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Spatiotemporal heterogeneity of antibiotic pollution and ecological risk assessment in Taihu Lake Basin, China

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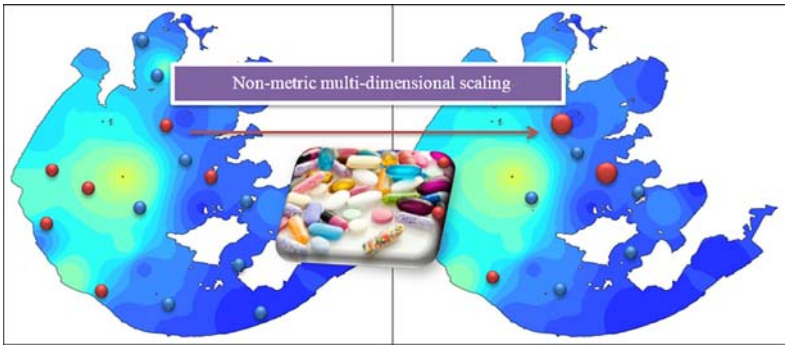
HIGHLIGHTS

- 22 antibiotics in water and sediment were measured in Taihu Lake Basin in 2017.
- Summed standardized concentrations represented an overall pollution of antibiotics.
- The overall pollution level of antibiotics generally presented spatial homogenous.
- Antibiotics pollution was more serious in spring, summer and winter than in autumn.
- Cumulative ecological risk for fish species in Taihu Lake can be neglectable.

GRAPHICAL ABSTRACT



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ABSTRACT

Natural lakes play a vital role as receiving system of a cocktail of antibiotics (ABs) which have triggered a major health concern. The comparisons of ABs concentrations have been substantially implemented throughout the worldwide range. However, from lake management, the questions are not yet adequately solved: "when and where does the overall pollution level of ABs present more serious, and what AB species dominate". In this study, we detected 22 ABs in water column and sediment bottom in Taihu Lake Basin in January, April, July and October in 2017. Non-metric multi-dimensional scaling (NMDS) was applied to characterize spatiotemporal dissimilarity of ABs concentrations. Combined with a method of summed standardized concentrations, analysis of variance was applied to evaluate the overall pollution level of ABs at different sites and time periods, instead of, traditionally, a comparison of concentration. The results showed that 90% CI of Macrolides, Sulfonamides, Tetracyclines and Quinolones were 0.020–5.646, 0.040–7.887, 0.100–13.308 and 0.130–9.631 ng/L in water column, respectively; and 0.005–1.532, 0.002–0.120, 0.010–0.902 and 0.006–3.972 μg/kg in sediment, respectively. ABs concentrations approximately presented spatial homogeneity in the whole basin which included all main inflow rivers, outflow rivers and the lake body itself. Species composition was seasonally distinct and the overall pollution level was significantly lower in autumn. A critical body residue analysis showed that ABs concentrations presented a neglectable cumulative risk for fish species. This research added to the body of knowledge to develop pollution management strategies on point and non-point source loads for Taihu Lake Basin, and also the methodology provided reference for spatiotemporal characterization of dissolved pollutant in other water bodies.

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1. Introduction

Antibiotics (ABs) have been widely used in human and veterinary health care, and also acted as growth-promoting drugs in livestock and poultry breeding and aquaculture (Chung et al., 2017) (Gothwal and Shashidhar, 2015). Generally, they are spreading in low concentration level across the receiving natural water bodies mainly via direct discharge, agricultural runoff and wastewater treatment plant (Aga et al., 2016; Hu et al., 2018). The main "side-effect" of ABs concerns their toxicity to inhibit the growth of aquatic organism, and the antibiotic resistance to undermine human health (Bielen et al., 2017; Liu et al., 2017). Particularly, along with long-term ABs input to aquatic ecosystem, the antibiotic-resistant bacteria and/or antibiotic-resistant pathogens carrying antibiotic resistance genes could "invade" into human body via direct contact and food chain transfer as function of horizontal gene transfer mechanism, and further potentially yield specific antibiotic resistance which causes medical treatment failure and even death (Ashbolt et al., 2013; Devarajan et al., 2017; Le Page et al., 2017; Shindo and Hasegawa, 2017; Tavernise and Grady, 2016). As a result, the water body polluted by ABs probably presents an important reservoir for the proliferation and transmission of "antibiotic resistance", which aggravates the potential to infect disease in a vulnerable population that is exposed to the water or aquatic products (Carvalho and Santos, 2016; Stoll et al., 2012). Furthermore, ABs might pose high cumulative risk to aquatic organisms due to its pseudo-persistent characteristics of long-term loading and degradation-resistant in environments (Z. Wang et al., 2017). Consequently, for improving the knowledge on "pollution source - fate and transport - toxic effects" of emerging ABs (Arnold et al., 2014), the primary goals are to substantially investigate the occurrence of ABs in the environments, identify the potential sources based on spatiotemporal distribution, and evaluate cumulative ecological risk.

China is the largest producer and consumer of ABs in the world, with different categories of ABs detected in environmental media (Li et al., 2017; Zhang et al., 2015). Lake ecosystems always present close relationship with regional anthropologic activities and economic development, particularly located in plain river network, and possibly provide sufficient water supply and aquatic resources for local residents (Wang and Bi, 2016). As a representative, Taihu Lake (with a drainage area of 36,900 km^2, a surface water area of 2338 km^2, mean water depth of 1.9 m) is the third large freshwater lake in China, which contributes to approximately 14% of gross domestic product of the nation (He et al., 2015; Li et al., 2016; C. Wang et al., 2017). Up to now, five reports were published on the field investigations on ABs concentrations in the Taihu lake (Hu et al., 2017; Xie et al., 2015; Xie et al., 2017; Xu et al., 2014; Zhou et al., 2016). Meanwhile, concentration levels in natural water bodies were substantially compared in nationwide and worldwide range. However, based on those results we found several potential inadequacies as follows: (1) these investigations were carried out on only one or two sampling dates. It was demanding to illuminate the difference or similarity of pollution level in different seasons. (2) The sampling sites were restricted to the lake body without considerations of inflow and outflow rivers. The question on "where the ABs in the most polluted sub-region of the lake are more likely to originate from?" was still unanswered. (3) The analysis on ABs concentrations was discussed based on respective category rather than considering the cocktail of ABs as a whole. As a result, it was intended to form a composite response variable of all detected ABs, in order to describe, in an overall sense, how "polluted" a sample was.

