

Original Research

Occurrence and Ecological Risk of Phenolic Compounds in the Taiyuan Section of Fen River in China

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Abstract

To evaluate the occurrence and ecological risk of phenolic compounds in the Taiyuan section of Fen River, nine water samples were collected, and the concentrations of 11 phenolic compounds were determined using gas chromatography after liquid-liquid extraction. The ecological risk was assessed using the risk quotient method. As a result, the total concentrations of phenolic compounds in the samples ranged from 1.17 to 14.48 $\mu\text{g}\cdot\text{L}^{-1}$, with a mean concentration of $5.27\pm 4.29 \mu\text{g}\cdot\text{L}^{-1}$. Non-chlorinated phenolic compounds were predominant, comprising 64.3% to 95.1% of the total concentration, with 2-nitrophenol exhibiting the highest concentration, followed by phenol. The ecological risk assessment indicated that 2-nitrophenol and 4-chloro-3-methylphenol were the primary contributors to ecological risks in the Fen River, while the other phenolic compounds did not pose significant ecological threats. In conclusion, non-chlorinated phenols outnumbered chlorinated phenols, with 2-nitrophenol and 4-chloro-3-methylphenol identified as the priority pollutants. These findings may provide information for improved monitoring, development of pollution control strategies, and implementation of advanced treatment technologies to mitigate the impacts of phenolic pollutants on the water quality of the Fen River.

Keywords: Fen River, phenols, ecological risk

Introduction

Phenolic compounds have been widely detected in various environmental media worldwide [1]. They originate from multiple sources, including industrial discharges, agricultural runoff, and domestic wastewater [2]. The extensive use of these compounds in chemical

manufacturing, pharmaceuticals, and synthetic fiber production has contributed to their prevalence in the environment [3]. More than 60 distinct phenolic compounds have been reported in ambient water globally, with concentrations ranging from less than 0.065 $\text{ng}\cdot\text{L}^{-1}$ to 179 $\text{mg}\cdot\text{L}^{-1}$ [4]. These compounds are persistent in the environment and can accumulate in the bodies of both humans and animals [5], posing significant risks to aquatic organisms and ecosystems. Concerns arise due to their potential for endocrine disruption, genotoxicity, and carcinogenicity [6].

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Consequently, 11 phenolic compounds were listed as priority pollutants by the U.S. Environmental Protection Agency [7]. Additionally, three specific phenols – 2,4-DCP, 2,4,6-TCP, and PCP – have also been listed as priority pollutants by the Environmental Quality Standards for Surface Water of China (GB 3838-2002) [8]. The World Health Organization has classified some phenolic pollutants as Group 3 carcinogens, while 2,4,6-TCP has been designated as a Group 2B carcinogen and PCP as a Group 1 carcinogen [9].

The Fen River, the second largest tributary of the Yellow River, is the largest in Shanxi Province in China. Although the water quality of the Fen River has improved over the years, some pollutants were still detected in the water, such as nitrate [10], heavy metals [11], estrogens [12], polyfluoroalkyl substances [13], and so on. Until recently, relatively few studies focused on the pollution of phenolic compounds in the Fen River. Consequently, it is essential to investigate the current levels of pollution caused by phenolic compounds and assess their ecological risks. Such research would directly impact the effectiveness of water ecological risk assessment and pollution management strategies, providing a scientific basis for managing the water environment in the Fen River basin.

In the present study, the objectives were to (1) determine the occurrence and concentrations of phenolic compounds in the Taiyuan section of the Fen River, (2) compare the pollution levels of phenolic compounds with those reported in other rivers, and (3) assess the ecological risks associated with these compounds using the risk quotient method.

