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# Occurrence, Distribution and Ecological Risk of Bisphenol Analogues in The Surface Water from A Water Diversion Project in Nanjing, China

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**Abstract:** Because of the widespread use of bisphenol analogues (BPs) as the alternatives to bisphenol A (BPA), they have attracted considerable attention for health risk in aquatic ecosystems. The occurrence and distribution of six BPs were researched in soluble phase (< 5 kDa), colloidal phase (5 kDa to 1 μm) and suspended particulate matter (SPM > 1 μm) in a water diversion project of Nanjing, China. Except for bisphenol Z, all BPs were detected in the two or three phases, the total concentrations of detected BPs were 161-613 ng/L, 5.19-77.2 ng/L and 47.5-353 ng/g for the soluble phase, colloidal phase and SPM, respectively. Among the detected compounds, BPA still the dominant BPs in the soluble and colloidal phases, followed by BPS, while BPAF was the major contaminant in SPM, followed by BPA. The mean contribution proportions of colloids were 1-2 orders of magnitude higher than SPM, suggesting that colloids have an obvious impact on regulating BPs' environmental behaviors. In terms of spatial distribution, although the water diversion project could reduce the pollution levels of BPs, which might further affect the ecological security of the Yangtze River.

**Keywords:** bisphenol analogues; colloids; suspended particulate matter; environmental risk; water diversion project

## 1. Introduction

Environmental hormone bisphenol-A (2,2-bis(4-hydroxyphenyl)propane; BPA) has varying degrees of influence on animals, such as fish [1], rats [2] and human health [3]. A number of countries and regions, including China [4], San Francisco [5], Canada and European Union [6], have issued policies banning BPA in products in response to the adverse effects of BPA. However, in order to meet the market demand for products, various alternatives to BPA were widely used in industrial production. Such as, BPA analogues bisphenol-S (4,4'-sulfonyldiphenol; BPS) is widely used in the manufacture of epoxy resins [7], bisphenol-E (bis(4-hydroxyphenyl)ethane; BPE) cyanate resin [8], bisphenol-F (bis(4-hydroxypheny)methane; BPF) epoxy resin reinforced with nano polyanilines [9] and Bisphenol-AF (4,4'-(hexafluoroisopropylidene) diphenol; BPAF) was applied to polycarbonate resin production [10]. However, are the products without BPA safe worry-free? Recent studies have shown that BPS, BPF and other BPA analogues were widely detected in the water environment, and the pollution levels have an increasing trend [11,12]. And the current researches have indicated that BPS, BPAF and BPF can be adsorbed by aquatic organisms, and pose a serious threat to the whole

ecosystem as BPA [13,14]. Research is still needed to better elucidate the environmental sources, distribution and fate of bisphenol analogues (BPs).

Most of the existing researches focus on the traditionally solubility phase, which was subdivided from natural water bodies according to the operationally defined limit (e.g., 0.7 or 1.0 μm). However, natural colloids (1.0 nm–1.0 μm) are ubiquitously present in the traditionally solubility phase. Due to its small size and large specific surface areas, colloids may have significance in terms of the proportion of bound pollutants and their subsequent behavior and fate [15]. Previous studies reported that the 76 percent of total organic carbon were contributed by colloidal organic carbon, these colloids are important sinks of BPs and pharmaceuticals active compounds [11,16]. And up to 50% of antibiotics and BPs were associated with natural colloids in surface water [11,17]. However, few studies on the distribution and toxicological characteristics of BPs in the soluble (<1 nm) and colloidal phases are investigated, further studies on exact distribution of BPs between different particle size factions in aquatic system are needed.

Qinhuai River is connected with Gucheng Lake, Shijiu Lake and Yangtze River, the chief receiving water body of domestic wastewater. Due to the rapid economic growth and high population density, the water quality of the Qinhuai River was gradually deteriorating. To improve water environmental quality of Qinhuai River, a project of Yangtze River Diversion was implemented. The route of water diversion is: the Yangtze River→the New Qinghuai River→the Qinhuai River→the Yangtze River. By mean of the project of Yangtze River Diversion, contaminants can be diverted and diluted, and the water flow can be promoted. However, there is noteworthy that the diversion is finally discharged into Yangtze River, which may pose a threat to the ecological health of the river. In this study, thirteen sampling sites were settled up along the route of water diversion and the Nanjing section of Yangtze River. The distribution of six BPs in multiple environmental media, including soluble phase, colloidal phase and suspended particulate matter of surface water were investigated. Based on the knowledge, the environmental risk of BPs was evaluated to determine potential implication to ecosystem of water diversion project.

