Archives of Environmental Protection
Vol. 49 no. 1 pp. 63–73

PL ISSN 2083-4772
DOI 10.24425/aep.2023.144738

Current status of phenolic pollution in urban lakes and its toxicity to cells – A case study of Xi'an, China

Min Wang*, Yutong Zhang, Jingxin Sun, Chen Huang, Hongqin Zhai

Xi'an University of Technology, China

*Corresponding author's e-mail: wangmin@xaut.edu.cn

Keywords: Urban lakes; phenolic compounds; pollution; ecological risk assessment

Abstract: Liquid chromatography-mass spectrometry was used to detect and analyze phenolic compounds in the surface waters of four urban lakes in Xi'an – Hancheng Lake, Xingqing Lake, Nanhu Lake, and Taohuatan Lake. A total of 5 phenolic compounds were detected from the water samples, with a concentration range of ND-100.32 ng/L, of which bisphenol A (BPA) and nonyl phenol (NP) were the main types of phenolic compounds pollution in the four lakes. Pearson correlation analysis was used to analyze the concentration of phenolic compounds in the lake waters of Xi'an City and the water quality indicators COD, TP, NH_3-N, DO, and pH during the same period. It was found that there was a significant positive relationship between the concentration of BPA and COD, the concentration of estradiol (17-beta-E2), estrone (E1) and TP and TN, the concentration of octylphenol (4-t-OP) and pH. The ecological risk assessment (ERA) shows that the concentration of BPA, 4-t-OP and NP in the lakes is at a medium risk level(is between 0.1–1), and that of E1 is at a high risk level (is greater than 1). Female cells (breast cancer cells) and male germ cells (testis cells) of mice were used as research objects to explore BPA and NP Toxic effect on mouse germ cells. BPA and NP at a concentration of 10^{-8} mol/L were found to have the most value-inducing effect on MCF-7 breast cancer cells positive for estrogen receptor. Obviously, both BPA and NP can induce the proliferation of testicular Sertoli cells.

Introduction

Phenolic compounds are a kind of synthetic raw materials, which are usually used in chemistry (Wang and Kurunthachalam 2018), dyeing(Yousefi et al. 2013) and textile industry (Tülay et al. 2002). Once released into the environment, they are easily absorbed and accumulated in organisms. These compounds are not only toxic, but also have a strong pungent smell, which will seriously affect the water quality after entering the water body (Takuo Kunio 1996). However, the amount of phenolic compounds used and discharged is increasing year by year, so they are often detected in surface water. Research on phenolic compounds in water bodies is relatively early. The relevant standards issued by EPA stipulate that phenols in water bodies will have chronic toxic effects on aquatic organisms at 2.56 mg/L and will cause harm to human health when the concentration exceeds 3.5mg/L (Tan et al. 2015). Bolz (Hodaka et al. 2004) tested phenolic interferences in river water bodies and sediments in southwestern Germany, and the results showed that the content of phenols in sludge was an order of magnitude higher than that in river sediments. Kawahata tested the phenols in the waters of Okinawa and Shijiao Island in Japan, and the levels of phenols in the waters were generally below the detection limit. Yin (Yin et al. 2020) detected that the total concentrations of phenolic compounds in Tianjin water sources and rivers were 116.72–220.24 ng/L and 114.55–448.12 ng/L, respectively. By species sensitivity distribution method and entropy method, they found that nonyl phenol poses unacceptable risks to water environment and sediments. Diao (Diao et al. 2017) identified the concentrations of 4-nonyl phenol, 4-tert-butyl phenol and bisphenol A in the surface water along the Pearl River Estuary ranging from 1.20 ng/L to 3,352.86 ng/L and found that 4-nonyl phenol had low potential risks to aquatic organisms and human health. Liu (Liu et al. 2017) found that the contents of 4-tert-butyl phenol, nonyl phenol and bisphenol A in the surface water of the Yangtze River Basin were 225–112100 ng/L, 1.40–8580 ng/L and 1.70–5630 ng/L, respectively.

Phenolic compounds are a kind of environmental endocrine disruptors. Once they enter the body, they will react with the corresponding receptors in the body and have a long-term and slow impact on the hormone balance in the body (Yang et al. 2022). According to animal experiments and human epidemiological investigations, environmental endocrine disruptors can affect male reproductive system and offspring reproductive system, causing such diseases as testicular cancer and testicular tumor (Namita et al. 2022).