In this study, we investigated spatiotemporal characteristics of the concentrations of Macrolides (MLs), Sulfonamides (SAs), Tetracyclines (TCs) and Quinolones (QNs) antibiotics in water column and sediment bottom in Taihu Lake Basin which included all main inflow rivers, outflow rivers and the lake body itself; and then using statistical techniques we characterized concentration differences at different sampling sites and four distinct seasons in the basin; Finally, cumulative ecological risk was estimated to comment on strategies for ABs pollution regulation.

2. Material and methods

2.1. Sample collection

Taihu Lake Basin, which is located between 30°56′-31°33′N and 119°53′-120°36′E in China, and in general the river-fed lake has 22 inflow and 7 outflow rivers (Wang et al., 2015a). The filed investigations of ABs concentrations were implemented in the middle month of January (winter), April (spring), July (summer) and October (fall) in 2017, respectively. We established one water sampling site at each inflow and outflow river, and 9 sites were respectively distributed into 9 sub-regions of the lake (see Fig. 1). Each sampling site with corresponding river and sub-region of Taihu Lake were listed in Table S1. At each site, water samples were collected at a depth of 0.5 m in surface water using stainless steel water sampler, and then sealed in brown glass bottles (1 L). Sediment samples were collected at a depth of 0-5 cm in sediment bottom using grab dredger, and then packaged with aluminum foil and stored in a sealed bag. Under the guide of navigation-GPS (the reference system is China Geodetic Coordinate System 2000), we collected a total of 304 samples for concentration determination (38 sites × 2 samples × 4 times, 152 water samples and 152 sediment samples). All samples were transported to laboratory on the same day and stored at 4 °C in fridge. Samples were analyzed for four categories of MLs, SAs, TCs and QNs which included 22 ABs (see Section Chemicals in SM).

2.2. Analytical methods for antibiotics

2.2.1. Pretreatment

Water sample was filtered by 0.45 μm glass fiber filter (Pall Corp., USA), and then 100 mL of the filtrate was adopted. With addition of 0.2 g Na_2EDTA, the solution was shaken up, and then the pH was adjusted to approximately 5.0 using formic acid. 5 μL of 1.0 mg/L mixed solution of internal standards (Roxithromycin-d7, Sulfamethoxazole-d4, Norfloxacin-d5, Demeclocycline, purchased from AccuStandard, New Haven, USA) was added by micro-syringe, and then we shook up the mixture well. The solution was enriched via Oasis HLB column (200 mg 6 mL, Waters Corp., USA). 8 mL of methanol was applied to elute all of the target analytes. The collected eluant was blown to dryness at 40 °C by a gentle stream of nitrogen (bath-typed nitrogen blowing instrument, DN-24 W, China), reconstituted in 1 mL of 20% methanol and acidified water (0.1% formic acid). Finally, it was filtered through 0.22 μm membrane (Pall Corp., USA) for instrumental analysis.

Sediment sample was grinded to pass 60 mesh sieve after freezing-drying (vacuum freeze dryer, BIOCOOL Corp., China), in order to remove large particle matters, e.g., plant debris and rubble. 5 g of the sample after treatment was put into 50 mL centrifuge tube, and then 5 μL of 1.0 mg/L mixed solution of internal standards (Roxithromycin-d7, Sulfamethoxazole-d4, Norfloxacin-d5, Demeclocycline, purchased from AccuStandard, New Haven, USA) was added by micro-syringe, and then we shook up the mixture well. With addition of 10 mL of phosphate buffer and 10 mL of acetonitrile, it was subjected to the vortex blending for 1 min (Vortex Genie II vortex mixer, Scientific Industries Inc., USA) and violently shaking for 3 min. An additional 2 g NaCl was added and mixed in a vortex blending for 1 min and then centrifuged for 6 min under 4000 rpm (Eppendorf Centrifuge, 5810R, Germany). The supernatant was set to a final volume of 10 mL using acetonitrile, and then 1 g $MgSO_4$ was added, with the following mixing. 3 mL of resultant solution was then purified by solid-phase extraction (SPE) clean-up (Oasis HLB cartridge, 500 mg/6 mL, Waters Corp., USA). 2 mL of the clear supernatant was blown to dryness by nitrogen blowing and reconstituted in 1 mL of 20% methanol and acidified water (0.1% formic acid). Finally, it was filtered by 0.22 μm membrane for instrumental analysis. Meanwhile, we also determined the density of