Materials and Methods

Main Instruments and Reagents

The primary instruments used in the experiment included a gas chromatograph (model 8890 from Agilent Technologies Ltd.), an analytical chromatography column, a nitrogen blower (model JHD-003 from Shanghai Jieheng Industrial Co., Ltd.), an analytical balance (model YH-M1003 from Wuxin Weighing Instrument Co., Ltd.), and a muffle furnace (model SX2-4-10NP from Shanghai Yihang Science and Technology Co., Ltd.). The reagents employed were sodium hydroxide (NaOH), hydrochloric acid (HCl), ethyl acetate, n-hexane, methanol, sodium chloride (NaCl), anhydrous sodium sulfate, and standard solutions of phenolic compounds. Before use, anhydrous sodium sulfate and sodium chloride were baked in a muffle furnace at 450°C for 4 hours to eliminate organic impurities. Additionally, dichloromethane and n-hexane were purified through a secondary re-evaporation process. The standard solution of phenolic compounds had a concentration of 2500 mg/L and contained a methanol solution with 11 target compounds.

Sample Collection

Water samples were collected from the Taiyuan section of the Fen River in China during April 2024. As shown in Fig. 1, the sampling locations included Shanglan Village (F1) in Jiancaoping District, Yingze Bridge (F2) in Yingze District, Xiangyun Bridge (F3) in Xiaodian District, Tongda Bridge (F4) and Yingbin Bridge (F5) in Jinyuan District, as well as Fenhe Erba Bridge (F6), Guanzhong Bridge (F7), Nan'an Bridge (F8), and Hanwu Village (F9) in Qingxu County. Nine samples were taken from the surface water column at a depth of 0.5 meters. After collection, the water samples were adjusted to a pH of less than 2 by adding an appropriate amount of HCl. The samples were then sealed and stored at 4°C, protected from light, with extraction completed within three days.

The determination of phenolic compounds in water was carried out following the methods outlined in the industry standard of China (HJ 676-2013) [14]. The liquid-liquid extraction was performed using a 1000 mL water sample in four batches to enrich the phenolic compounds. Initially, 250 mL of the water sample was measured and transferred to a 500 mL separating funnel. The pH of the sample was adjusted to above 12 using a NaOH solution, and 10 g of NaCl was added and mixed thoroughly until dissolved. Subsequently, 80 mL of a dichloromethane/n-hexane mixed solvent (2:1) was added for extraction. This extraction process was done twice, with the aqueous phase collected after each extraction. The collected aqueous phase was adjusted to a pH below 2 using HCl. Following this adjustment, 40 mL of a mixed dichloromethane/ethyl acetate solvent (1:1) was added, and two more extractions were performed, with the combined organic phase extracts collected. After dehydration, the extract was transferred to a concentration flask and concentrated to 0.5-1.0 mL using nitrogen blowing. Finally, 3.0 mL of the dichloromethane/ethyl acetate mixed solvent (1:1) was added, and the solution was further concentrated to a final volume of 1.0 mL for analysis.

Chemical Analysis

The content of phenolic compounds was determined using gas chromatography under the following conditions: The initial temperature was set at 50°C for 5 min, followed by a temperature increase of 6°C·min⁻¹ up to 150°C. Next, the temperature was further increased at a rate of 20°C·min⁻¹ to 280°C and finally at a rate of 30°C·min⁻¹ to 300°C, where it was held for 2 minutes. The inlet temperature was maintained at 250°C, and the flame ionization detector (FID) was set to 300°C. The carrier gas flow rate was 1.5 mL·min⁻¹, with the rates of hydrogen and air flow set at 40.0 and 450.0 mL·min⁻¹, respectively. The tail gas flow rate was 30.0 mL·min⁻¹. A splitless flow injection mode was utilized for sample injection, and a purging process occurred after 1.0 minutes at a purge gas flow