Xi'an is one of the cities with severe water shortage in China. Moreover, the wastewater discharged from the industrial areas in the eastern and western suburbs has also polluted the rivers in the city. Li (Li et al. 2019) took Qujiang Pool, Taiye Pool, Xingqing Lake, Guangyuntan Lake and Weiyang Lake in Xi'an as the research subjects, and found that the heavy metal content in the sediments of each lake was higher than the soil background of Shaanxi Province. Zhang (Zhang et al. 2017) conducted a comprehensive investigation on the water environment of Hancheng Lake and found that some water quality indicators have exceeded the minimum requirements of Class A standards for landscape and recreational water quality. At present, there are ecological problems in lakes in Xi'an, but research has focused on pollutants such as nitrogen and phosphorus, and it is not clear whether new pollutants will cause serious pollution and ecological risks. A possible impact of novel pollutants in landscape lakes on organisms is also unclear. The study selected four typical lakes in Xi'an (Hancheng Lake, Xingqing Lake, Nanhu Lake and Taohuatan Lake), analyzed the types and distribution characteristics of phenolic compounds and their correlation with water quality indicators, and evaluated the ecological risks of phenolic compounds. What is more, toxicological experiments of phenolic compounds on murine female cells and male cells were carried out to provide reference for further exploration of the toxicity mechanism of phenolic compounds on the reproductive system. It is expected to provide some reference for ensuring the ecological security of urban lakes.

Materials and methods

Overview of the study area

Xi'an is in the middle of Guanzhong Plain, bordering the Weihe River in the north and Qinling Mountains in the south. The climate there is warm temperate semi-humid continental monsoon with distinct seasons. At the end of 2019, the resident population was 10,203,500, the urbanization rate was 74.61%, and the population density reached 1,010 people per km^2, much higher than the national average of 143 people per km^2. There are 54 rivers in Xi'an, forming its complicated waterways. However, with the urban expansion, population explosion and land reclamation, the wetland area is greatly reduced, which is only 8.9% of the total area. In this paper, Hancheng Lake in the northern suburb of Xi'an, Xingqing Lake in the eastern suburb, Nanhu Lake in the southern suburb, and Taohuatan Lake connected with the Bahe River, have been selected for detection and analysis. Lakes locations are shown in Fig. 1.

Hancheng Lake was built in April 1971, with a total length of 6.27 Km and a total capacity of 137×10^4 m^3. When it is filled with water, it forms a lake surface of 0.57 Km^2. The lake body flows into the Weihe River from south to north, and the make-up water comes from the Fenghe River. The designed water diversion flow is 1.7 m^3/s, and the residence time is about 9.3d. Before the urban river channel reconstruction in 2009, Hancheng Lake was the main sewage outlet. After that, a reinforced concrete box culvert was designed at the bottom of the pool for sewage discharge and rain & flood discharge of Xingqing Lake, the moat, the old city and the northwest suburb (Liu et al. 2017).

Xingqing Lake Park was built in 1975, which had been the largest water area in Xi'an for a long time. It is a flood control buffer in the eastern suburbs.

Nanhu Lake, also known as Qujiang Pool, was built in July 2008. The pool water is from the Heihe River, which is Class IV water quality (Zhang 2016). Qujiang Pool is long from north to south and narrow from east to west, because of its excavated terrain.

Taohuatan Lake is located at the widest part of the lower reaches of the Chan River. There are two sources of pollution near Taohuatan – the sewage discharge from Xi'an No. 3 Wastewater Treatment Plant and the sewage discharge from iron & steel processing plant. The overall water quality of Taohuatan lies between Class III and Class V surface water (Hu et al. 2016).

Sample collection and processing

In May 2019, samples were taken from the inlet, center, and outlet of Xingqing Lake, Taohuatan Lake, Hancheng Lake and Nanhu Lake in Xi'an, at the same time, and the mixed samples were analyzed. During the sampling period, the weather was fine, and the collected water samples were all 50 cm surface