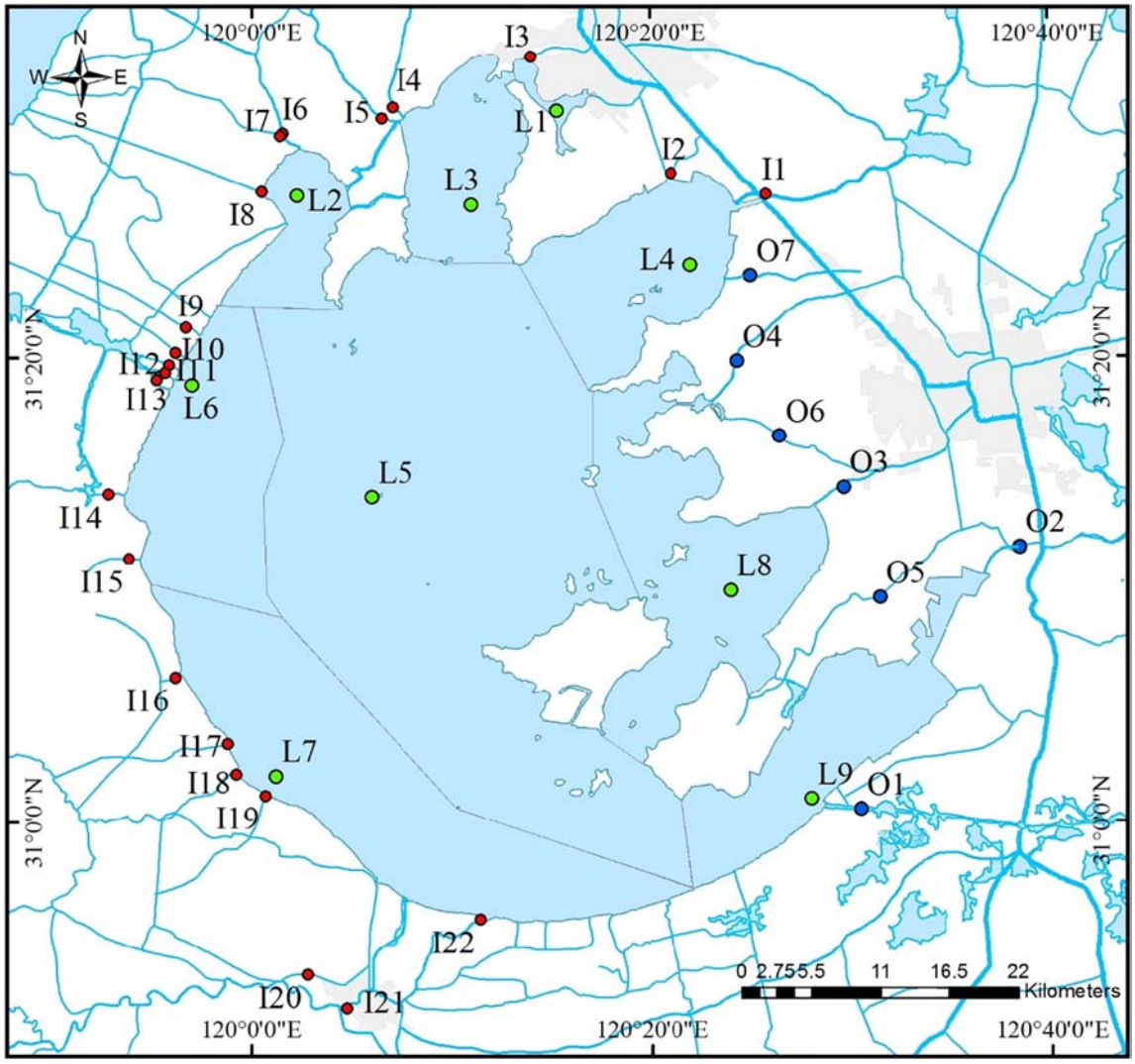

Fig. 1. Taihu Lake Basin and 38 sampling sites (22 main inflow rivers: I1-I22; 7 main outflow rivers: O1-O7; 9 sub-regions in the lake: L1 in Wulihu Bay (WB), L2 in Zhushan Bay (ZB), L3 in Meilianghu Bay (MB), L4 in Gonghu Bay (GB), L5 in Center Zone (CZ), L6 in West Zone (WZ), L7 in South Zone (SZ), L8 in East Zone (EZ) and L9 in Dongtaihu Bay (DB)).

sediment bottom for all sampling locations using ring shear test (Wang and Sassa, 2002), in order to transfer the concentration unit of ABs (μg/kg to ng/L).

2.2.2. UHPLC-MS/MS determination

The ABs concentrations were determined by an ultra-high performance liquid chromatography (UHPLC) (ExionLC AD -QTRAP5500) coupled with 5000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (AB SCIEX, Redwood City, CA, USA) equipped with an electrospray ionization (ESI) source using multiple reaction monitoring (MRM) positive mode. The UHPLC was equipped with Kinetex F5 PFP core-shell LC columns (50 mm × 3.0 mm, 2.6 μm, Phenomenex, Torrance, CA, USA) and Security Guard ULTRA Cartridges system (UHPLC PFP for 3 mm ID columns, Phenomenex, Torrance, CA, USA). The sample injection volume was 10 μL. Column temperature was maintained at 40 °C and the separation was carried out at a flow rate of 0.4 mL/min. The mobile phase gradient was run with 0.1% formic acid in water (v/v) (mobile phase A) and acetonitrile (mobile phase B). The gradient was 5% B for 1 min, 5%–15% B for 1–1.1 min, 15%–75% B for 1.1–9.5 min, 75%–95% B for 9.5–9.6 min, 95% B for 9.6–11.5 min, 95%–3% B for 11.5–11.6 min and then maintained for 11.6–13.5 min. The optimum MS parameters of the ion source were: curtain gas pressure (CUR) 30 psi; ion spray voltage: 5500 V; ion source temperature (TEM): 500 °C; atomization air pressure (GS1): 50 psi; auxiliary gas (GS2): 60 psi; collision gas: medium; scheduled MRM (basic); MRM detection window: 40s; Target scan time: 0.3 s; Q1 resolution unit; Q3 resolution unit. The optimal MRM parameters for retention time (RT), decluttering potential (DP) and collision energy (CE) for each chemical were summarized in Table S2.

2.2.3. Quality assurance and quality control

All determined data were subjected to a strict quality assurance and quality control (QA/QC) analysis, and corresponding concentrations were calculated based on an internal standard method (Ding et al., 2016; Hoff et al., 2015). The estimated limit of detection (LOD) and limit of quantification (LOQ) for signal/noise, 3 and 10 respectively were listed in Table S3, based on the work of (Couperus et al., 2016). For each AB, we added the mixed standard solution with corresponding internal standard into collected water and sediment sample respectively to implement recovery test. Under consideration of large difference in concentration of collected samples, for each chemical high (5.0 μg/L), moderate (1.0 μg/L) and low (0.1 μg/L) concentration level of surrogate standard were spiked for recovery, respectively, and on each level six parallel samples were specified. The optimized method as mentioned above was adopted to determine ABs concentrations. The detailed information of precision and accuracy results of 22 ABs in water column and sediment bottom were referred of Table S4 and Table S5.

2.3. Statistic analysis methods

2.3.1. Non-metric multidimensional scaling and permutation test

Non-metric multi-dimensional scaling (NMDS) is an ordination/dimensionality-reduction technique (Vathy-Fogarassy and Abonyi, 2009; Wagner et al., 2007) that uses rank order information of a computed matrix of dissimilarity/similarity metrics (such as the Brays-Curtis distance (Ahrens et al., 2011)) between collected pairs of samples. In this study, the total measured concentrations of MLs, SAs, TCs and QNs in water column and sediment bottom at each sampling site and date were used to compute the similarity matrix on which the NMDS analysis was based. The NMDS reduces the 22-dimensional data to a much smaller number (typically two or three dimensions) for ease of plotting and interpretation, while attempting to preserve the rank order of dissimilarity between pairs of samples – that is, the pair of sites with the largest calculated dissimilarity should have the largest Euclidean distance between them in the NMDS space and the pair of sites with the seventh-largest dissimilarity should have the seventh-largest Euclidean distance in the NMDS space, etc.

The extent to which the NMDS analysis is successful in keeping the rank-order of site-pair dissimilarities consonant with the Euclidean distance ranks in NMDS space is measured by a metric called *stress*. As the iterative NMDS procedure runs, stress declines towards zero as the fit improves. Stress values <0.15 are acceptable, but stress <0.1 is preferred. Stress can be reduced by including additional NMDS dimensions, but adding too many defeats the purpose of the technique and provides diminishing returns. The analyst typically begins by examining two NMDS dimensions, adding a third or fourth if the stress of the lower-dimensional solution is too high.