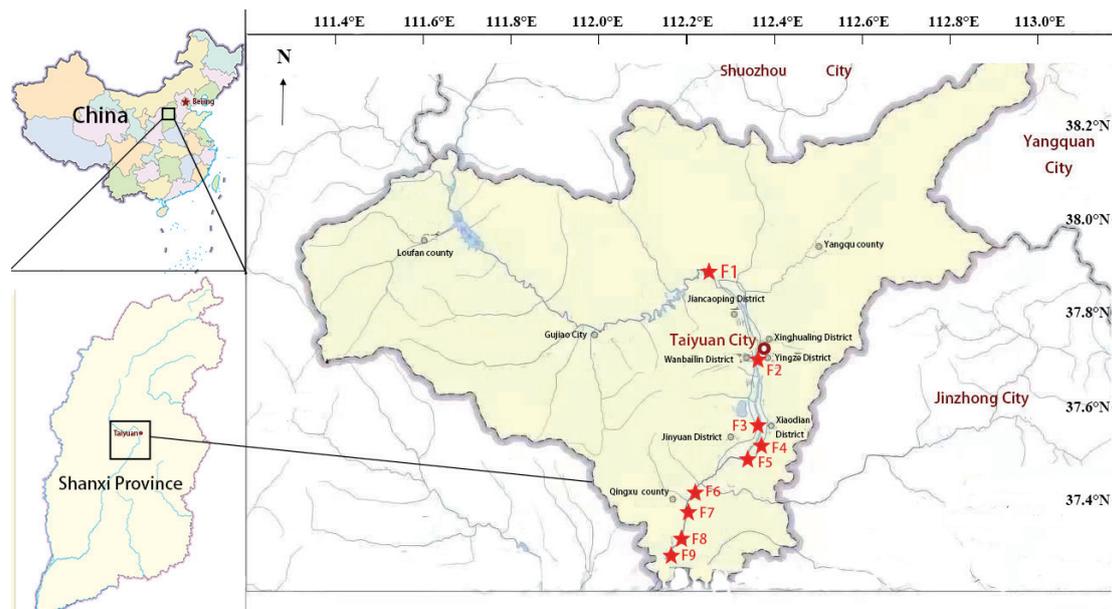


Fig. 1. Distribution of sampling sites along the Taiyuan section of the Fen River in China.

rate of $30.0 \text{ mL}\cdot\text{min}^{-1}$. The sample injection volume was $1.0 \text{ }\mu\text{L}$.

Ecological Risk Assessment

The risk quotient (RQ) method was used to assess the ecological risk of phenolic compounds, defined as follows:

$$\text{RQ} = \text{MEC}/\text{PNEC} \quad (1)$$

Where RQ is the risk quotient, MEC is the measured environmental concentration of each phenol, and PNEC is the predicted no-effect concentration of the corresponding phenolic compound. The PNEC values were obtained from a literature report on the ecological risk assessment of phenols in the Weihe River (the first major tributary of the Yellow River) [15]. The PNEC values were derived from the Species Sensitivity Distribution (SSD) method [16]. Briefly, acute toxicity data and chronic toxicity data for phenolic compounds affecting three groups of aquatic organisms – algae, invertebrates, and vertebrates – were collected from the Ecotoxicology Database of the United States Environmental Protection Agency (<https://cfpub.epa.gov/ecotox/>). The chronic toxicity data were used to construct SSD model curves for five phenolic compounds: phenol, 2,4-DNP, 2,4-DCP, 2,4,6-TCP, and PCP. Acute toxicity data were used to construct the SSD model curves for the remaining compounds. From the SSD curve, the HC_5 (hazardous concentration of 5% species) value was calculated. Subsequently, the PNEC value was derived by dividing the HC_5 by an assessment factor (AF). The AF was set at 10 for chronic toxicity and 1,000 for acute toxicity assessments. The risk levels associated with the RQ

values were categorized as follows: an RQ value of less than 0.1 indicated low risk, an RQ value between 0.1 and 1 suggested medium risk, and an RQ value greater than 1 signified high risk.

Quality Assurance and Quality Control

The flasks, funnels, test tubes, droppers, and anhydrous sodium sulfate used in the experiments were heated in a muffle furnace at 400°C for 4 hours to decompose any residual organic chemicals. These precautions ensured that no organic contamination affected the samples during the experiment. No target compounds were detected in the blank samples, and the recoveries of the spiked standard samples ranged from 83.6% to 105.9%. The phenolic compounds were quantified using the external standard method, with a standard curve established in the range of $25\text{-}400 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ and an R^2 value between 0.988 and 0.999. The method's detection limit was determined to be $0.025 \text{ }\mu\text{g}\cdot\text{L}^{-1}$.