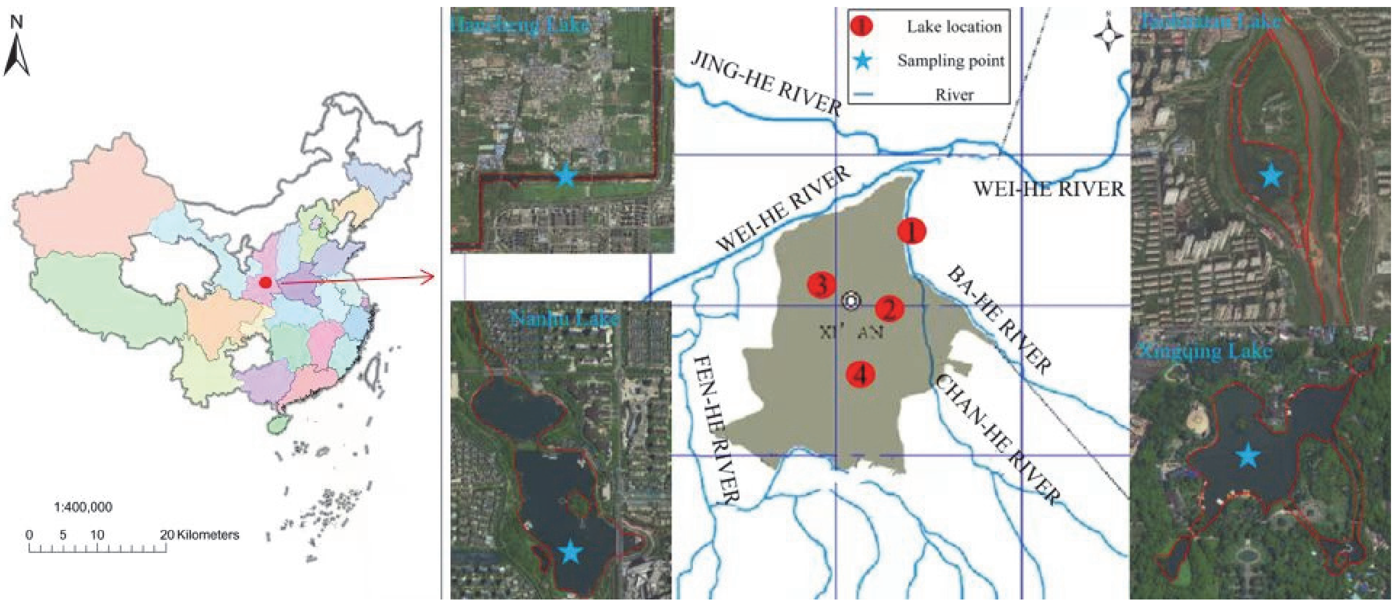

Fig. 1. Location of four lakes

water with a volume of 5 L by simple water collector. The samples were stored in brown glass sampling bottles, sealed immediately after adding a proper amount of copper sulfate, transported back to the laboratory, and sealed for reservation at 4°C. All this was conducted under the National standard of the People's Republic of China – «Water quality sampling – technical regulation of the preservation and handling of samples» (HJ 493-2009)(MEEPRC 2009).The water quality analysis methods are shown in Table 1. And the abbreviations and prototypes involved in the article are shown in Table 2.

Sample analysis

Phenolic compounds in water were separated and determined by ultra-high performance liquid chromatography (Wates HPLC, FLD detector) and ACQUITY UPLCHSS T3 column (1.7 μm). The column temperature was 30°C, Mobile phase (A) was a mixture of 0.2% formic acid and 2 mmol/L ammonium acetate, and mobile phase (B) was acetonitrile. The gradient elution procedure was as follows: 0 min 90% A, 5 min 85% A, 7 min 80% A, 11 min 60% A, 15 min 40% A, 16 min 5% A, and 25 min 5% A. The flow rate was 0.2 mL/min, the injection amount was 5.0 μL, and the wavelength was 280 nm (Zhou et al. 2012; Wei et al. 2017).

Ecological risk assessment

Ecological risk assessment (ERA) is a process of analyzing and evaluating the possibility of adverse ecological effects caused by environmental pollutants and quantifying the ecological harm of pollutants to the environment through concentration threshold or risk entropy (Kong et al. 2021). The specific steps are as follows: (1) Acquisition and analysis of toxicity data, (2) curve fitting of species sensitivity distribution (SSD) and calculation of HC5, (3) calculation of Predictive No Effect Concentration (PNEC) and Risk Entropy (RQ)(Wang et al.2017), (4) characterization of ecological risks of pollutants.

According to the classification method proposed by Hernado (Hernando et al. 2006), RQ_S can be classified into three categories: high risk (RQ_S>1), medium risk (0.1<RQ_S<1) and low risk (0.01<RQ_S<0.1).

Toxicological test analysis method

Studies have shown that BPA and NP can cause gonadal dysplasia and sperm decrease in male mammals. Also, it can cause morphological or functional changes in vagina, uterus and ovary of female mammals through signal pathways such as binding to estrogen receptor (ER). Many studies at home and abroad demonstrate that BPA (Tao et al. 2019) and NP (Dong et al. 2009) have reproductive toxicity to mice. Therefore, the toxicological experiments of phenolic compounds on female and male cells of mice were carried out in this study, which provides reference for further exploring the toxic mechanism of phenolic compounds on reproductive system.