After the NMDS solution is achieved, a permutation test (Nichols and Holmes, 2002) can be invoked to examine whether covariates and/or factors (sampling site and date for our purposes) significantly affect positioning of samples in NMDS space. For each test, the null hypothesis is that the factor, e.g., monitoring site, has no effect on how data are positioned in NMDS space. The test works by randomly re-assigning concentration data to each sample, then calculating the separation between group centroids. For our study, this was done 10,000 times. The separation between group centroids from actual data is then compared to the distribution of 10,000 randomly-generated centroid differences. The proportion of random differences larger than the actual centroid difference serves as the significance level (p-value) of the test. The NMDS analysis was implemented using the VEGAN package available for the statistical software R (Team, 2016).

2.3.2. Analysis of variance and Tukey's multiple comparison test

Analysis of variance (ANOVA) compares the equivalence of group means (Sabo and Boone, 2013) and is applied here to determine which sampling sites and dates show the greatest levels of ABs pollution. We used standardization method to process ABs concentrations for each sampling date and site so that no one chemical would dominate the analysis, in case of only the information in the chemicals of high concentration would factor into the model. Therefore, we aimed to give equal weight to the concentrations of each chemical so that the chemicals found at higher concentrations would not dominate those found at lower concentrations. First, the summation of concentrations of each AB in water column and sediment bottom was obtained. Then, the concentration was standardized by subtracting the mean concentration of that chemical across all dates and sites and dividing by the standard deviation, and finally, the 22 standardized values were then summed for each sample. This value as an overall indication of ABs pollution for each sample. Generally, high positive sum represented high ABs concentrations, while a low negative sum denoted low ABs concentrations. ANOVA was run on the summed standardized concentrations, with sampling site and collection date examined as potential explanatory factors. A multiple comparison test (Tukey's Honest Significant Difference, HSD) was used to test which pairs of standardized and summed means were statistically different (Abdi and Williams, 2010). It protects the family-wise error rate at 5% if invoked only after an overall significant ANOVA result ($p < 0.05$) is achieved. Analyses were performed in R.

Table 1
Statistics for antibiotics concentrations detected in water column and sediment bottom across 33 sites in Taihu Lake Basin.

Antibiotics		CAS	$LogK_{ow}$[a]	Water column (ng/L)						Sediment bottom (μg/kg) (dw)					
				Freq (%)[b]	LOD	LOQ	Min[c]	Median	Max	Freq (%)[b]	LOD	LOQ	Min[c]	Median	Max
MLs	ATM	83905-01-5	4.02	100.0	0.03	0.13	0.03	0.72	35.28	88.2	0.005	0.02	0.003	0.09	14.74
	SPM	8025-81-8	1.87	100.0	0.03	0.11	0.11	0.94	9.20	69.7	0.01	0.04	0.005	0.01	1.88
	TMC	108050-54-0	3.8	80.9	0.07	0.26	0.04	0.20	9.30	88.8	0.01	0.04	0.005	0.11	3.29
	ODM	3922-90-5	1.69	59.9	0.04	0.15	0.02	0.07	4.50	98.0	0.005	0.02	0.003	0.125	22.89
	ETM	114-07-8	3.06	100.0	0.04	0.17	0.07	1.77	272.29	94.7	0.008	0.032	0.004	0.075	2.03
	TYL	1401-69-0	1.63	100.0	0.04	0.15	0.11	0.91	35.23	99.3	0.008	0.032	0.004	0.175	5.12
	KIT	18361-45-0	2.59[d]	80.9	0.03	0.13	0.02	0.14	4.50	99.3	0.008	0.032	0.004	0.21	8.84
	RTM	80214-83-1	2.75	100.0	0.02	0.07	0.03	0.67	60.21	93.4	0.005	0.02	0.003	0.155	8.21
	JSM	16846-24-5	2.39[e]	82.9	0.03	0.10	0.02	0.08	2.35	84.2	0.007	0.028	0.004	0.02	0.38
SAs	SDZ	68-35-9	−0.09	100.0	0.04	0.14	0.07	0.555	14.99	52.0	0.004	0.014	0.002	0.01	0.39
	SMR	127-79-7	0.14	86.8	0.03	0.13	0.02	0.145	0.58	52.6	0.003	0.013	0.002	0.01	0.23
	SMZ	57-68-1	0.14	98.7	0.03	0.11	0.02	0.355	10.89	56.6	0.003	0.011	0.002	0.01	1.9
	SMX	723-46-6	0.89	100.0	0.06	0.22	0.06	5.695	490.64	72.4	0.006	0.022	0.003	0.02	0.61
	STZ	72-14-0	0.05	100.0	0.03	0.13	0.04	0.555	36.74	71.1	0.003	0.013	0.002	0.02	5.9
	SPD	144-83-2	0.35	90.8	0.04	0.17	0.02	0.265	4.7	57.2	0.004	0.017	0.002	0.01	0.32
	SQX	59-40-5	1.68	71.1	0.12	0.48	0.06	0.21	19.25	32.2	0.012	0.048	0.006	0.006	0.17
TCs	CTC	57-62-5	−0.62[f]	96.7	0.25	1	0.13	2.6	83.8	69.7	0.025	0.100	0.013	0.045	4.31
	DCC	564-25-0	−0.02	50.0	0.28	1.12	0.14	0.21	18.96	47.4	0.028	0.112	0.014	0.014	22.27
	OTC	79-57-2	−0.9	67.8	0.19	0.76	0.1	0.74	11.69	63.2	0.019	0.076	0.01	0.03	1.04
	TCC	60-54-8	−1.3	96.1	0.26	1.04	0.13	1.065	35.23	38.2	0.026	0.104	0.013	0.013	1.04
QNs	NFX	7045896-7	−1.03	92.1	0.11	0.45	0.06	1.12	31.26	82.9	0.011	0.045	0.006	0.07	38.3
	OFX	82419-36-1	−0.39	100.0	0.06	0.25	0.07	1.45	15.31	94.1	0.006	0.025	0.003	0.21	11.8

[a] $logK_{ow}$ data were derived from online PubChem database (https://pubchem.ncbi.nlm.nih.gov).
[b] Detection frequency (%).
[c] Measurements below the limit of detection were set to half the LOD.
[d] Estimated by EPI Suite using canonical SMILES of chemical.
[e] (Tomasz et al., 2010).
[f] (US, 2012).

