supporting info to justify the conclusion i.e. the farm wastewater as the main source of pollution

Keywords: Water physio-chemical parqameters; benthic fauna; Zebrafish embryotoxicity; Yellow River Basin; Urban-rural reaches

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1. Introduction

The global climate change, combining with the increase in global population and rapid urbanization, resulting in increasing water pollution and biodiversity loss at an unprecedented rate in rivers around the world to face [1,2]. ~~Wastewater~~ from industrial and personal use ~~is~~ discharged directly into rivers and lakes, leading to severe pollution with heavy metals and organic waste [3]. It has been reported that 90% of global rivers flowing through towns and cities are heavily polluted [4], the pollution is highly correlated with human activities in the watershed, with variations on major pollutants and toxic effects to ecosystems due to different human activities [5]. In addition, there are often significant differences in the pollution status of rivers in different reaches such as urban and village areas [6], and water quality in streams around urban with high population density is often worse than in areas away from urban [7].

Until the 1990s, river health assessment relied mainly on water quality, which was usually conducted by analyzing conventional physico-chemical indicators. However, they could not reflect the full ecological status of the water body [8]. The European Water Framework Directive (WFD) clarified the need for integrated water quality, water quantity and water ecosystem management [9]. The organisms-oriented approaches was then integrated to water quality monitoring. The analysis based on biological effects is the key to water environment monitoring and suggested measuring the acute toxicity of organisms [10]. Jeppesen et al. [11] proposed to apply relevant indices of aquatic communities, including biodiversity index, index of biological integrity (IBI), etc. to assess the health of aquatic ecosystems. The latter index integrates information reflected by various biological indicators, including community composition, species abundance, dominance, and diversity levels, which have been widely used to evaluate benthic organisms [12,13]. However, there is still a gap between toxics and their effects to different aquatic organisms, in particular for emerging pollutants that presented as mixture in aquatic ecosystems.

In order to illuminate the ecological risk and water scarcity, dilution become a necessary approach. Nowadays, inter-basin water transfer between larger rivers has become frequently in countries around the world [9,14]. It is also one important source of production and domestic water in water-scarce areas such as the middle and lower reaches of the Yellow River Basin of China [15,16]. Of which, the Juancheng County, ~~where locates~~ along the lower reaches of the Yellow River, is one of the cities that half of the water resources rely on the Yellow River. The amount of available water resources is 282 million m^3 per year, of which the Yellow River transfer accounts for 57%. The water transfer could not only improve water quality during the transfer period in the transferred lakes, especially total nitrogen and ammonia nitrogen, but also affect aquatic organism community structure and diversity [9]. The structure of macroinvertebrate community changes in response to environmental perturbations [17]. The ecological protection and high-quality development of the Yellow River Basin have been elevated to a major national strategy [18]. Therefore, to ensure the ecological health of the Yellow River watershed, it is necessary to evaluate the ecological health of water quality in towns and cities based on the Yellow River water intake.

In this study, we jointly explored the relationship between water quality-hydraulic ecology and water security in cities of the lower reaches of the Yellow River through a multi-indicator approach, including traditional indicators, benthic integrity index and toxicity test, with a view to providing important support for water ecological health protection and restoration.

2. Materials and Methods

2.1 Study area

The sampling sites are located in the Juancheng County of Shandong Province, where ~~is~~ on the lower reaches of the Yellow River. The water of the Yellow River flows through the water intake site into the Jishan River of the County. Based on the variations of human activities along the longitudinal gradient of the Jishan River, 4 sampling sites were selected on the main stream, i.e., site 1 was in water intake point of the Yellow River (35°30′4.56″N, 115°21′18.28″E). The site 2 was in Shaheqiao (35°26′45.12″N, 115°32′44.08″E), where is the drinking water source reservoir for the County. The site 3 was in the urban area of the County, where the effluent from a sewage treatment plant is discharged into the river (35°34′36.96″N, 115°31′26.06″E). The site 4 was in the reaches where is the rural area (35°39′18.53″N, 115°40′35.48″E). In addition, 6 sites were set up in a tributary where a goose farm lies on nearby. Tributary site 1 (TS1, 35°67′96.38″N, 115°57′07.23″E) was 10 km away from the estuary, TS2 was inside the goose farm (35°68′00.49″N, 115°58′07.93″E), TS3 (35°67′08.37″N, 115°59′01.68″E) and TS4 (35°67′71.39″N, 115°59′91.72″E) were 8.7 and 7.4 km away from the estuary, respectively. TS6 was in the estuary (35°66′18.11″N, 115°68′00.29″E), and TS5 (35°65′15.46″N, 115°61′01.47″E) was in another tributary as a reference, where no goose farm within the basin of this tributary. The locations of sampling sites chosen for the study are shown in Figure 1.