## 2. Materials and methods

### *2.1. Chemicals*

Bisphenol contaminants including BPA, BPS, BPE, BPF, BPAF and bisphenol Z (BPZ) were purchased from J&K Chemical Co., Ltd. (Shanghai, China). Methanol, formic acid and ammonia were obtained from Merk Corporation (Darmstadt, Germany).

### *2.2. Sample collection*

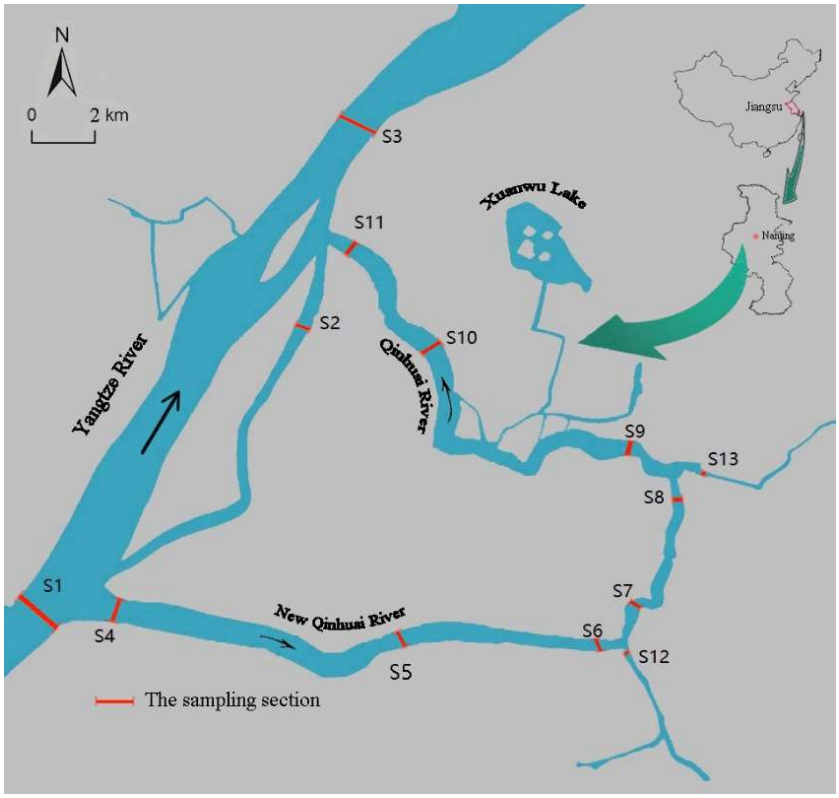



**Figure 1.** The locations of 13 sampling sites in the survey region.

As shown in Figure 1, a total of thirteen sampling sites were selected along the Yangtze River (S1-S3) and urban river (S4-S13). At each sampling site, 2 L of water sample was collected three times in July 2018, according to the requirements for monitoring of surface water [11]. The water samples collected were stored in containers containing dry ice, and quickly transported to the laboratory for further processing. According to the pretreatment method reported by previous study [16], the water samples were divided into three parts (i.e., SPM, colloidal and soluble phase) by vacuum filter unit and cross-flow cell, using 1 μm glass fiber filters and 5 kDa polyether sulfone membrane, respectively.

### *2.3. Sample extraction and instrument analysis*

The methods for extraction and analysis of BPs in the soluble and colloidal phases were described in our previous study [11]. Briefly, solid-phase extraction (SPE) system was used to extract the exposure water samples through an Oasis HLB (6 mL, 200 mg) cartridge (Waters, Massachusetts, USA), and 9 mL methanol with 2% ammonia was used to elute. Based on the previous study [18], the extraction methods of BPs in SPM samples were developed. Briefly, the SPM samples were extracted using 20 mL of mixture solvent of methanol/acetone (50:50 v/v) with sonication for 30 min, and then centrifuged at 4000 r/min for 10 min. The above steps were repeated two times; the supernatant was merged and concentrated to 2 mL. Then, the supernatant was added into 200 mL ultra-pure water, and further cleanup was done using SPE method similar to the method of water samples.

UPLC-MS-MS (LC-Waters Acquity ultra-high performance liquid chromatograph, MS-Waters Acquity Xevo TQ triple quadrupole mass spectrometer) was used to identify and quantify the BPs with multiple response monitoring. The mobile phase consisted of 0.01% ammonium hydroxide and 100% acetonitrile. The column temperature, the injection volume and the flow rate were 40 °C, 5 μL and 0.3 mL/min, respectively. Other instrumental parameters of UPLC/MS/MS were shown in supplementary information (SI, Table S1 and S2).