Detection of female cell activity in mice

Human breast cancer MCF-7 is the most commonly used cell line for detecting estrogen-like activity. Studies have found that this cell is abundant in estrogen receptor and proliferates under the action of estrogen. This method for detecting estrogen is called E-Screen test. MCF-7 cells were cultured in DMEM containing phenol red at 37°C in 5% CO_2 incubator. Before the experiment, the cells were cultured in DMEM without phenol red (containing 5% de-hormone fetal bovine serum) for two days (starvation culture) to improve the sensitivity of cells to estrogen. Then, MCF-7 cells were diluted to 5×10^4 cells/mL and inoculated into 96-well plates according to 5000 cells/well, with 100 μL added to each well. The next day, 100 μL of fresh medium containing different concentrations of drugs and corresponding solvents was added to each well (DMSO final concentration less than 0.1%). According to the set dose group, each group was provided with at least three parallel wells, and 20 μL of 5 mg/mL MTT solution was added to each well at

Table 1. Water quality index analysis method

Monitoring indicators	Analytical method
COD (mg/L)	Potassium dichromate rapid digestion-photometric method (HJ 828-2017)
TP (mg/L)	Ammonium molybdate spectrophotometry (HJ 671-2013)
TN (mg/L)	Potassium persulfate oxidation ultraviolet spectrophotometry (HJ 636-2012)
NH_3-N (mg/L)	UV spectrophotometry (HJ 535-2009)
DO (mg/L)	Portable Dissolved Oxygen Meter Method (HJ 506-2009)
pH	Portable pH meter method (HJ 1147-2020)

Table 2. The limits of detection (LOD) the limits of quantification (LOQ) and the recovery rate of phenolics in different matrices

	LOD (ng/L)	LOQ (ng/L)	Recovery rate(%)
BPA	0.43	0.62	91.2%
4-t-OP	0.13	0.14	90.8%
NP	0.02	0.83	97.08%
17-beta-E2	0.12	0.2	91.2%
E1	0.06	0.18	90.6%

24 h, 48 h, 72 h, and 96 h after the addition of test substance. The absorbance value (A) of each well was measured at 490 nm wavelength and was compared with the solvent control group. The maximum multiple of MCF-7 cell proliferation in the experiment group is the proliferation effect (PE):

$$\text{Proliferation effect (PE)} = \frac{\text{average OD in the experiment}}{\text{average OD in the control group}} \quad (1)$$

Detection of mouse male cells

The experimental dose of a typical environmental estrogen was selected as (pC=5,6,7,8,9, pC means -logC, mol/L). Sertoli cells have the function of synthesizing estrogen, which begin to proliferate on the 16th day of rat embryo, reach the peak on the 2nd day before birth, and start to differentiate on the 15th day after birth. The number of Sertoli cells in testis remains stable during sexual maturity.

(1) MTT assay was used to determine the toxic effect of environmental estrogen on Sertoli cells of testis.

MTT is a type of yellow dye. Succinate dehydrogenase in mitochondria of living cells can metabolize and reduce MTT. Meanwhile, under the action of cytochrome C, blue (or blue-purple) formazan insoluble in water can be produced, and its amount can be determined at 490nm by microplate reader. Under normal circumstances, the amount of formazan produced is directly proportional to the number of living cells, so the number of living cells can be estimated according to the optical density OD value (Optical Density). There is no succinate dehydrogenase in dead cells, so there is no reaction when MTT is added. The specific operation steps are the same as those of E-Screen experiment.

(2) Cell cycle changes were measured by flow cytometry. After starvation culture for 48 hours before the experiment, the cells were inoculated into a 25 cm^2 culture bottle with density of 1×10^5 cells per bottle. After 24 hours, the fresh culture medium containing different concentrations of drugs and corresponding solvents was added, and three parallel samples in each group were cultured for 48 hours. The cells were collected, centrifuged at 2000 r/min to discard the culture solution, washed once with PBS, and centrifuged again to discard PBS. Then, 70% precooled ethanol was added and it was fixed at -20°C for 24 hours. They were rinsed with PBS once, suspended again in PI dye solution (0.1% NP40, 0.1% sodium citrate, 50 μg/mL PI, 100 μg/mL RNase A), marked for 30 min away from light,

Table 3. Detected concentration and rate of phenolic compounds in urban lakes of Xi' an