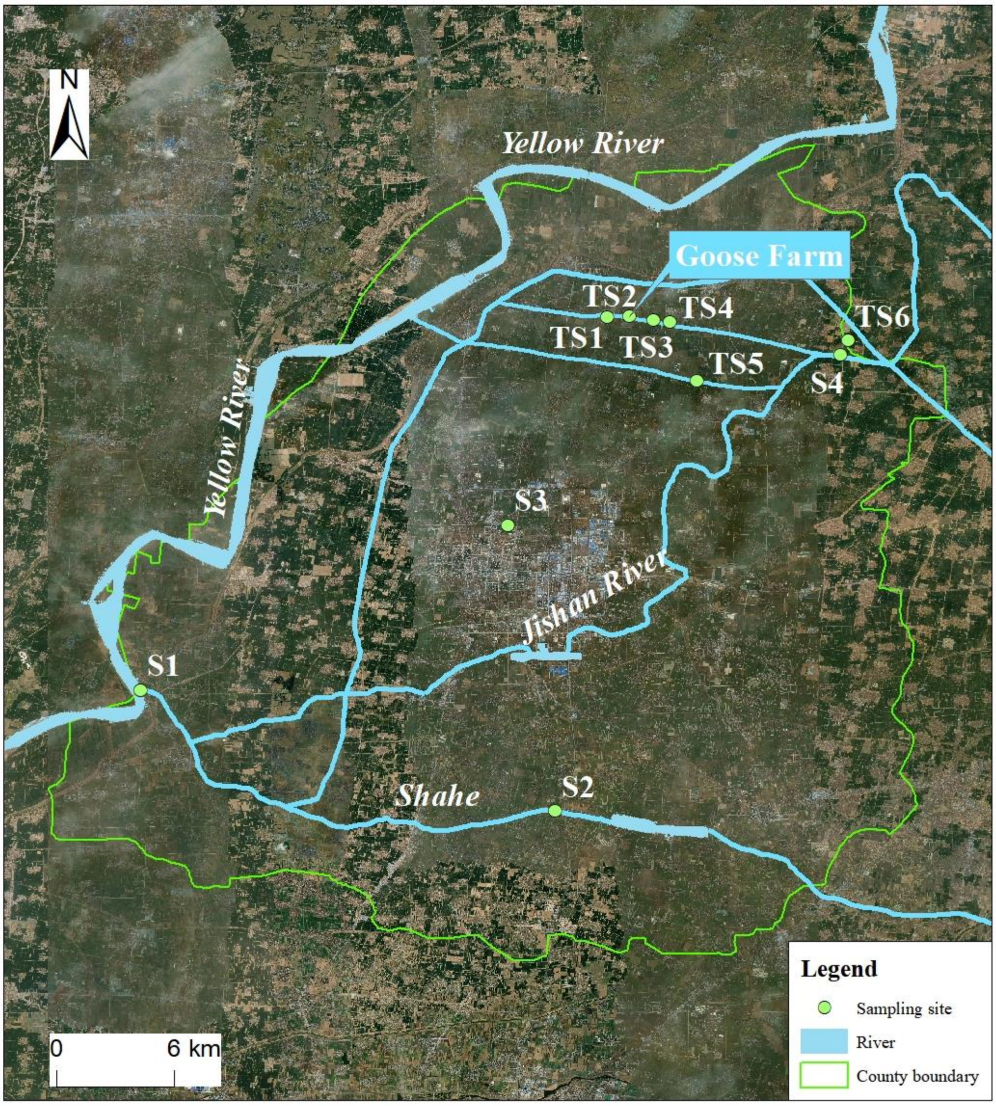

Figure 1. Location of the study area and distribution of sampling sites in Juancheng of Shandong, China. Among them, S1-4 (site 1-4) are located in the main stream and TS1-6 are located near the goose farm in the tributary of the Jishan River.

2.2 Sampling and sample treatments

During field investigation, three pre-cleaned 100 mL glass vials were used to collect surface water samples at each site (S1-4). Sediments were collected in three times by a 1/40 Peterson grab sampler, mixing into one portion with approximately 500 g at each sampling site. Then each sediment sample was divided into two parts, one was prepared for toxicity tests and substance identification which was stored in tin foil bags at 4°C condition, the other one was for benthic fauna identification and was stored in formalin solution.

After being taken back to the laboratory, surface water samples were filtered by 0.45-μm syringe filter (Jinteng, Tianjin, China) and were stored at 4 °C in glass vials. Sediment samples for toxicity test were freeze-dried, grounded to pass through a 60-mesh sieve, and followed by weighting 20 g pre-treated sediment sample for Soxhlet extraction with 120 mL solvent mixture (*n*-hexane: acetone; *v:v* = 1:1) for 12 h. Afterwards, the extract was concentrated by a vacuum rotary evaporator (R201D, Yinggu, China) under the conditions of 40 °C and 60 rpm to 1-2 mL. The extracts were further dried by nitrogen blowing and transferred to a liquid phase vial, and 1 mL Dimethyl sulfoxide (DMSO) was added to dissolve it for subsequent biological experiments [19]. Another part of the sediment samples was sealed and stored in 7% buffered formalin. Taxonomic identification of benthic organisms was carried out by a microscopy. The same taxonomic unit was weighed on an electronic balance and the final result was converted to biomass per unit area. Benthic identification with reference to data described by Zhang et al. [20].