Results and Discussion

Phenolic Concentrations in the Taiyuan Section of the Fen River

As shown in Table 1, all 11 phenolic compounds (ΣP11) were detected at nine sampling sites along the Taiyuan section of the Fen River. Among these compounds, seven phenolic compounds, including phenol, 2-NP, 4-NP, 4C-3-MP, 2,4-DMP, 2,4,6-TCP, and PCP, were identified with detection rates exceeding 50%. Conversely, 2,4-DNP, 2-CP, and 2,4-DNP displayed lower detection rates. The similarity in pollution

Table 1. Statistical description of phenolic concentrations from the Taiyuan section of Fen River in China in 2024 ($\mu\text{g}\cdot\text{L}^{-1}$, $n = 9$).

	Acronym	Compound	F1	F2	F3	F4	F5	F6	F7	F8	F9	DF ^a	Range	Mean	SD ^b
1	Phenol	Phenol	0.42	0.17	0.59	2.28	2.48	0.95	2.04	0.56	2.04	100	0.17–2.48	1.28	0.91
2	2,4-DMP	2,4-Dimethylphenol	BRL ^c	BRL	0.03	0.05	0.04	0.03	0.12	0.05	0.12	78	BRL ^c –0.12	0.06	0.04
3	2-NP	2-Nitrophenol	0.74	0.75	0.68	3.97	5.29	0.77	1.41	4.1	1.41	78	0.68–5.29	2.12	1.81
4	4-NP	4-Nitrophenol	0.14	0.06	0.06	1.32	1.25	BRL	1.55	0.26	1.55	89	BRL–1.55	0.77	0.70
5	2,4-DNP	2,4-Dinitrophenol	BRL	BRL	BRL	0.03	BRL	BRL	BRL	BRL	BRL	11	BRL–0.03	0.03	—
6	2M-4,6-DNP	2-Methyl-4,6-Dinitrophenol	BRL	BRL	0.03	BRL	0.25	0.4	BRL	BRL	BRL	33	BRL–0.40	0.23	0.19
7	2-CP	2-Chlorophenol	BRL	BRL	BRL	BRL	BRL	BRL	0.28	BRL	0.28	22	BRL–0.28	0.28	0.00
8	4C-3-MP	4-Chloro-3-methylphenol	0.1	0.09	0.09	0.72	4.94	BRL	0.2	1.01	0.2	88	BRL–4.94	0.92	1.66
9	2,4-DCP	2,4-Dichlorophenol	BRL	BRL	BRL	BRL	0.14	BRL	0.11	BRL	0.11	33	BRL–0.14	0.12	0.02
10	2,4,6-TCP	2,4,6-Trichlorophenol	0.03	0.04	0.26	0.08	0.03	BRL	0.03	0.06	0.03	89	BRL–0.26	0.07	0.08
11	PCP	Pentachlorophenol	0.12	0.06	0.14	0.03	0.06	0.11	0.03	BRL	0.03	89	BRL–0.14	0.07	0.04
		$\sum\text{CP}_5$	0.25	0.19	0.49	0.83	5.17	0.11	0.65	1.07	0.65		0.11–5.17	0.95	1.52
		$\sum\text{NCP}_6$	1.30	0.98	1.39	7.65	9.31	2.15	5.12	4.97	5.12		0.98–9.31	4.22	2.98
		$\sum\text{P}_{11}$	1.55	1.17	1.88	8.48	14.48	2.26	5.77	6.04	5.77		1.17–14.48	5.27	4.29
		$\sum\text{NCP}_6\%$	83.9	83.8	73.9	90.2	64.3	95.1	88.7	82.3	88.7				

^a DF: detected frequency in percentage.^b SD: standard deviation.^c BRL: blow reported limits. Reported limits for phenolic compounds were 0.025 $\mu\text{g}\cdot\text{L}^{-1}$.

levels across the sampling sites suggested the presence of various pollution sources, likely linked to industrial discharges or agricultural runoff.