### *2.4. Quality assurance and quality control*

In the pretreatment process of field sample, solvent blank, standard and procedure blank were run successively to check the background of BPs. Limit of quantitative (LOQ)was used to evaluate sensitivity of the method. The LOQs of BPs in water samples and SPM samples are 0.53-11.1 ng/L and 0.25 -1.5 ng/g, respectively, and the linear range is 1-200 ng/L ($R^2$>0.991). The recovery rates of BPs in water samples and SPM samples are 70.3%~108% and 73.5%~110% (Table S3), respectively. The relative standard deviation is less than 20%. The detail information was presented in SI.

### *2.5. Parameter measurement and statistical analysis*

Using the risk quotient (RQ) of algae, daphnias and fish to conduct an ecological risk assessment of BPs detected in the traditional solubility phase [19]. According to oestrogen equivalent factor (EEF), 17β-oestradiol equivalency quantity (EEQ) was calculated based on the measured concentrations of BPs [16]. The results were described using mean, median, and concentration ranges. The detail information was showed in SI.

**Table 1.** Concentrations of BPs detected in the SPM, colloidal and phases.

| Compounds | Soluble Phase (ng/L) | | | | Colloidal Phase (ng/L) | | | | SPM (ng/g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Median | Range | DF[a] (%) | Mean | Median | Range | DF (%) | Mean | Median | Range | DF (%) |
| BPA | 253 | 222 | 120-554 | 100 | 27.4 | 22.3 | 4.54-66.7 | 100 | 38.8 | 42.8 | 28.2-89.8 | 100 |
| BPS | 39.2 | 38.3 | 2.24-73.3 | 100 | 4.21 | 4.20 | 0.14-10.2 | 100 | 12.6 | 14.5 | 2.78-19.0 | 100 |
| BPAF | 5.10 | 3.47 | 1.50-16.2 | 100 | 1.12 | 0.97 | 0.12-2.47 | 100 | 46.7 | 38.1 | 28.2-89.8 | 100 |
| BPF | 2.20 | 1.90 | 0.00-4.76 | 61.5 | 0.35 | 0.33 | 0.00-0.82 | 69.2 | 2.10 | 0.00 | 0.00-17.3 | 23.1 |
| BPE | 0.83 | 0.94 | 0.00-2.12 | 53.8 | 0.25 | 0.23 | 0.00-1.11 | 53.8 | 0.00 | 0.00 | 0.00-0.00 | 0.00 |

[a] DF: detection frequency.

## 3. Results and discussion

### *3.1. Soluble phase of surface water*

Table 1 shows the concentrations of BPs in surface water, including min-max, mean, and median values. In the six target BPs, only five were detected in soluble phase, with detection frequencies of 53.9%-100%, while no BPZ was detected. BPA was the predominant compound (mean concentration: 253 ng/L), followed by BPS (39.2 ng/L) > BPAF (5.10 ng/L) > BPF (2.20 ng/L) > BPE (0.83 ng/L). As shown in Figure 2, the total concentrations of detected BPs (ΣBPs) are from 161 to 613 ng/L (mean concentration: 300 ng/L). Spatially, the tributary Yunliang River (S13) had the highest mean concentrations of ΣBPs, followed by Qinhuai River outlet (S11), downstream of the Yangtze River (S3), and Qinhuai River. The Yunliang River as an urban receiving river, receives effluent of a sewage treatment plant (STP, Sewage treatment capacity: 20 0000 $m^3$/d) [16,20], overflow water of rainwater and some untreated domestic sewage, which may be the main reason for high ΣBPs in S13. The lowest ΣBPs were found in upstream of the Yangtze River, which ensure the availability of water quality of the Yangtze River Diversion. Compared to pollution levels in upstream of the Yangtze River, the concentrations of ΣBPs in downstream of the Yangtze River (S3) and Qinhuai River outlet (S11) are

2.45 and 2.94 times higher, respectively. These results suggests that the water diversion project from the Yangtze River to urban river mitigated the adverse effects of BPs in the aquatic environment of urban river, however, it could also increase the ecological risk of BPs in downstream of the Yangtze River.