2.3 Zebrafish embryotoxicity test (ZET)

The ZET was initiated at 4-5 h post fertilization (hpf) at the gastrulation period and ended at 96 hpf, as this covers the entire organogenesis in a zebrafish embryo [21]. Zebrafish embryos were collected into petri dishes containing recombinant water, which prepared by mixing $CaCl \cdot 2H_2O$ (10 mL, 294.0 mg/L), $MgSO_4 \cdot 7H_2O$ (10 mL, 123.3 mg/L), $NaHCO_3$ (10 mL, 63.0 mg/L) and KCl (10 mL, 5.5 mg/L) in 960 mL double distilled water to make a 1 L solution, and screened out under a microscope at 8 cells stage. Solvent controls (recombinant water), positive controls (100 mg/L 3,4-dichloroaniline) and negative controls (DMSO) were included in each independent experiment. The exposure medium of the water samples was raw water, and the exposure concentration of the sediment samples was 100 mg/mL. Then, zebrafish embryos were exposed in 24-well plates, which can provide more oxygen supply for the growth of zebrafish [22]. Every 10 wells of the 24-well plates were used to expose a substance to be tested or a negative control, with the positive control set up with 20 wells. Add 1 zebrafish embryo (pipetted with 1 mL of pre-exposure solution) to each well with a pipette gun, 1 mL of pre-exposure solution, for a total volume of 2 mL per well. Hatching rate, teratogenic rate and lethal rate are recorded using a microscope every 24 hours after exposure up to 96 hpf [23].

2.4 Biodiversity index

In this study, biodiversity of benthic fauna was evaluated using the following four indicators: Richness index, Shannon Wiener index, Simpson diversity index and Pielou evenness index, which are calculated as follows:

Richness index equals to the richness of the species contained in the community;

$$\textbf{Simpson diversity index} = 1 - \sum_{i=1}^{S} \left(\frac{N_i}{N}\right)^2;$$

$$\textbf{Shannon Wiener index} = -\sum_{i=1}^{S} (p_i * \ln p_i);$$

$$\textbf{Pielou evenness index} = \frac{H}{\ln S'};$$

In the above formula S refers to the total number of all kinds of species, N is the total number of individuals of all species, N_i is the number of individuals of species "i", p_i is the proportion of species "i" to the total number of species [24,25].

2.5 Benthic Index of Biological Integrity (B-IBI)

In order to analyze the benthic index of biological integrity (B-IBI), setting references sites are necessary. The reference sites mean there was no obvious human interference, good water quality, and high habitat quality. Based on the results of water quality and previous studies on zooplankton integrity index in Dongping Lake [9], the reference sites were selected (S1 and S2), and the rest were identified as impaired sites with high disturbance.

Based on relevant studies, a comprehensive selection of 15 candidate indicators sensitive to environmental changes was used to assess water samples (Table S1), and the indicators were classified into three categories: community diversity, community composition, and community trophic structure [26]. A preliminary boxplot analysis (Figure S1) of the above indicators is then performed. Correlation analysis (Table S2) was performed on the screened indicators to reflect the mutual independence of indicators [8]. $|r|$ greater than 0.750 means much overlapping information between two indices, and for highly correlated indices, one of them can be taken [17]. The ratio method is more accurate among the commonly used parameter standardization methods [27]. The 95% quantile value was selected as the best value for the parameter whose value became smaller due to enhanced interference. The parameter fraction for each sampling site is the parameter value divided by the best value. For those metrics positively correlated with disturbances, the optimal value is the 5% quantile of all sample parameters, and the index score is calculated as follows:

$$\mathbf{B-IBI} = (\mathbf{maximum\ value} - \mathbf{parameter\ value})/(\mathbf{maximum\ value} - \mathbf{optimal\ value});$$

After calculation, the scores ranged from 0 to 1. The final result of B-IBI is the sum of all the parameters. The 25% quantile of the reference point was selected as the health threshold, while those below the 25% quantile value could be scored in 4 equal segments, so the standards for evaluating the different health levels had 5 levels: excellent, good, moderate, poor, and bad.

2.6 Physico-chemical properties analysis

We analyzed 8 physico-chemical parameters aggregately. Temperature (T), Pressure (P), potential of hydrogen (pH), dissolved oxygen (DO), specific conductance (SPC) and oxidation reduction potential (ORP) are determined on-site using an YSI sensor (Professional Plus, USA). The total nitrogen (TN) and total phosphorus (TP) were determined according to APHA (2012) [20].