Phenolic compound	Lake	Detected concentration	Detection rate (%)
BPA	Taohuatan Lake	11.92±2.59	100%
	Nanhu Lake	22.21±5.31	
	Xingqing Lake	19.04±4.99	
	Hancheng lake	16.14±6.12	
4-t-OP	Taohuatan Lake	3.44±1.11	100%
	Nanhu Lake	0.80±0.19	
	Xingqing Lake	2.70±0.58	
	Hancheng lake	0.89±0.22	
NP	Taohuatan Lake	100.32±14.68	100%
	Nanhu Lake	7.85±2.63	
	Xingqing Lake	13.90±3.53	
	Hancheng lake	10.99±2.71	
DES	Taohuatan Lake	ND	0%
	Nanhu Lake	ND	
	Xingqing Lake	ND	
	Hancheng lake	ND	
17-beta-E2	Taohuatan Lake	ND	25%
	Nanhu Lake	ND	
	Xingqing Lake	1.70±0.39	
	Hancheng lake	ND	
E1	Taohuatan Lake	0.2±0.05	100%
	Nanhu Lake	0.55±0.14	
	Xingqing Lake	5.67±1.81	
	Hancheng lake	0.51±0.08	
EE2	Taohuatan Lake	ND	0%
	Nanhu Lake	ND	
	Xingqing Lake	ND	
	Hancheng lake	ND	

and detected with flow cytometry to measure the excitation of argon ion at 488 nm. Finally, we collected PI red fluorescence with 610 nm band-pass filter, analyzed the number of 10^4 cells in each sample, and calculated the cell proliferation index (PI). Among them, S, G1, and G2 represent the cell distribution in the DNA synthesis stage, the early stage of synthesis, and the later stage of synthesis, respectively.

$$PI = \frac{S + G2}{G1 + S + G2} \times 100\% \quad (2)$$

(3) WESTERN BLOT method was used to determine the proteins related to the interference effect of environmental estrogen.

The principle of WESTERN BLOT method is as follows (Biam et al. 2022): Differentiate different components by electrophoresis, transfer them to solid support, and use specific reagent (antibody) as probe to detect target substances. Western Blot technology of protein combines high resolution of gel electrophoresis and specific sensitivity of solid immunoassay, and can detect target proteins of medium size as low as 1–5 ng (the lowest being 10–100 pg). In this experiment, the estrogen receptor α protein in breast cancer MCF-7 cells was determined by WESTERN BLOT, which is helpful to judge whether environmental estrogen exerts its estrogenic activity through classical pathway (nuclear pathway) or non-classical pathway (cytoplasmic pathway). The method can also determine the related proteins of MAPK signal transduction pathway in MCF-7 cells and Sertoli cells, so as to clarify the mechanism of environmental estrogen injury on female and male.

(4) RT-PCR method was used to determine the genes related to the interference effect of environmental estrogen.

RT-PCR is a technology combining reverse transcription of RNA (RT) and polymerase chain amplification of cDNA (PCR) (Atieh et al. 2022). First, cDNA is synthesized from RNA by reverse transcriptase, and then the target fragment is amplified and synthesized by using cDNA as template. RT-PCR technology is sensitive and widely used, it can detect the gene expression level in cells and quantify expression information. Transcription factors C-fos and C-jun were determined in this experiment. C-fos and C-jun genes were not transcribed in static cells, but when stimulated by outside, they induced gene expression and participated in cell proliferation and differentiation.

Results and discussion

Types and concentration of phenolic compounds in lake water of Xi'an City

In this study, five types of phenolic compounds were detected in four urban lakes in Xi'an. The specific detection concentration and detection rate are shown in Table 4. The detection rate of four phenols reached 100%, which were bisphenol A (BPA), octylphenol (4-t-OP), nonyl phenol (NP) and estrone (E1). The detection range of five phenolic compounds in water was ND-100.32 ng/L. The total concentration of phenolic compounds in the four lakes was as follows: nonyl phenol (4-NP, 133.06 ng/L) > bisphenol A (BPA, 69.31 ng/L) > octylphenol (4-t-OP,7.82 ng/L) > estrone (E1,6.93 ng/L) > estradiol (17-beta-E2, 1.70 ng/L). Detected concentration and rate of phenolic compounds in urban lakes of Xi'an.

Comparison of types and concentrations of phenolic compounds in the four lakes is shown in Table 4. The main phenols in Hancheng Lake, Xingqing Lake and Nanhu Lake are BPA and NP, while the main phenols in Taohuatan Lake are NP, and the concentration of NP far exceeds other phenols, reaching 100.32 ng/L. From the perspective of the detected concentration and detection rate, BPA and NP are the main pollutants in the four lakes of Xi'an.