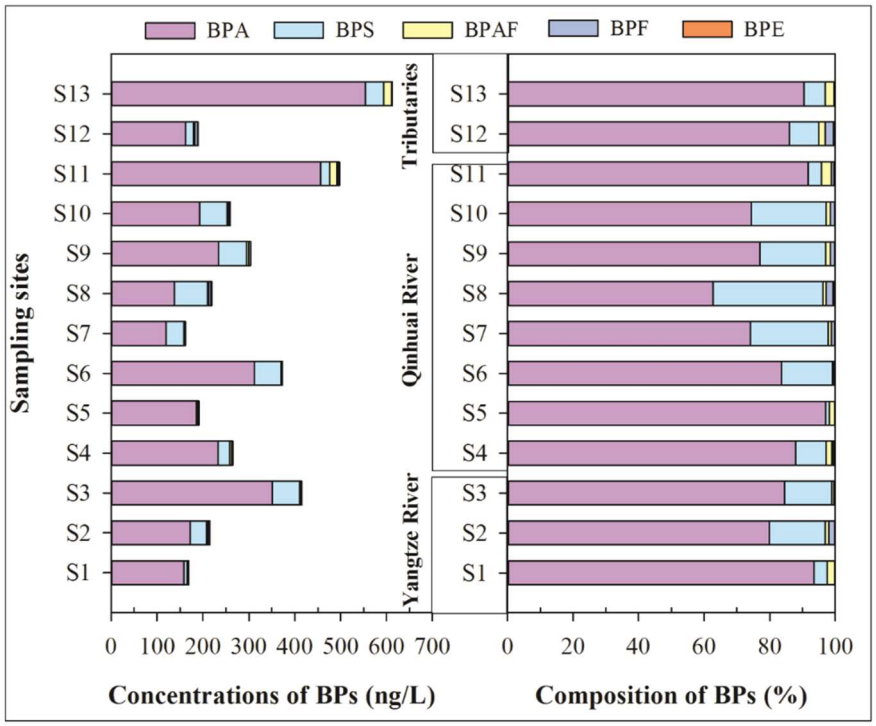



**Figure 2.** Concentrations and composition of BPs in the soluble phase.

In terms of monomer composition, the compositions of BPs in the soluble phase at each sampling site are generally similar, the dominant BP was BPA with the mean composition of 83.4%, followed by BPS (13.5%), BPAF (1.8%) and BPF (1.0%) (Figure 2). In river-lake system of Taihu Lake basin [11], the mean contribution rates of BPA, BPS and BPF were 81.8%, 12.5% and 4.7%, respectively, which were similar to our results. The pollution levels of BPA in sampling sites S3, S6, S11 and S13 were above 300 ng/L, while BPS in S3, S6, S8, S9, S10 and S13 were higher than 40 ng/L, which were similar with Taihu Lake basin [11]. Among the alternatives to BPA, BPS was the most abundant alternative to BPA in Nanjing section of the Yangtze River and Taihu Lake basin [21], which were clear different from the Pearl River Delta, where BPF has 78.8% of contribution rate as the main BP. These results suggest that BPS is being widely used in the Yangtze River basin.

### *3.2. Colloidal phase of surface water*

Colloids, as important sink of emerging contaminants is a widely existing in aquatic environment [22], which can cause several negative influences on the growth of aquatic organism [23,24]. As shown in Figure 3, five of six BPs in the colloidal phase were widely detected with the detection rates of 53.8%-100%, among them, the detection rates of BPA, BPAF and BPS were 100%. The mean concentrations of ΣBPs were from 5.19 ng/L (site S1) to 77.2 ng/L (site S4), which were significantly lower than soluble phase, indicating that the biological availability of BPs was high. Similar to the monomer distribution of BPs in the soluble phase, BPA remains the primary BP, followed by BPS and BPAF, with the mean concentrations of 27.4, 4.21 and 1.12 ng/L. These results were consistent with Taihu Lake basin, where BPA and BPS were the dominant BPs [11]. In terms of monomer composition, the compositions of BPs in colloidal phase were generally similar to that in soluble phase, the mean contribution rate of BPA is 80.6%, followed by BPS (13.6%), BPAF (4.0%) and BPF (1.2%), which was also similar to that of monomer distribution of BPs in Taihu Lake basin [11].