2.7 Data analysis

The Origin Pro 2021 for statistical processing and graphical files of test data. The results were statistically evaluated using analysis of variance (ANOVA). The level of significant difference was set at 5% ($p < 0.05$). All statistical analyses were performed with the software package SPSS 25.0 (IBM SPSS Statistics 25 IBM Corp., USA). Dose-effect curve was drawn by GraphPad Prism 8.0.

3. Results

3.1 Physico-chemical parameters

The water quality parameters of the sampling sites are shown in the Figure 2. Temperature and water pressure exhibit spatial differences among these sites. The pH values varied from 8.30 to 9.06, of which S2 was the highest and significantly higher than S3 and S4 ($p < 0.05$). The lowest concentration of DO (0.11 mg/L) was found at S1, followed by S3 (1.243 mg/L), S4 (2.693 mg/L) and S2 (4.23 mg/L). Both SPC and ORP showed a trend of fluctuation, with S4 being the largest and S3 was the smallest. The concentration of TN at all sites was higher than 2 mg/L, which is the maximum limit of agricultural water. Besides, the concentration of TP in S2 was much higher than that in other sites ($p < 0.05$).

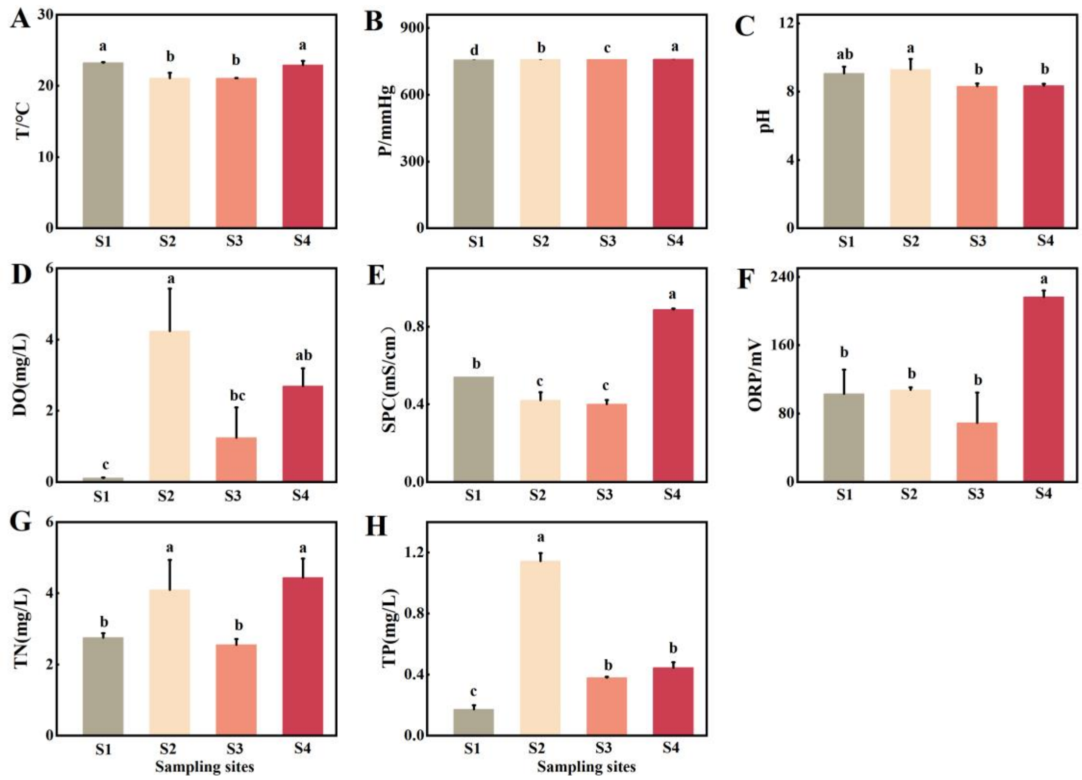

Figure 2. The physico-chemical factors of raw water in the main stream of the Jishan River of the Juancheng County, China. The different letters above the column indicate significant differences at the $p < 0.05$ level.

3.2 Benthic diversity

3.2.1 Species composition, distribution and diversity

A total of 21 benthic species were identified (Table 1), belonging to molluscs, arthropods and linkages. Among which, arthropods are the most common species, accounting for 67% of total species. The linkages (3 species) accounted for 14%, and molluscs (4 species) amounted to 19%. With each site, the largest number of species were found at S2 (57.8%). Regarding the abundance of species, Chironomus sp. was the most abundant species found at S2 and S3. The lowest number of benthic fauna was found at S1, with only 6 individuals (1.5%). The relationship between the biomass (B) and sampling sites is consistent with the number of species. The highest biomass contribution were from Plectotropis.

Table 1. Number of benthic species (N) and biomass (B) in the main stream of the Jishan River of the Juancheng County, China.