2.4. Ecological risk assessment

A critical body residue (CBR) method (McCarty et al., 2013; Wang et al., 2015b) was applied to assess the cumulative ecological risk associated with estimated ABs concentrations in fish species in Taihu Lake Basin using detected total concentrations in water column and sediment bottom at each sampling date and site. Estimated whole body concentrations (WBC, mmol/kg) were calculated as shown in Eqs. (1) and (2). For conservative assessment, a threshold of CBR for significant deleterious effects on fish species was specified as 0.2 mmol/kg (McCarty and Mackay, 1993). It implied that the fish species in the lake were at high risk when the WBC exceeded this threshold. For the 304 samples in which all 22 ABs were measured, the WBC (summing across four categories of ABs) was estimated and then used to evaluate risk:

$$WBC = \sum_{i=1}^{n=22} C_i \times \frac{BAF_i}{MW_i} \times 10^{-6} \quad (1)$$

$$\log BAF = 0.12 + 0.86 \times \log K_{ow} \quad (2)$$

C_i [ng/L], BAF_i [L/Kg] and MW_i [g/mol] represented the total concentration in water column and sediment bottom for each AB, and the molecular weight of the i^{th} chemical was obtained from the online PubChem database (https://pubchem.ncbi.nlm.nih.gov) (also see Table S6). *BAF* values were estimated using the K_{ow} of chemical (Arnot and Gobas, 2006).

3. Results and discussion

3.1. Determination of antibiotics

All samples were analyzed for 22 ABs which were classified into four categories: MLs (9 chemicals) which included Azithromycin (ATM), Spiramycin (SPM), Tilmicosin (TMC), Oleandomycin Phosphate (ODM), Erythromycin (ETM), Tylosin (TYL), Kitasamycin (KIT, Leucomycin A5), Roxithromycin (RTM) and Josamycin (JSM, Leucomycin A3); SAs (7 chemicals) which included Sulfadiazine (SDZ), Sulfamerazine (SMR), Sulfamethazine (SMZ), Sulfamethoxazole (SMX), Sulfathiazole (STZ), Sulfapyridine (SPD) and Sulfaquinoxaline (SQX); TCs (4 chemicals) which included Chlortetracycline (CTC),

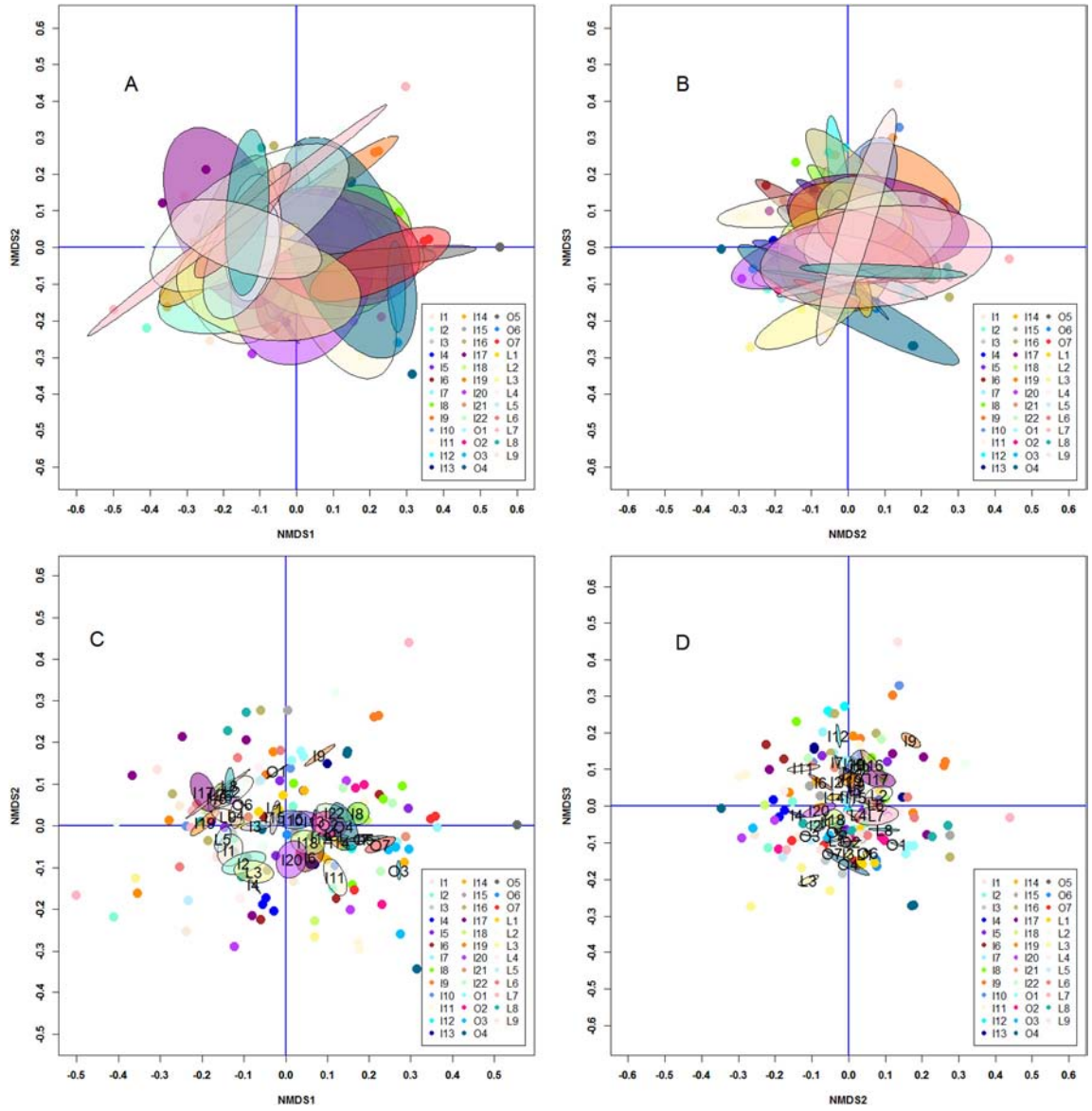

Fig. 2. NMDS plots of antibiotics concentration data. Confidence ellipses for the 38 site centroids are based on the 95% (A: NMDS1-NMDS2; B: NMDS2-NMDS3) and 10% (C: NMDS1-NMDS2; D: NMDS2-NMDS3) confidence levels.