Comparing the detected concentration of phenolic compounds in the urban lakes of Xi'an with that in other lakes of China, we obtained the results shown in Table 5. The concentration of BPA detected in the four lakes of Xi'an is 11.92–22.21 ng/L, which is the same order of magnitude as that of Tianjin surface water and Taihu Lake, far lower than that of Nanjing section of the Yangtze River, the Yellow River and 62 drinking water sources in 31 cities in China. The detected concentration of 4-t-OP in the four lakes is 0.80–3.44 ng/L, which is the same order of magnitude as Taihu Lake and the Yellow River, far lower than the concentration in the Pearl River Estuary and Nanjing Section of the Yangtze River. The concentrations of NP in the four lakes range from

Table 4. Concentration distribution of phenolic compounds in different waters in China

lake	BPA (ng/L)	4-t-OP (ng/L)	4-NP (ng/L)
Hancheng lake	16.14±6.12	0.89±0.22	10.99±2.71
Xingqing Lake	19.04±4.99	2.70±0.58	13.90±3.53
Nanhu Lake	22.21±5.31	0.80±0.19	7.85±2.63
Taohuatan Lake	11.92±2.59	3.44±1.11	100.32±14.68
Pearl River Estuary (Winter) (Dong et al. 2009)	–	85.5–581	810–3340
Tianjin surface water (Yin et al. 2020)	ND-16.26	–	47.80–358.40
Taihu Lake (Chen et al. 2017)	ND-55.1	ND-8.14	ND-121
Nanjing section of Yangtze River (Liu et al. 2017)	ND-563	ND-100	1.4–858
Yellow River (Duan et al. 2014)	12.5–171.5	2.4–14.5	165.8–1187.6
Water from 62 drinking Water sources in 31 cities in China (Fan et al. 2013)	ND-512	–	8.20–918

ND: None Dectected; –: not detected.

7.85 ng/L to 100.32 ng/L, lower than those in the Pearl River Estuary, Tianjin surface water, Taihu Lake, Nanjing section of the Yangtze River, the Yellow River and 62 drinking water sources in 31 cities in China. To sum up, compared with other lakes, the four lakes of Xi'an have lower concentration of phenolic compounds.

Relationship between detected concentration of phenolic compounds and water quality indicators

The water quality data in May 2019 is shown in Table 6. Pearson correlation analysis is used to analyze the concentration of phenolic compounds in the Xi'an lakes and the water quality indexes COD, TP, TN, NH_3-N, DO and pH in the same period. The results are shown in Table 7, where $P<0.01$ means extremely significant correlation, and $P<0.05$ means significant correlation.

It can be seen from Table 7 that there is a significant relationship between BPA concentration and COD value, between 17-beta-E2 concentration and TP and TN values, between E1 concentration and TP and TN values, between 4-t-OP concentration and pH value. It was shown that TN, TP and pH were important factors in adjusting the concentration of phenolic compounds and showed a significant positive correlation. However, the NH_3-N and DO values have no significant correlation with phenolic compounds concentration in the Xi'an lakes, indicating that NH_3-N and DO have few influences on the occurrence of phenolic compounds.

Ecological risk assessment of phenolic compounds and antibiotics in surface water of lakes in Xi'an

Table 8 shows that the RQ_S values of BPA, 4-t-OP and NP in the four lakes are between 0.1 and 1, which are at a medium risk level. The RQ_S values of E1 in the four lakes were higher than 1, which is at a high risk level. The risk entropy value of E1 in Xingqing Lake is the highest, which is 35.42 ng/L.

Numerous studies have shown that BPA, 4-t-OP, NP and E1 will be accumulated in organisms, migrate in the environment, have strong toxicity and are difficult to biodegrade. These characteristics can further endanger the environment (Standnicka et al. 2012). Lu (Lu et al. 2019) found that fish plasma has high bioaccumulation potential for BPA, 4-t-OP and E1. Qiu (Qiu et al. 2015) found that 4-t-OP and NP have potential bioaccumulation effects, and the higher the trophic level, the more obvious the biomagnification effect. Therefore, the fish in urban lakes also faces the pollution and harm from phenolic compounds which cannot be ignored, and may pose some health risks to the population exposed to this environment.