Classification	Latin name	S1	S2	S3	S4

		N	B	N	B	N	B	N	B
Linkage	*Limnodrilus hoffmeisteri*	1	0.0001	20	0.0208	1	0.0001		
	Branchiura sowerbyi			1	0.0001				
	Tubifex sinicus			2	0.0812				
Mollusc	*Plectotropis*			10	27.936				
	Gyraulus compressus							7	0.0377
	Physa acuta					1	0.0208		
	Parafossarulus striatulus			2	0.4716				
Arthodpod	*Macrobrachium nipponense*			1	0.0317				
	Exopalaemon modestus	1	0.2185						
	Chironomus sp.	1	0.0001	95	0.7597	149	0.7058		
	Polypedilum sp.			6	0.0003	1	0.0058		
	Dicrotendipes sp.			1	0.0001				
	Tanytarsus sp.			1	0.0001				
	Cryptotendipes sp.			25	0.0006				
	Cricotopus sp.					1	0.0001	1	0.0001
	Propsilocerus sp.	3	0.0006	26	0.0595	1	0.0002		
	Orthocladius sp.								
	Cryptochironomus sp.			1	0.0001				
	Clinotanypus sp.			5	0.0034				
	Tanypus sp.			12	0.0249				
	Procladius sp.			2	0.0041				
	Total	6	0.2193	229	29.3942	153	0.7328	8	0.0378

The results of species diversity are shown in Figure 3. Comparing these indexes, S2 was significantly higher than S1, S3 and S4, while there was no significant difference within other sites, with Pielou evenness index as exception.

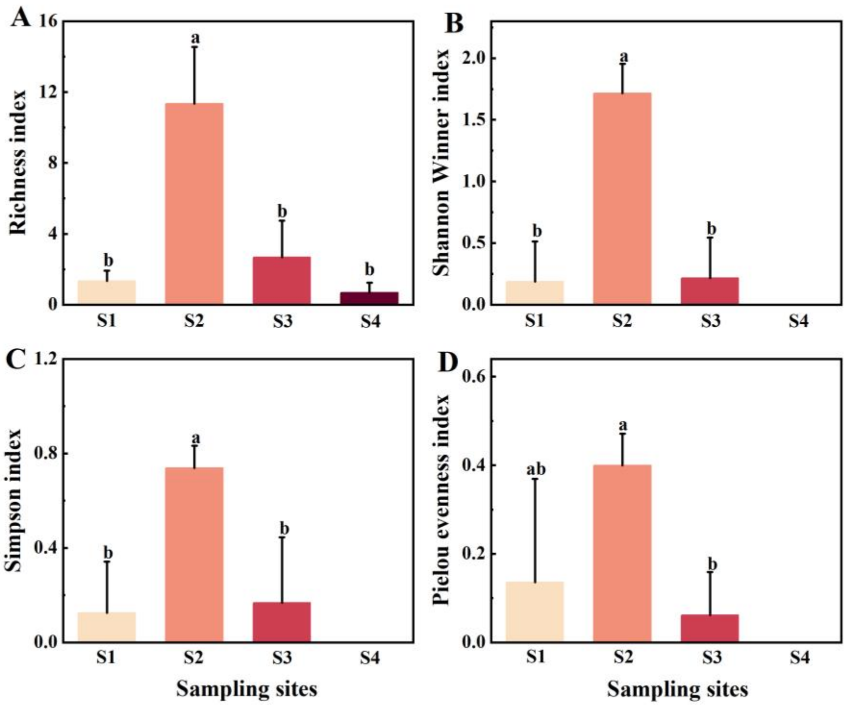

Figure 3. Richness index (A), Shannon Winner index (B), Simpson index (C) and Pielou evenness index (D) in S1, S2, S3, S4 in Juancheng, China. Different letters above each column indicate significant differences at $p<0.05$ level.

3.2.2 Ecological health evaluation using B-IBI

Through the Boxplot and Pearson correlation analysis, the final evaluation index of the B-IBI evaluation system was screened. Moreover, the B-IBI health evaluation criteria is shown in Table S3. As shown in Table S4, the B-IBI values of all sampling sites ranged from 0.741 to 2.963, with a mean value of 1.650. Hence, the overall health level of the tributaries of the Juancheng County is moderate. However, the spatial variability of the health status was observed, the highest B-IBI value was found at S2, which was 2.963. And the B-IBI values for S1, S3, S4 were 1.288, 1.740 and 0.583 respectively, referencing to poor, moderate, and bad status of ecosystem health. The ecological health of S4 was the worst among the four sites.

3.3 Effects of ZET

3.3.1 Effects of raw water and sediments

Figure 4A shows embryotoxic effects of zebrafish in raw water samples. As shown in Figure 4A, the raw water samples of all four sites exhibited teratogenic effects. Besides, S3 presented the most significant teratogenic effect on zebrafish embryos, which showed a significant difference in comparison to the negative control (NC) ($p < 0.05$).