The concentrations of phenolic compounds and their statistical description for nine samples are presented in Table 1. The total concentrations of the 11 phenolic compounds ranged from 1.17 to 14.48 $\mu\text{g}\cdot\text{L}^{-1}$, with a mean value of $5.27\pm 4.29 \mu\text{g}\cdot\text{L}^{-1}$. Among the samples, the highest concentration of total phenols, 14.48 $\mu\text{g}\cdot\text{L}^{-1}$, was observed at Yingbin Bridge (F5), while the lowest value, 1.17 $\mu\text{g}\cdot\text{L}^{-1}$, was found at Yingze Bridge (F2). These results indicated a trend where the total concentration of phenolic pollutants was lower at the upstream and downstream sites but higher at the intermediate site. This pattern suggested that the intermediate location may have been influenced by urban anthropogenic activities, resulting in additional inputs of phenolic compounds.

Moreover, the total concentration of five chlorophenols ($\sum\text{CP}_5$) ranged from 0.11 to 5.17 $\mu\text{g}\cdot\text{L}^{-1}$, with a mean value of $0.95\pm 1.52 \mu\text{g}\cdot\text{L}^{-1}$. In contrast, the total concentration of six non-chlorophenols ($\sum\text{NCP}_6$) varied from 0.98 to 9.31 $\mu\text{g}\cdot\text{L}^{-1}$, with a mean of $4.22\pm 2.98 \mu\text{g}\cdot\text{L}^{-1}$. Non-chlorinated phenols accounted for 64.3% to 95.1% of the overall concentration. 2-NP and phenol were the primary compounds, accounting for 40.4% and 24.3% of the total average concentration, respectively. These findings indicated that the contamination levels of non-chlorinated phenols were higher in this study compared to chlorophenols, contrasting with previous research that reported a predominance of chlorophenols [4]. This shift suggests that the environmental pollution of water bodies has improved considerably over the years. The types of phenolic compounds released into the environment have changed. Chlorophenols, previously the most toxic, were no longer the dominant compounds detected.

Analyzing the standard deviation of the concentration of various phenolic compounds in the Fen River revealed that some of these compounds were at relatively stable levels. For instance, the standard deviations for 2,4-DMP, 2,4-DCP, 2,4,6-TCP, and PCP were all below 0.1, suggesting that the concentrations of these compounds were stable and likely free from new inputs. In contrast, 2-NP and 4C-3-MP exhibited higher standard deviations exceeding 1.5, suggesting that these phenolic contaminants may have distinct sources or exhibit different environmental behaviors.

A comparison of phenolic pollution levels in the Fen River with existing surface water quality standards, as shown in Table 2, indicated that the maximum concentrations of most phenolic compounds were below the thresholds established by the Chinese Environmental Quality Standards for Surface Water [8]. Additionally, these levels fell within the nationally recommended water quality standards for human health criteria [17] and organoleptic effects [18], as reported by the U.S. Environmental Protection Agency (USEPA). However, the concentration of 2-CP exceeded the USEPA's

recommended standards for organoleptic effects [18], while the concentration of PCP surpassed the standards for human health criteria [17]. Although most phenolic compounds in the Fen River did not exceed regulatory limits, certain compounds, such as PCP – classified as a Class 1 carcinogen [9] – could pose a potential risk. Consequently, it is crucial to continue monitoring phenolic compound pollution and to implement appropriate control strategies.