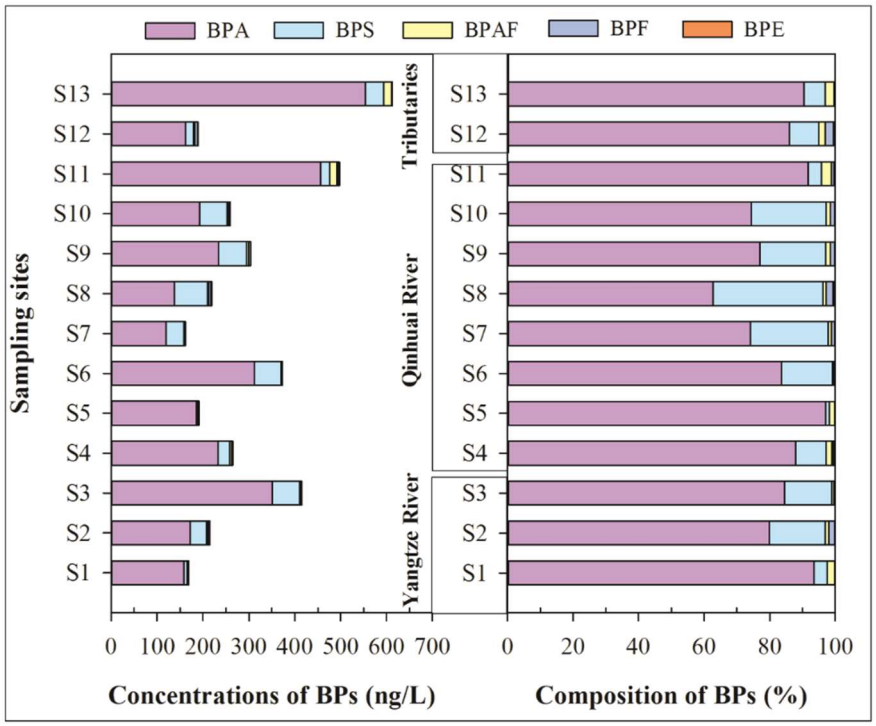



**Figure 3.** Concentrations and composition of BPs in the colloidal phase.

### *3.3. Suspended particulate matter of surface water*

Figure 4 presents the concentrations composition of BPs in the SPM samples. In the SPM, four of the six BPs were detected, and detection frequencies were 23.1%-100%. Neither BPZ nor BPE was detected in the all of sampling sites. The mean concentrations of ΣBPs were from 47.5 ng/g to 353 ng/g. Among them, BPAF was the predominant BP, with the mean concentration of 46.7 ng/g, followed by BPA (38.8 ng/g), BPS (12.4 ng/g) and BPF (2.10 ng/g). In SPM samples of Taihu Lake basin, the concentrations of BPA were from ND to 877 ng/g dw (mean: 76.8 ng/g dw) [25], which were comparable to that of our study and the Yangtze River (Nanjing section), ranged from ND to 364 μg/g (mean concentration 51.8 μg/g) [18]. However, only a few researches were focus on the other BPs' residues in SPM.

The compositions of BPs in SPM phase have been given in Figure 4. In contrast to the soluble and colloidal phases, BPAF exhibited relatively higher concentrations, the mean contribution of BPAF to the ΣBPs (46.7%) was much higher than that in the soluble phase (1.8%) and colloidal phase (4.0%). While the mean contribution of BPA to the ΣBPs (38.8%) in SPM phase was lower than that in the soluble phase (83.4%) and colloidal phase (80.6%), which may be due to its powerful binding SPM capability owing to the relatively high $K_{ow}$ of BPAF. Especially, the mean contributions of BPAF in S6, S7 and S8 reached more than 72%, the three sites were located at the downstream of Jiangning STP, which may be related to the contribution variation of BPAF.