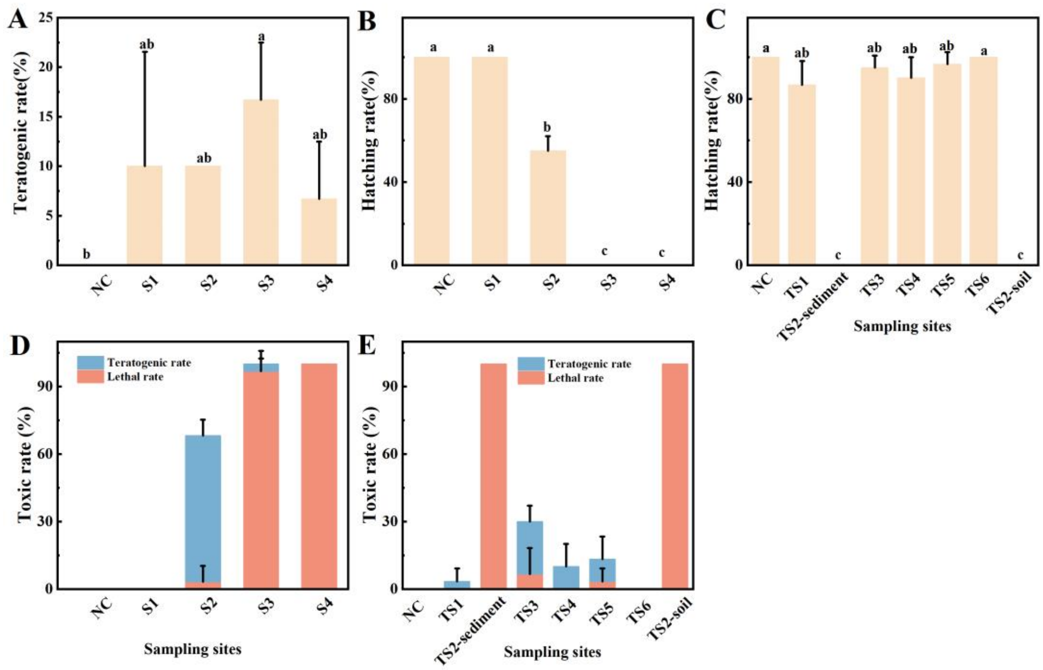

Figure 4. Zebrafish embryos toxic effects of raw water in Juancheng, China (A). Hatchability of zebrafish embryos in sediment samples of Main Stream (B) and Tributary (C) and toxic effects of zebrafish embryos in Main Stream (D) and Tributary (E).

The results of embryotoxic effects of zebrafish in sediment samples (100 mg/mL, Figure 4B, C, D, E) showed that 96 hpf hatching rate of zebrafish embryos in S1 is consistent with NC, implying that S1 has no significant effect on the development of zebrafish embryos. Zebrafishes were stunted in growth and development when exposed to S2, and only 55% hatched at 96 hpf. Simultaneously, a variety of malformation effects could be produced, specifically reflected as the formation of death, tail bending, yolk sac edema and pericardial edema (96 hpf). Completely death on zebrafish embryos were observed from exposing to S3 and S4 at 24 hpf. Considering the impact of farm wastewater discharge around the S4 on water quality, the same analysis of zebrafish embryotoxicity was done on the collected farm sediments in this study. The results showed that both TS2-sediment and TS2-soil, located inside the goose farm, showed significant differences in hatching rate and mortality comparing to NC ($p < 0.05$).

3.3.2 Dose-response relationship and LC_{50}

The four samples with 100% lethal effect were gradient diluted and the dose-effect curve was made through a series of concentration gradients tests to obtain the LC_{50} values. These results (Figrue 5) showed that exposure to S3, S4, TS2-sediment and TS2-soil induced concentration-dependent effects on the embryo survival in the ZET (96 hpf). In contrast, the LC_{50} values of the in-farm samples were smaller, implying greater toxicity and greater sensitivity of the embryos. More specifically, a 1.4-fold higher potency for S3 compared to S4 as reflected by the of the LC_{50} values of 45.36 and 32.05 mg/mL. In the farm samples, the LC_{50} value of the sediment was larger than that of the soil sample.