Doxycycline (DCC), Oxytetracycline (OTC) and Tetracycline (TCC); QNs (two chemicals) which included Norfloxacin (NFX) and Ofloxacin (OFX). The statistics of those concentrations were listed in Table 1 and Table S7. In general, the detection frequency of ABs in water column was relatively higher than that in sediment bottom which however was subjected to more serious pollution based on concentration (transfer the unit to ng/L in sediment). It was worth to note NFX, ODM, DCC in sediment bottom across 22 detected ABs, as the maximum concentration of 38.3, 22.89 and 22.27 μg/kg was detected in Taihu Lake Basin, respectively. The average concentrations of MLs, SAs, TCs and QNs were: 1.588, 2.169, 3.141 and 3.016 ng/L in water column; and 0.365, 0.043, 0.216 and 1.111 μg/kg in sediment bottom across 38 sampling sites in 2017. In general, the spatiotemporal distribution of ABs in Taihu Lake Basin was influenced by pollution source, physicochemical properties, meteorological condition and lake dynamics.

3.2. Antibiotics at sampling sites

The three-dimensional (3D) NMDS solution was acceptable (stress = 0.15), indicating that NMDS was a useful ordination approach for extracting structure from the observed data. However, NMDS analysis generally did not show significant separation between 38 sampling sites, indicating that ABs pollution in Taihu Lake Basin showed a relatively homogenous distribution of sampling sites in ordination space. Fig. 2A and B depicted the 95% confidence ellipses for each site's centroid (the mean of NMDS scores for all samples taken at a given site) and they overlapped a great deal. The Fig. 2C and Fig. 2D showed the 10% confidence ellipses, presented only to give a general sense of the position of each site's centroid in NMDS space. In general, many sites were primarily separated by their values on NMDS1, the x-axis. Particularly, site #O3 was situated as far right as the other sites, but also separated from the other sites along NMDS2, the y-axis. The site #O1 and #I9 had a relatively high values in NMDS2. Site #I9, #I12 and #L3 were slightly separated along NMDS3. The permutation test *p*-value for sampling site as a factor was significant (<0.001) which provided an evidence for site-specific differences in ABs pollution.

The result of the ANOVA on the summed standardized concentrations indicated that then difference on overall pollution level of ABs were highly significant (<0.001), which meant the factor "site" had effect on overall ABs pollution. However, Tukey's HSD revealed that the concentration difference between site-site pair was not statistically significant if confidence level was specified as 0.05, even the average of pollution level could be ranked in order by site (see Fig. S1). Three pairs of monitoring sites differed in overall pollution level at a significance level of 0.1, namely, site #O7 was relatively greatly polluted than #L4 (difference: 24.18, $p = 0.080$), #L5 (difference: 24.17, $p = 0.081$) and #L8 (difference: 23.73, $p = 0.099$). The mean of summed standardized concentrations at site #O7 were higher than all other sites, and presented the lowest concentration at site #L4.

In general, there were intensive industrial and agricultural actives in the Taihu Lake Basin which had a large population of 60.28 million, according to "Taihu Basin and Southeast Rivers Water Resources Bulletin" issued in 2016 (MWR, 2016). Point-source (PS) and non-point source (NPS) loading's major contribution to ABs pollution attracted great attention from local authorities and much public concern. We investigated 4889 industrial discharges and 217 wastewater treatment plants (WWTPs) load which was considered as the main source of human-use ABs surrounding the Taihu Lake during 2013 through 2017 (Kerrigan et al., 2018). The main 317 state-controlled pollution sources (100 industrial discharges and 217 WWTPs) which in particularly included 10 pharmacy enterprises were illustrated in Fig.S2. Unfortunately, we cannot have access to the detailed calculation on how much ABs was yielded from PS loads. Moreover, NPS load always played a significant role as a large amount of crop farming (orchard, tea, vegetable garden), livestock and poultry breeding (e.g., chicken, pig, cow, duck), and aquaculture (e.g., fish, shrimp, crab) were assembled around the lake (Pollard and Morra, 2018; Saxena et al., 2018; Zhang et al., 2016). Both "scattered" PS and "patched" NPS load implied that they potentially provided a "seedbed" of ABs and probably caused approximately homogeneity distribution. Local authority should manage and control on ABs pollution from a systematic and holistic perspective due to its homogenous characteristic.

3.3. Antibiotics on sampling dates

NMDS results grouped by sampling date appeared in Fig. 3. Only the 95% confidence ellipses were shown because separation between dates was more distinctive. Samples taken in January (winter) were isolated in the top right corner of NMDS1-NMDS2 space (see Fig. 3A). April samples (spring) had good separation from samples taken in July (summer) and October (autumn) which somewhat overlapped with each other. It could also been seen that the last two sampling dates, July and October, were positioned between the two above groups in NMDS2-NMDS3 space (see Fig. 3B). Particularly, a great variability – that was, a large

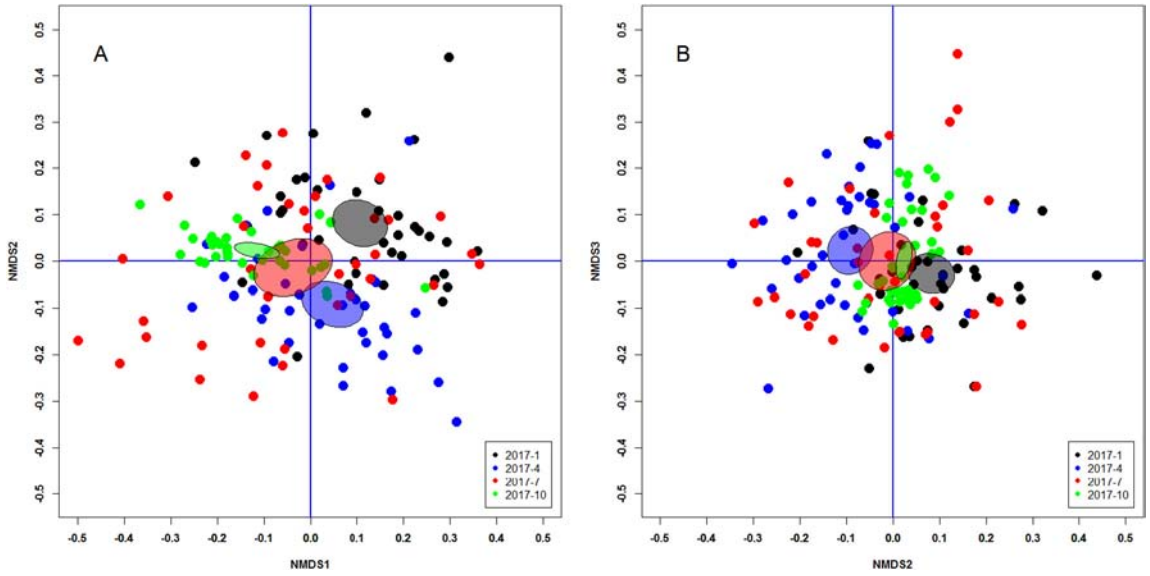

Fig. 3. NMDS plot for antibiotics concentration data, showing the 95% confidence ellipses for the centroids of samples taken on four monitoring dates (A: NMDS1-NMDS2; B: NMDS2-NMDS3).