Worldwide, most phenolic compounds in the Fen River were lower than those in the Minas Gerais River of Brazil except for 2-NP and 4C-3MP [19] and in the Doce River of Brazil except for 2-NP [20]. Similarly, phenolic compounds in the Fen River were significantly lower than those observed in the Msunduzi River in South Africa [1] and the Epe and Osun Rivers in Nigeria [21]. In contrast, all phenolic compounds in the Fen River were in higher concentrations than those in the Alexandria River in Egypt [22]. Furthermore, except for phenol, most phenolic compounds in the Fen River concentrations exceeded those in the Namhan, Nakdong, Geum, and Yeoungsan Rivers in South Korea [23]. The lower concentrations of phenolic compounds in certain rivers likely reflected stricter environmental regulations and advanced water treatment technologies. Conversely, the higher concentrations detected in other rivers indicated a need for improved pollution management. The variation in phenolic compound concentrations across different rivers could be attributed to local industrial activities, agricultural practices, and the effectiveness of wastewater treatment facilities. This situation underscores the urgent need for effective pollution control and management strategies in regions with heavily polluted rivers.

In China, concentrations of all phenolic compounds in the Fen River were higher than those in the Yinma River in Jilin Province [24]. However, these concentrations in the Fen River were lower than those in the Dagu River in Tianjin City [25]. Additionally, most phenolic compounds in the Fen River showed lower levels than in the Weihe River in Shaanxi Province, except for 2-NP and 4-NP [16]. Conversely, the phenolic compounds in the Fen River levels were approximately equivalent to those in Taihu Lake [26]. Overall, this indicated that pollution levels in the Fen River were moderate compared to other rivers in China. This suggests that the watershed in the section of Fen River in China has implemented relatively effective pollution control measures for both industrial and agricultural activities.

Ecological Risk Assessment of Phenolics in the Taiyuan Section of the Fen River

2M-4,6-DNP was excluded from the ecological risk assessment due to the absence of a PNEC value. Among the remaining ten phenolic compounds, eight exhibited low (RQ<0.1) or medium (RQ: 0.1-1) ecological risk across all sampling sites (Table 3). This finding indicated

Table 2. Maximal concentration of phenolic compounds in rivers compared to those in other countries ($\mu\text{g}\cdot\text{L}^{-1}$).

River	Year /Reference	Samples	1	2	3	4	5	6	7	8	9	10	11
China ^a	[8]		Phenol — ^b	2,4- DMP	2-NP	4-NP	2,4- DNP	2M-4,6- DNP	2-CP	4C-3- MP	2,4- DCP	2,4,6- TCP	PCP
US EPA ^c	[17]		4000	100	—	—	10	2	30	500	10	1.5	0.03
US EPA ^d	[18]		300	400	—	—	—	—	0.1	3000	0.3	2	30
Brazil	2021 [19]	12	—	2.19	0.37	25.4	—	—	2.43	0.52	10.03	38.6	—
Brazil	2021 [20]	3	—	0.76	1.63	7.36	—	—	—	26.2	1.55	—	—
Egypt	2022 [22]	10	0.109	0.118	0.087	—	—	—	0.091	0.052	0.066	0.046	0.06
South Africa	2023 [1]	5	—	—	96.9	—	—	—	—	47.8	11.2	—	7.37
Nigeria	2023 [21]	31	261	—	—	—	553.4	—	—	—	—	275	—
South Korea	2023 [23]	13	4.96	—	0.008	—	—	—	0.033	—	—	0.004	—
China	2017 [24]	17	0.12	—	0.084	—	—	—	—	—	0.055	0.029	0.005
	2018 [25]	37	15.5	—	17.6	1.95	—	—	—	—	1.21	—	—
	2020 [26]	165	1.29	—	—	—	—	—	—	—	0.44	0.55	—
	2021 [16]	32	3.84	0.84	0.38	1.15	2.69	—	1.74	—	3.76	2.48	5.96
	2024/ This study	9	2.48	0.12	5.29	1.55	0.03	0.40	0.28	4.94	0.14	0.26	0.14

^a Environmental Quality Standards for Surface Water of China (GB 3838-2002).

^b Not reported.

^c National Recommended Water Quality Criteria: Human Health Criteria.

^d National Recommended Water Quality Criteria: Organoleptic Effects.

Table 3. Ecological risk assessment of phenolic compounds from the Taiyuan section of the Fen River in China in 2024.