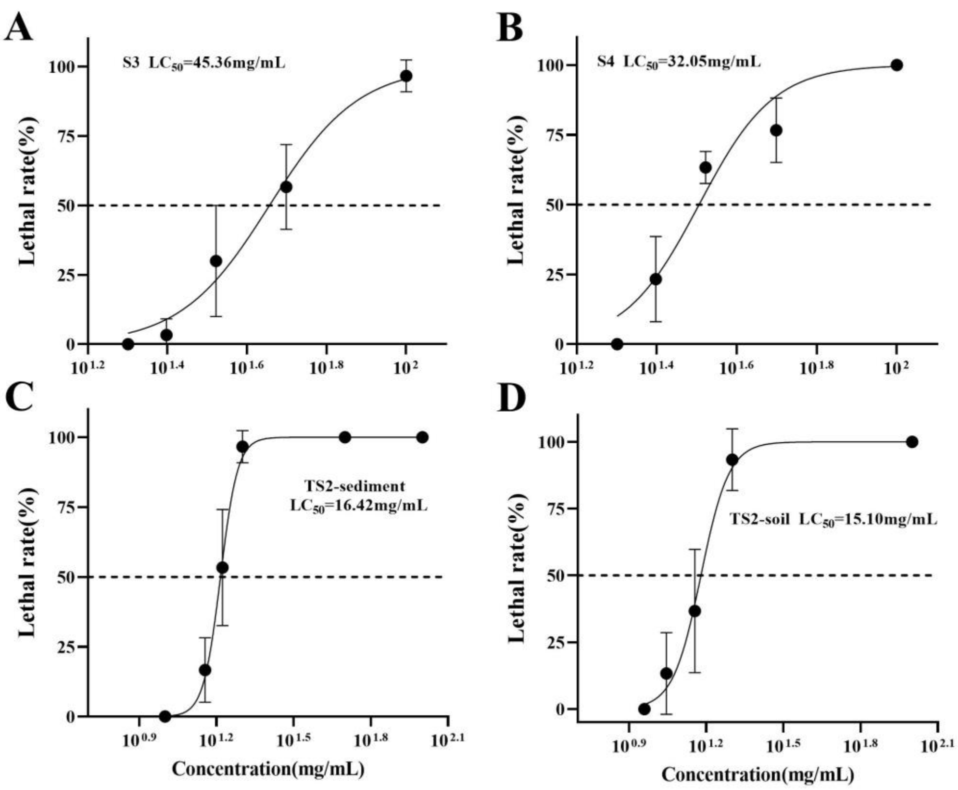

Figure 5. Dose-effect curve and LC_{50} values of S3 (A), S4 (B), TS2-sediment (C), TS2-soil (D).

3.4 Correlation analysis

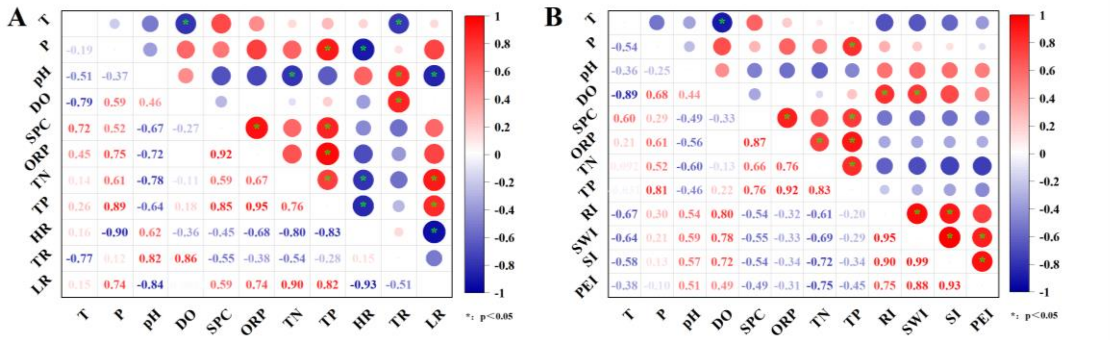

Figure 6. Correlation analysis between physico-chemical factors and toxic effects on zebrafish embryos (A) and physico-chemical factors and species diversity index (B). (HR: hatching rate, TR: Teratogenic rate, LR: Lethal rate, RI: Richness index, SWI: Shannon Winner index, SI: Simpson index, PEI: Pielou evenness index)

Pearson correlation analysis (Figure 6A) showed that toxicity indexes of fish all showed correlation with physico-chemical factors ($p < 0.05$). HR and LR showed significant correlation with both TN and TP, with the former being negatively correlated and the latter being positively correlated respectively. TR was not significantly related to TN and TP, but had a significant positive relationship with pH and DO. Besides, a negative correlation was observed between CHR and P, TR and T, and LR and pH. In Figure 6B, both RI and SWI were positively correlated with DO ($p < 0.05$). However, there was no significant correlation between SI, PEI and physico-chemical factors.