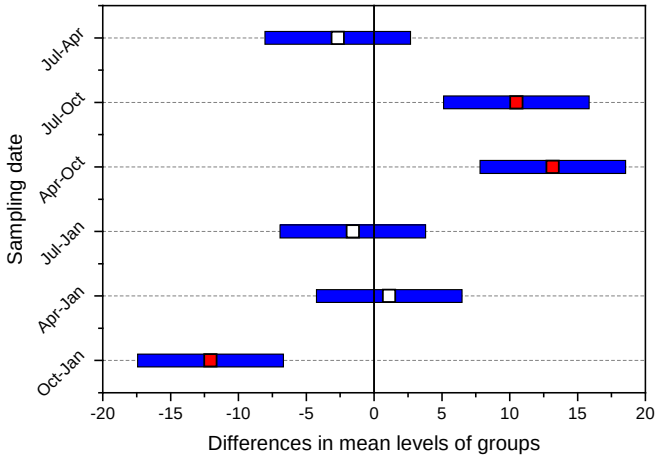

Fig. 4. Tukey pairwise comparison of the standardized and summed average concentrations of antibiotics on the four sampling dates. Squares represent the difference between the sample means and the red squares show significant ($p < 0.001$) differences.

confidence ellipse relative to the other dates, — was revealed in July samples. The sampling date was located near the middle of the NMDS plot. The permutation *p*-value (<0.001) clearly showed sampling date was related to location of these samples in NMDS space.

ANOVA on standardized and summed chemical concentrations also indicated that the overall ABs pollution varied significantly between sampling dates ($p < 0.001$). ABs concentrations were lowest in October, as illustrated in Fig. 4 (also see Fig. S3). Each significant pairwise comparison involved the date. In other pairwise comparisons, no significant differences were displayed. However, with such a small number of sampling dates across a long period of time, and without data on day-to-day variability in pollutant concentrations in this system, definitive conclusions cannot be drawn.

Examining a plot of chemical "hotspots" in NMDS space can be instructive: Fig. 5 showed the location of the centroid for each AB, designated by chemical name. The location of each centroid was the weighted mean of NMDS1 and NMDS2, and NMDS2 and NMDS3 scores for all samples, where weights were concentration of the chemical in the sample. A chemical centroid should thus be located near samples with the highest concentrations of that chemical. If all samples had equal concentrations of a chemical, that chemical centroid would be at the geographic center of every point on the NMDS plot. By averaging observation locations in NMDS space, it was possible for a chemical centroid to appear in a region of NMDS space where no sample occurred. This can make interpretation of the centroids more difficult, and only general conclusions should be drawn.

As illustrated in Fig. 5A and B, most of MLs had centroids to the right of NMDS1 axis. An obvious exception was TMC which had remarkable higher value on NMDS2. The NMDS2 values for SAs showed much greater variability. Particularly, SMR and SPD had a large negative NMDS2 scores, indicating that samples with higher these two ABs concentrations tended to occur on the down side of NMDS1-NMDS2 plot. Similarly pattern could be found in TCs alongside NMDS3 in which CTC and DCC had large positive scores. We also found that a large negative score for DCC in NMDS1. For QNs, high concentration would drive the centroid towards the right side of NMDS1 axis, and particularly, NFX had a high score in NMDS3.

Comparing chemical centroids in Fig. 5 with the sampling date centroid confidence ellipses (see Fig. 3), we deduced that the samples taken in January (black ellipse) occurred in the same region as SPM, SMZ, TYL and NFX. In April (blue ellipse), the location of ellipse might depended on ODM, ETM, OTC, SPD and OFX. July (red ellipse) appeared in the middle of the NMDS plot where centroid for most of MLs and TCs, as well as some SAs and OFX. The one sampling date with the lowest pollution level (October, based on ANOVA results) coincided with the centroids for SDZ, STZ, SQX, SMX and JSM. The overlap in sampling site confidence ellipses (Fig. 2A and B) limited conclusions regarding site samples and chemical hotspots. We could only note that some sites (e.g., site #O7) appeared on the right side of NMDS1, corresponding to the location of MLs and QNs centroids.

In general, the complex component of ABs varied in different seasons, possibly due to abiotic factors, e.g., hydrodynamic condition, wind direction and speed. The overall pollution level of ABs might depended on the net effect of many co-exist external drivers. E.g., the positive role of rainfall was that the more precipitation drove the more surface runoffs which potentially carried ABs into receiving rivers and the lake. The rainfall served as negative effect might also reduce ABs concentrations as function of water dilution. It seemed that the positive effect might dominate during January, April and July in which the