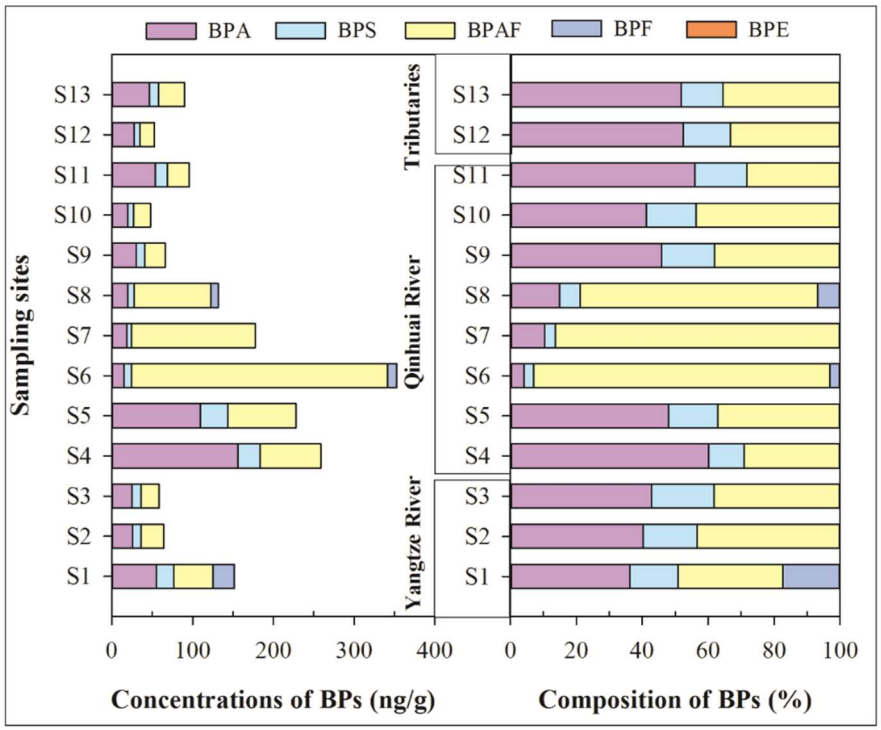



**Figure 4.** Concentrations and composition of BPs in suspended particulate matter.

### *3.4. Partitioning among SPM, colloidal and soluble phases of surface water*

In order to assess the potential importance of particulate matter to environmental behavior of BPs in the aquatic environment, figure 5 calculated the proportions of adsorption contribution of SPM and colloids to BPs. On average, 90.0% of BPA, 89.9% of BPS, 65.3% of BPAF, 84.2% of BPF and 76.5% of BPE were found in the soluble phase. These finding were similar to those reported in the Pearl River [26], where between 24.4% and 94.1% of BPA were detected in the soluble phase. These results suggested that the majority of BPs were existing as dissolved state in surface water. In colloidal phase, 9.7% of BPA, 9.6% of BPS, 14.4% of BPAF, 13.5% of BPF and 23.5% of BPE were associated with colloids, which were covered in the results reported by Gong et al. [26], where colloid-bound BPs were from 3.6% to 52.4% for BPA, 16.7% to 63.1% for 4-nonylphenol ($\log K_{ow}$ = 4.48). Si et al. found that 9.5% of BPS, 10.9% of BPA, 25.2% of BPF and 50.4% of BPAF were bound with colloids in surface water from Taihu Lake basin (Wujin district) without taking SPM into account [11]. In the present study, 0.2% of BPA, 0.5% of BPS, 2.3% of BPF and 20.3% of BPAF were bound with SPM, which were much lower than that of colloids with the except of BPAF. The findings indicate that colloids were potential sinks for BPs, which might influence the environmental behaviors of BPs due to its ubiquity, abundance and mobility in aquatic systems.

Information about the occurrence of BPs is mainly concentrated in the traditionally soluble phase, and the pollution levels of BPs in the soluble and colloidal phases are little. In the present study, the mean concentrations of BPs in the traditionally soluble phase were from 174 ng/L to 642 ng/L (mean concentration: 344 ng/L). The mean contribution rates of the traditionally soluble phase were 99.8% of BPA, 99.5% of BPS, 79.7% of BPAF, 97.7% of BPF and 100% of BPE. The pollution levels in the traditionally soluble phase reported in surface water worldwide are shown in Table S4. Based on frequent detection of BPs (BPA, BPF, BPS and BPAF), the total concentrations of BPs in our study area were similar to those from Taihu Lake basin investigated in 2018 [11] and the Pearl River Delta investigated in 2015 [27], with the concentration ranges of 98.8–726 ng/L and 107–987 ng/L, respectively. However, the contrast between composition of BPs by BPA and BPF between Taihu Lake basin/Yangtze River (Nanjing section) and Pearl River Basin (China) /Tamagawa River (Japan)/Han River (Korea) showed the obvious difference in the use and discharge of BPs in these

regions/countries [21,27]. BPA was still the predominant in Taihu Lake basin and Yangtze River (Nanjing section) with mean concentrations of 217 ng/L and 291 ng/L, respectively [11], while BPF was the abundant in the Pearl River basin, Han River and Tamagawa River (mean concentrations > 630 ng/L) [27].