	Compound	PNEC [15] ($\mu\text{g}\cdot\text{L}^{-1}$)	RQ Range	RQ%		
				<0.1	0.1-1	>1
1	Phenol	17.36	0.01-0.14	56	44	0
2	2,4-DMP	10.48	0-0.01	78	22	0
3	2-NP	1.58	0.43-3.35	0	67	33
4	4-NP	6.06	0.01-0.26	44	56	0
5	2,4-DNP	0.87	0.03-0.03	11	89	0
6	2M-4,6-DNP	—	—	—	—	—
7	2-CP	18.99	0.01-0.01	22	78	0
8	4C-3-MP	3.68	0.02-1.34	56	33	11
9	2,4-DCP	4.08	0.03-0.03	33	67	0
10	2,4,6-TCP	2.82	0.01-0.09	89	11	0
11	PCP	1.08	0.03-0.13	56	44	0

that these eight compounds did not pose a significant environmental threat to aquatic organisms in the Fen River. However, the RQ values for 2-NP and 4C-3-MP exceeded 1.0 at specific locations. Specifically, 2-NP displayed an RQ range of 0.43 to 3.35, with no samples classified as low risk, 67% categorized as medium risk, and 33% as high risk.

In contrast, 4C-3-MP had an RQ range of 0.02 to 1.34, with 56% in the low-risk category, 33% in the medium-risk category, and 11% in the high-risk category. In summary, 2-NP and 4C-3-MP were identified as the priority phenolic compounds contributing to ecological risks in the Fen River.

The ecological risk assessment identified high-risk compounds in the Taiyuan section of the Fen River. This discovery led to the implementation of targeted pollution control strategies. These strategies included enforcing industrial discharge standards, upgrading wastewater treatment facilities, and reducing agricultural runoff. These measures aimed to decrease the levels of phenolic compounds entering the river.

Future research should concentrate on additional phenolic compounds not examined in this study, such as bisphenol A, octylphenol, and nonylphenol [27]. Furthermore, detecting phenolic compounds in the Fen River highlighted the necessity of monitoring trends and seasonal variations in their concentrations to inform adaptive management strategies. Additionally, efforts should be directed toward exploring methods for removing phenolic pollutants from wastewater. This could include advanced oxidation processes [28], photocatalysis [29], membrane distillation [30], nanofibers membranes [31], and both aerobic and anaerobic biodegradation processes [32].

Conclusions

In the present study, eleven phenolic compounds were detected, and their ecological risks were assessed in the Taiyuan section of the Fen River in China. All identified phenolic compounds were found in the water, with non-chlorinated phenolic compounds predominating, consisting of 64.3% to 95.1% of the total concentration. Notably, 2-NP emerged as the compound with the highest concentration, followed closely by phenol. Moreover, an ecological risk assessment was conducted using the risk quotient method, which indicated that 2-NP and 4C-3-MP posed a high risk to aquatic organisms. At the same time, the other phenolic compounds did not present significant ecological risks. The findings of this study provide valuable insights for informed decision-making and policy development aimed at protecting the aquatic ecosystem of the Fen River.

Abbreviations

2,4-DMP: 2,4-Dimethylphenol; 2-NP: 2-Nitrophenol; 4-NP: 4-Nitrophenol; 2,4-DNP: 2,4-Dinitrophenol; 2M-4,6-DNP: 2-Methyl-4,6-Dinitrophenol; 2-CP: 2-Chlorophenol; 4C-3-MP: 4-Chloro-3-methylphenol; 2,4-DCP: 2,4-Dichlorophenol; 2,4,6-TCP: 2,4,6-Trichlorophenol; PCP: Pentachlorophenol.

Declaration of Competing Interest

The authors declare that they have no competing financial interests.

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Author Contributions

Bo Qu participated in the study's design and drafted the manuscript; Yongjing Zhang helped draft the manuscript; Hongxue Qi designed and coordinated the study and critically revised the manuscript; Fengjiao Zhao carried out the statistical analyses; and Sihan Zhao revised the manuscript in detail. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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