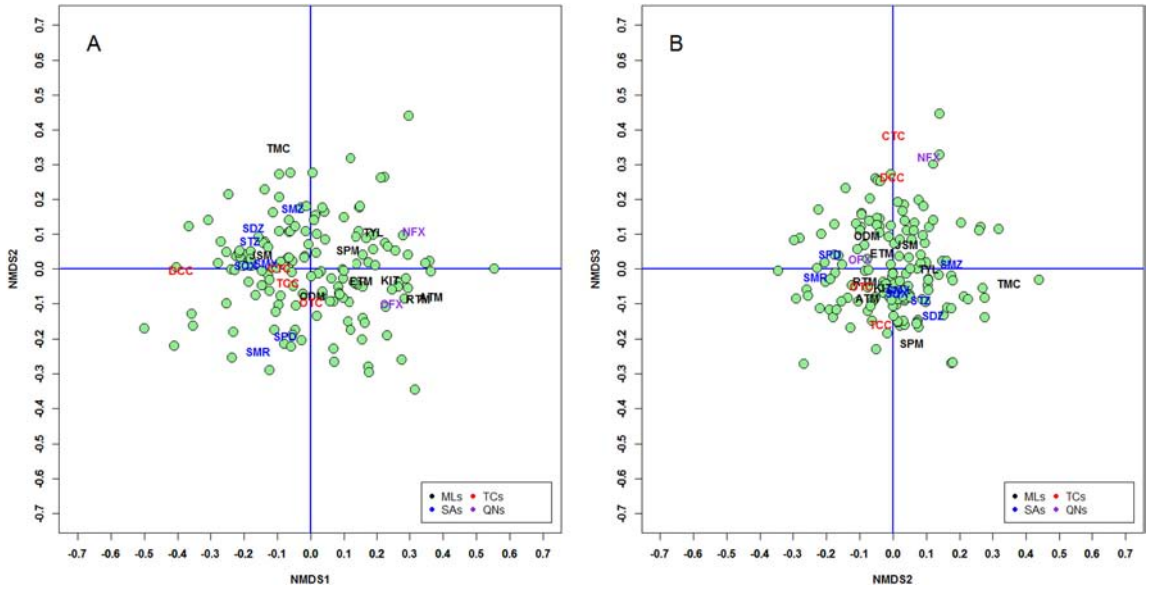

Fig. 5. Centroid locations for the 22 antibiotics determined by weighting NMDS1 and NMDS2 scores by chemical concentrations in each sample (A: NMDS1-NMDS2 and B: NMDS2-NMDS3).

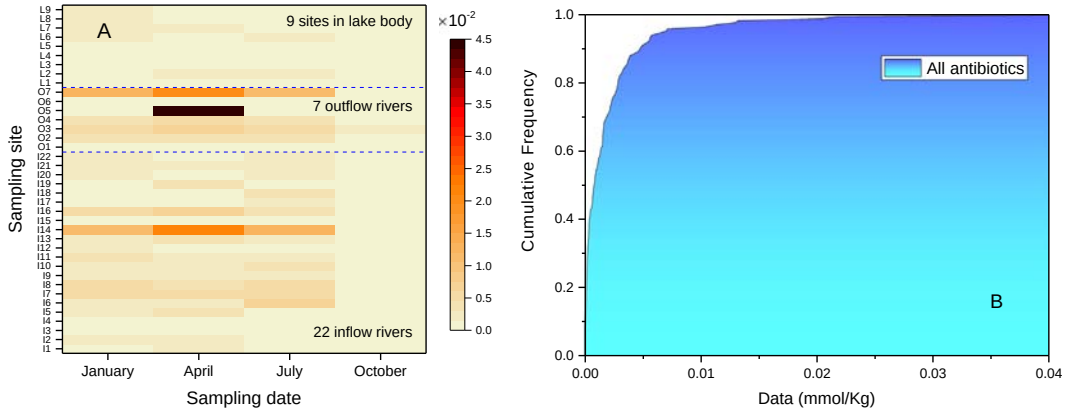

Fig. 6. Estimated whole body concentrations (WBC) across 22 antibiotics for fish, based on measured total concentrations at sampling sites and dates (A); the cumulative distribution function of the estimated WBC values in fish species (B).

monthly total amount presented relatively heavier than in October (see Fig. S4) and significantly high pollution levels were present. Particularly, biodegradation and photolysis for ABs in reduction were more forceful in summer than in the other seasons (Hu et al., 2010; Luo et al., 2010), however, the usage amount and frequency of ABs might also be increased for promoting growth or prevent and cure diseases of living organism in livestock and poultry breeding, and aquaculture. After all, pathogenic microorganism were more energetic in high temperature during summer. As a result, in our study, we did not found that the overall pollution level of ABs was statistically significant low in summer in Taihu Lake Basin.

3.4. Cumulative ecological risks of antibiotics

Up to date, there are no specific water quality criteria (WQC) for every antibiotic compound in surface waters of China, so that it is difficult to comparing WQC and ABs concentration in Taihu Lake Basin. Estimated whole body concentrations (WBC) for each collection date and site were shown in Fig. 6A, while Fig. 6B depicted the cumulative frequency of these values across the 304 qualifying samples. The highest WBC was estimated in April 2017 samples at site #O5; estimated WBC's ranged from 7.68×10^{-5}–4.38×10^{-2} mmol/kg across all qualifying dates and sites. We could not find any sample of concentration above the CBR of 0.2 mmol/kg, indicating that fish were not subjected to threatening pollutant concentrations. The average WBC at site #O7 (1.141×10^{-2}), #I14 (1.138×10^{-2}) and #O5 (1.112×10^{-2}) were relatively higher than counterparts. For 90% of the samples, the estimated WBC was less than the 4.8×10^{-3} mmol/kg (see Fig. 6B). However, we cannot accurately determine biomagnification and potential toxicological effects on humans, since we did not have ABs concentrations in tissues or internal organs of local residents who consumed fish in the Taihu Lake.

3.5. Limits of results and future research

For a broad range of ABs, more attention related to concentration level and toxic effect in Taihu Lake Basin should continue in regular intervals (e.g., monthly), although some concentrations detected in water column and sediment bottom were in trace level. With limited observed data we were able to make general conclusions concerning the spatial-temporal variability of the overall pollution level of ABs, however, we could not closely observe any meaningful interpretations knowing what happened at the locations of each site, and anything about what was going on the dates when the samples were collected, due to insufficient information on local industrial and agriculture activities.

To estimate the ecological risk of ABs on aquatic organisms, an ecotoxicological dataset should be established for the dominant species in different trophic levels. Meanwhile, we also need to pay close attention to the potential of ABs to inhibit the growth of living aquatic organisms. For more assessments, we need a better understanding of the potential risk of antibiotic resistance gene and bacteria accumulated on aquatic organisms.

4. Conclusions

A substantial field investigation on 22 ABs belonging to four categories was implemented in Taihu Lake Basin during 2017. In general, the concentration level was higher in sediment bottom than in water column. The statistics on total measured ABs (water column + sediment bottom) of MLs, SAs, TCs and QNs were 269.39 ± 687.76, 33.96 ± 191.46, 153.66 ± 756.35 and 865.82 ± 3268.61 ng/L, respectively. NMDS reduced 22-dimension of ABs concentrations to 3-dimension for ease to interpret that species composition was illegible if a significance level of 0.05 was specified, and site #O7 located at Xintongan River represented the direst site (all negative differences when site #O7 is subtracted from other sites) based on Tukey multiple comparison. Relatively, a clear separation was present for four sample dates. Particularly, the overall pollution level of ABs in the basin was significantly lower than spring, summer and winter based on ANOVA results. We also performed a cumulative ecological risk assessment of 22 ABs based on a single aquatic organism (fish) and one ecotoxicological endpoint (bioaccumulation) using a critical body residue method. Our findings provided that the cumulative risk was far less than the threshold. Controlling PS and NPS loads in Taihu Lake Basin will improve water quality and mitigate potential risk that further transmits to human body.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.06.175.

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