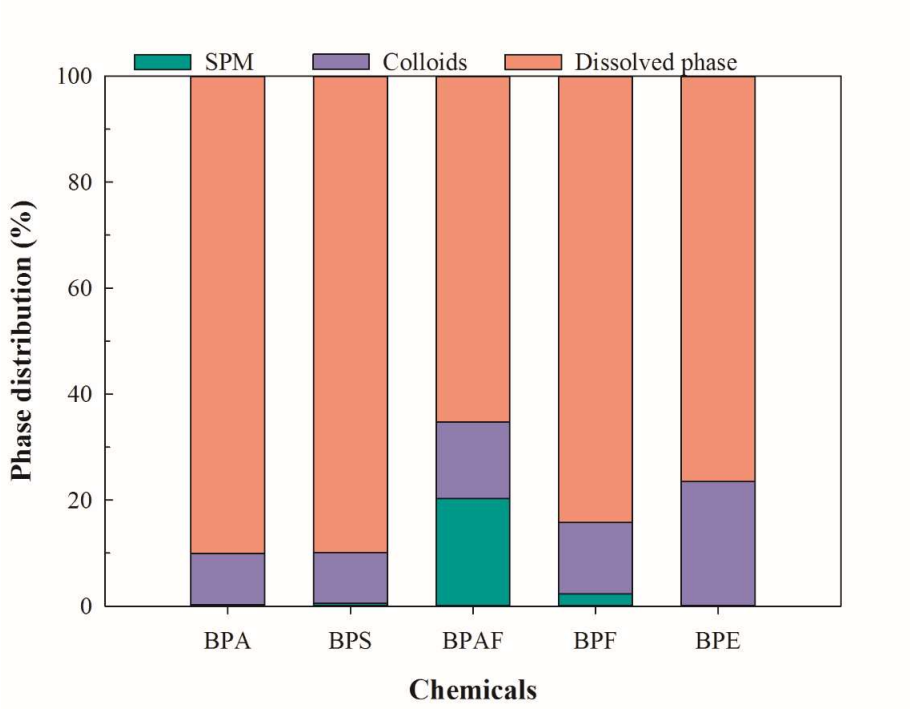
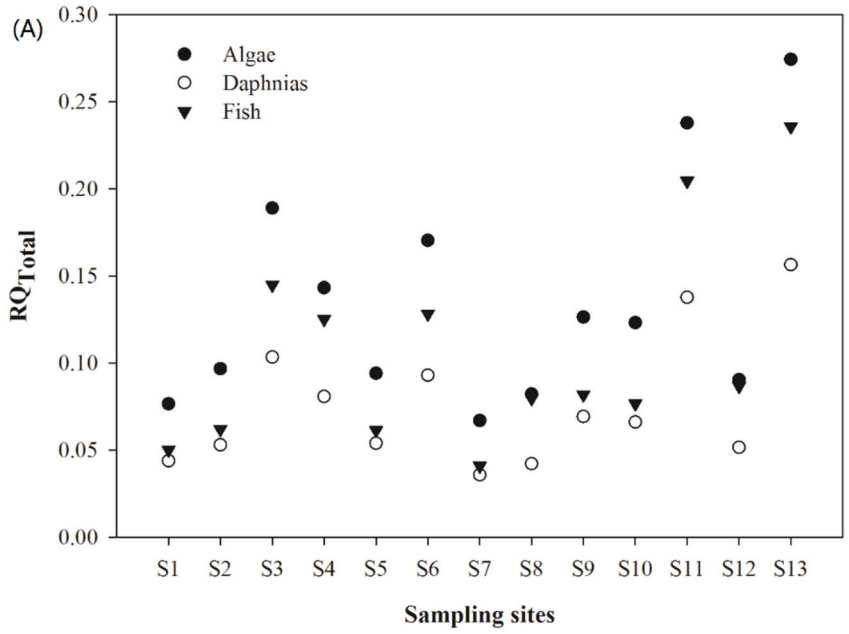



**Figure 5.** The BPs' distribution in the SPM, soluble and colloidal phases.

*3.5. BPs flux and environmental implication*

On the basis of flow rates and mean concentrations of BPs, the flux of BPs through water diversion project into the Yangtze River were estimated. The total flow rate of water diversion project was $1.5 \times 10^8$ $m^3$/month during July. Based on the mean concentrations of BPs measured in the surface water of sampling site S11, the flux of BPs was calculated as 75.6 kg/month for BPA, 3.05 kg/month for BPS, 2.75 kg/month for BPAF, 0.59 kg/month for BPF and 0.36 kg/month for BPE through the water diversion project. The total flux of BPs was 82.4 kg/month through the water diversion project.

Because of the increased emission and widespread presence of BPs in water bodies, which may have adverse effects on the ecosystem. Screening levels risk assessment of BPs were carried out based on the BPs' concentrations in the traditionally soluble phase. The toxicity data of BPs were presented in Table S5 for the most sensitive aquatic organisms. Based on the evaluation criteria of risk levels (Low risk: $0.01 \le RQ < 0.1$; medium risk: $0.1 \le RQ < 1$; high risk: $1 \le RQ$), the RQ values for detected BPs (except of BPA) were mostly below 0.01, suggesting no high risk was existing to the related aquatic organisms. The RQ values of BPA in most sampling sites exceeded 0.1 for algae, indicating medium risk for growth status of *Selenastrum capricornutum* might exist [29]. Due to co-existence of BPs and their similar action patterns, the total RQ ($RQ_{Total}$) of the detected BPs was evaluated to describe the worst-case scenario (Figure 6A). The $RQ_{Total}$ of algae were from 0.067 to 0.274, daphnias from 0.036 to 0.156, and fish from 0.041 to 0.236, respectively. In this study, fish was the most sensitive species, followed by daphnia and algae. In general, the mixture risk contribution rate of each BP decreased in the order as following: BPA (87.4%) > BPE (5.9%) > BPAF (3.8%) > BPS (2.0%) > BPF (0.9%).