Table 5. Water quality indexes of the four lakes in May 2019

Index	Hancheng Lake	Xingqing Lake	Nanhu Lake	Taohuatan Lake
COD (mg/L)	15±3.1	14±1.8	14±2.6	16±2.9
TP (mg/L)	0.047±0.011	0.088±0.019	0.046±0.009	0.035±0.005
TN (mg/L)	6.56±1.09	5.54±1.13	6.50±1.61	6.87±1.45
NH_3-N (mg/L)	0.378±0.25	2.74±0.73	0.067±0.012	1.17±0.39
DO (mg/L)	9.53±1.42	10.13±0.96	11.97±0.39	9.00±0.66
pH	9.28±0.34	8.26±0.22	8.93±0.17	8.08±0.37

Table 6. Correlation between phenolic compounds concentration and water quality index

	ρ (COD)	**ρ (TP)**	**ρ (TN)**	**ρ (NH_3-N)**	**ρ (DO)**	**ρ (pH)**
ρ (BPA)	0.045	0.574	0.508	0.888	0.069	0.589
ρ (4-t-OP)	0.499	0.852	0.901	0.306	0.383	0.044
ρ (NP)	0.130	0.498	0.455	0.905	0.372	0.315
ρ (17-beta-E2)	0.478	0.028	0.041	0.079	0.986	0.553
ρ (E1)	0.433	0.016	0.026	0.103	0.972	0.603

Table 7. Toxicological data and ecological risk assessment results of phenolic compounds

Antibiotics	Toxicity type	Safety margin	PNEC (ng/L)	RQ_S			
				Hancheng Lake	Xingqing Lake	Nanhu Lake	Taohuatan Lake
BPA (Schultis and Metzger 2004)	II	50	118	0.14	0.16	0.19	0.10
4-t-OP (Tanaka et al.2001)	IV	1000	13	0.07	0.21	0.06	0.26
NP (Legler et al. 2002)	I	10	500	0.02	0.03	0.02	0.20
E1 (Sun et al. 2010)	–		0.16	3.20	35.42	3.44	1.27

–:No data.

Toxic effects of phenolic compounds on mouse germ cells

Marin (Liang and Gu 2021) found that exposure to low-dose BPA during pregnancy can change the expression of miRNA in testes of offspring male mice. Therefore, in this paper, female cells (breast cancer cells) and male germ cells (testicular cells) of mice are taken as the research subjects to discuss the toxic effects of phenolic compounds on mouse germ cells.

Analysis of the effect of phenolic compounds on female germ cells

(1) Effect of bisphenol A (BPA) on female cell

As an environmental estrogen, BPA has affinity for estrogen receptor and can activate estrogen receptor and cause endocrine disorders. BPA with a concentration of 10^{-5} ~ 10^{-9} mol/L (22.4 ng/L-2.24 × 10^5 ng/L) was selected to act on breast cancer cells MCF-7 with estrogen receptor positive for 24 h, 48 h, 72 h and 96 h, respectively. The cell proliferation activity of cells was observed, the endocrine interference effect of BPA was detected, and the dose-effect relationship of estrogen activity induced by BPA was observed.

Fig. 2 shows that after BPA acts on MCF-7 cells for 24 h, BPA of 10^{-6} ~ 10^{-8} mol/L proliferates. Although the proliferation index is not high, BPA induces proliferation very quickly. BPA of 10^{-8} mol/L (224 ng/L) induced the highest proliferation intensity in 24 hours. After 48 hours of treatment, all dosage groups of BPA showed proliferation effect, and BPA of 10^{-8} mol/L had the strongest proliferation effect, which conformed to the parabolic dose-effect relationship of estrogen at 24 h and 48h. However, at 72 h, the proliferative effect decreased with the decrease of dosage. BPA of 10^{-5} mol/L has the strongest ability to induce proliferation, and the ability decreased with reduced concentration. At 96 h, only 10^{-9} mol/L BPA can induce cell proliferation, and other concentrations have no effect on cell proliferation. The effect of 10^{-5} mol/L BPA on cells becomes more obvious with prolonged contact time, reaching the maximum at 72 h and disappearing at 96 h. The effect of BPA with other concentrations on cell proliferation is that the proliferation index reaches the maximum at 48 h. With the increase of contact time, the proliferation index decreases, indicating that the time for BPA with different concentrations to induce proliferation is different. The greater the concentration, the more obvious its cumulative effect, and the lower the concentration, the weaker its cumulative effect and the obvious rapid toxicity. It can be seen from the figure that BPA with a concentration of 10^{-8} mol/L (224 ng/L, while the actual highest concentration in water body is 314.1 ng/L), has the most obvious effect on inducing proliferation.