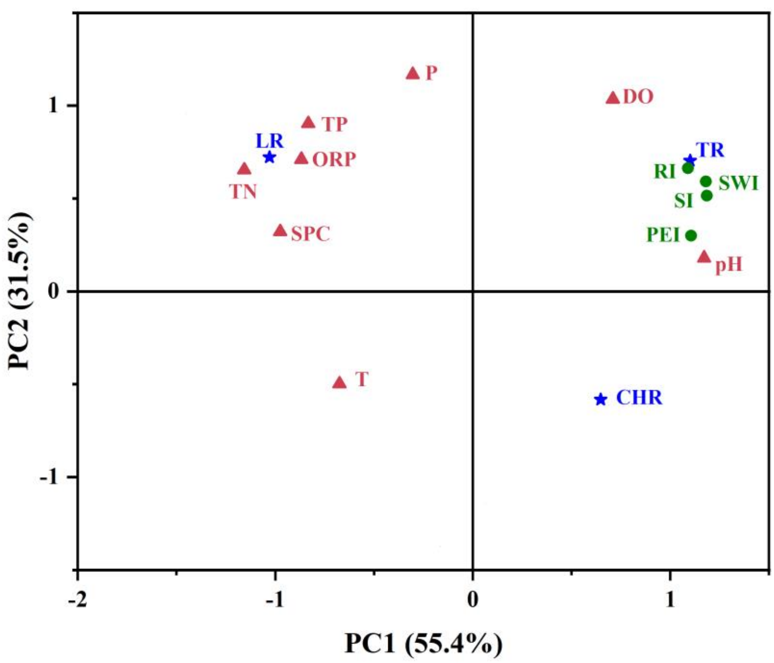

Figure 7. Principal component analysis (PCA) of physico-chemical factors, toxic effects in fish and species diversity index in the Juancheng County, China. The factor loadings of PCA-axes 1 and 2 are shown and explained 86.9% of the total variation. The angles between vectors indicate the degree of independence of individual variables.

The first two axes of the PCA explained 86.9% of the variation in each factor between the different sites (Figure 7), suggesting that the first two factors contain most of the data information, can accurately reflect the water quality condition. Most physico-chemical factors were clustered along the third quadrant. Species diversity index were clustered along the second quadrant, and clustered with DO and pH since their arrows on the X-axis pointed in the same direction.

4. Discussions

4.1 Water quality and benthic fauna

It has been known that benthic community reflect ecological quality and integrate the effects of different stressors providing a broad measure of their impact [28]. Therefore, benthic fauna is widely used as water quality indicators [8,29]. Our results showed that the number of species and biomass of all four sites showed a maximum at S2, which implies a high biodiversity. Chironomus sp. are the most abundant species in S2, which is an indicator organism for clean water [30]. S2 also has the most abundant taxa, and decreasing downstream to S3 and S4. This is in line with previously studies by Huh et al. [31] and Nestlerode, J.A.[32]. Compared to the other sampling sites, S1 had the least taxa, probably because the river channel narrows sharply after the flow of the Yellow River into the S4, resulting in the scouring force higher [32].

4.2 Toxic effects to zebrafish embryos and ecosystem risk

In this study, we assessed the ecological risk by zebrafish embryos, which is an important model organism for toxicological studies [34]. In general, except for S1, the sediment toxic effect was more significant compared to the teratogenic effect presented by the raw water. S1 did not show significant toxic effects when the sediment exposure concentration reached 100 mg/mL ($p < 0.05$). In contrast, S2 was more teratogenic, and a variety of deformities were found in the observation. The possible reason is that river siltation often occurs here [35], leading to the lack of oxygen and accumulation of harmful substances [36]. Both S3 and S4 showed very strong lethal effects, and further analysis of the samples collected from the goose farm revealed that the effect of farm wastewater on zebrafish embryos was significantly high ($p < 0.05$), with the LC_{50} values accounting for 1/3-1/2 of those at S3 and S4, which were more toxic [37]. This may due to the high level of contamination of organic matter, especially microorganisms (E. coli) in aquaculture wastewater [38]. It shows that the sources of ~~polluting substances~~ mainly come from human activities, including urbanization, industrialization, etc. This is consistent with the results of previous studies of endocrine disruptors in the region [39], which poses a challenge to the protection and conservation of drinking water resources.

5. Conclusions

In this study, the water quality safety of the downstream Yellow River transfer-type towns was analyzed by combining analysis of physico-chemical index, biological integrity index and zebrafish toxicity tests. It was found that even raw surface water could exhibit toxic effects. The tests on zebrafish embryos indicated that sediments from rural reaches were more toxic than in urban, similar results were obtained from aquatic organism community. Therefore, it is of importance to protect watershed environment in rural reaches of the river. This study can provide scientific basis and regulatory reference for the sustainable management of urban-rural rivers.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Preliminary analysis method of boxplot of candidate indicators；Table S1:

Candidate indicators and codes for benthic integrity evaluation system; Table S2: Pearson correlation between biological indicators; Table S3: B-IBI health evaluation criteria; Table S4: Calculation results and levels of core indicators for each sampling site; Figure S1: Boxplots of the candidate biological indicators at the reference point and the damage point.

Author Contributions: Wenhua Shao: Investigation, Writing – original draft preparation. Zhongli Chen: Supervision, Writing – review and editing. Ying Shao: Supervision, Writing – review and editing.

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Data Availability Statement: The data that support the findings of this study will be provided upon reasonable request to the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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