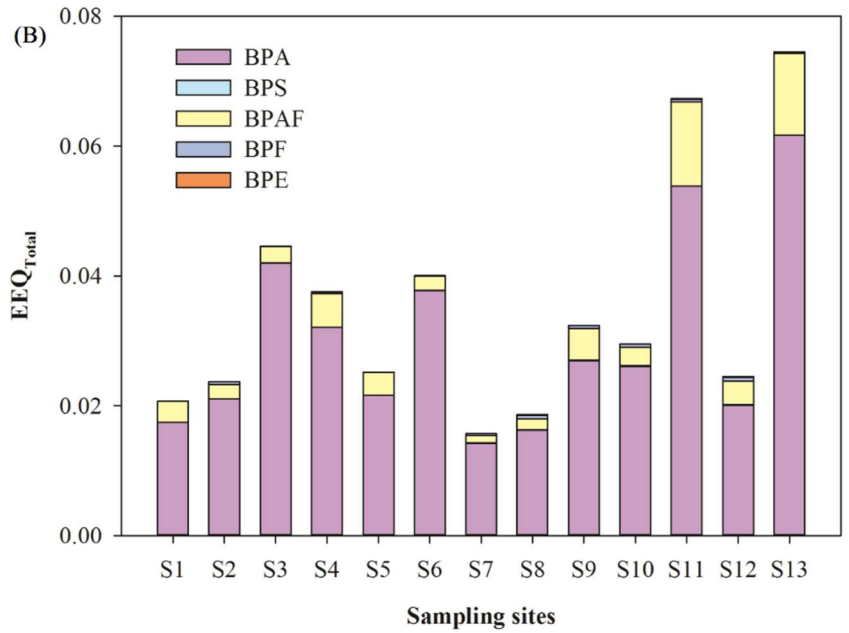




**Figure 6.** The $RQ_{Total}$ (A) and $EEQ_{Total}$ (B) of BPs for aquatic organisms in the surface water.

Studies have shown that BPs can cause oestrogenic effect on aquatic organisms by combining estrogen receptors. According to the EEF value in Table S5, 17β-oestradiol equivalency quantity (EEQ) method was applied to calculate the estrogen activity of BPs. When the total of EEQ ($EEQ_{Total}$) > 1.0 ng $E_2$/L, it was shown that the chemical could have a negative effect on the endocrine system of aquatic organisms. In our study, the results showed that $EEQ_{Total}$ in the waters was 0.0157-0.0745 ng $E_2$/L (Figure 6B), all the sampling points were no more than 1.0 ng $E_2$/L. The contribution rate of BPA to estrogen activity was the highest, which was 86.2%, followed by BPAF (12.9%), BPF (0.8%) and BPS (0.1%). The higher environmental risk of BPs were found in sampling sites S11 and S13. These two sampling sites are located in the estuarine of water diversion project into Yangtze River and ravine stream into water diversion project. These suggest that the flushing and dilution effects of water diversion project were existing for the mitigation of BPs contamination in urban river, however,

potential environmental risks transferring into the Yangtze River also needs to be taken into consideration.

## 4. Conclusions

The occurrence and distribution of six BPs in surface water were researched in the water diversion project. The results indicated that BPA, BPAF, BPS and BPF were existed widely in SPM, soluble and colloidal phases, with the total concentration ranges of 47.5−353 ng/g, 5.19−77.1 ng/L and 161−613 ng/L, respectively. In terms of medium partitioning, the majority of BPs were existing as dissolved state in surface water, followed by colloids and SPM adsorption. The mean distribution ratios of the three medium were 0.7%, 9.9% and 89.4% for the SPM, colloidal and soluble phases, respectively. Among the detected BPs, BPA and BPS were most abundant in the soluble and colloidal phases, while BPA and BPAF were the primary pollutants in the SPM. Particulate matter showed significant binding capacity for BPs, especially for colloids, which maybe further influence the environmental behaviors of BPs. The spatial distribution of BPs in surface water showed that BPs' concentrations was high and environmental risks of BPs were found in the estuarine of water diversion project into Yangtze River, which may further affect the ecological security of the Yangtze River.

**Supplementary Materials:** Table S1: The mobile phase compositions of the separation methods. Table S2: The six BPs of the optimized MS/MS parameters. Table S3: Recovery values obtained for the three independent concentrations of spiked quality control samples. Table S4: Concentrations of BPs reported as mean (median) and minimum-maximum observed globally. Table S5: The BPs' aquatic toxicity data of the most sensitive aquatic species.

**Author Contributions:** Conceptualization, J.L.; Investigation, C.Z., J.S., R.X. and J.R.; Writing—Original Draft Preparation, C.Z., J.F. and J.L.; Writing—Review & Editing, all authors.

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

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