(2) Effect of nonylphenol (NP) on female cell

Feminization of male fish has been found in the rivers downstream of some sewage treatment plants in Britain. Studies have shown that NP in sewage leads to this abnormal consequence. Studies on a Daphnia demonstrate that NP might indirectly exert its endocrine disrupting effect by blocking the process of elimination of testosterone metabolism.
Therefore, whether NP acts directly as an exogenous estrogen or by changing the concentration or efficacy of endogenous hormones remains to be further confirmed.

It can be seen from Fig. 3 that 10^{-5} mol/L (2.20×10^5 ng/L) NP had no proliferation effect on MCF-7 cells at four time points and showed mild inhibition at 24 h and obvious inhibition at 72 h and 96 h. After working 24 hours, 10^{-8} (220 ng/L) and 10^{-9} mol/L (22.0 ng/L) NP could slightly induce the proliferation of cells. After 48 h, 10^{-6} ~ 10^{-9} mol/L (22.0~2.20×10^4 ng/L) NP enhanced the induction ability of cells compared with 24 h, but compared with BPA with the same concentration, the induction ability of cell proliferation was obviously weakened. At 48 h, the proliferation index increased with the decrease of NP concentration. At the concentration of 10^{-8} mol/L, the proliferation was the highest. After 72 h treatment, the proliferation of 10^{-6} and 10^{-7} mol/L NP disappeared, and the induction of 10^{-8} and 10^{-9} mol/L NP was weaker than that at 48 h, but it could still induce cell proliferation. After 96 hours, all concentrations of NP could not induce proliferation, and the proliferation index was significantly lower than that of the solvent control group. The treatment rule of NP with 10^{-8} mol/L concentration is like that of BPA with the same concentration. It can induce cell proliferation at 24 h, reach the maximum at 48 h, and weaken the proliferation ability at 72 h. NP of 10^{-8} mol/L (220 ng/L, the highest concentration in water is 718.81 ng/L) has the most obvious effect on inducing proliferation.

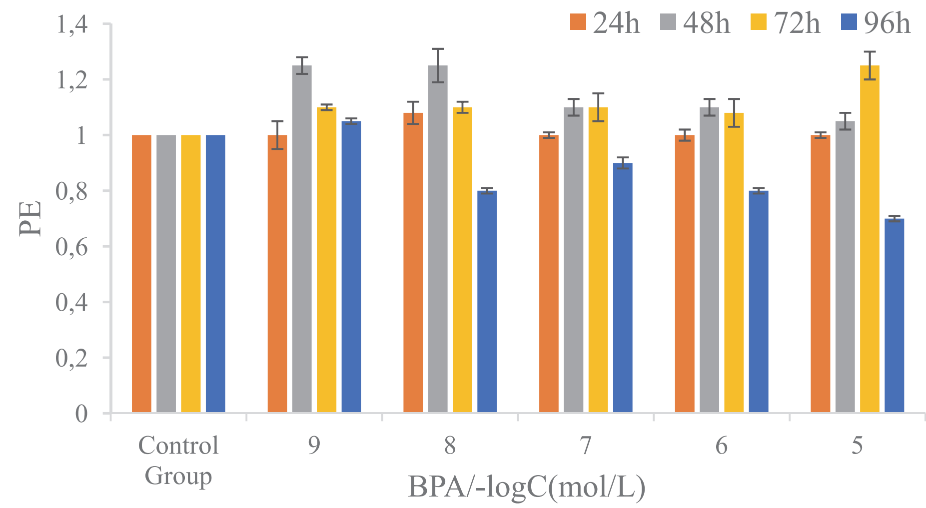

Fig. 2. Effects of BPA with different concentrations on MCF-7 cell proliferation

Analysis of the effect of phenolic compounds on male germ cells

(1) Effect of bisphenol A (BPA) on male cell

BPA has great impact on male cells, so the effect of BPA on Sertoli cells of testis was studied. Some studies have pointed out that environmental estrogen can affect male reproductive function in the following ways: (1) changing the bioavailability of hormones *in vivo* to regulate hormone levels, (2) changing hormone metabolism and its own metabolism, (3) mediating by receptor binding, and (4) acting on cell signal transduction pathway (Peranandam et al. 2014).

In male mammalian testicles, MAPK signal can regulate cell proliferation, differentiation, and apoptosis. Abnormal Sertoli cells in testicles will inevitably affect the normal spermatogenic process, resulting in dysgenesis at all levels of spermatogenic cells. Therefore, MAPK signal transduction pathway is considered as one of the important determinants of sperm development. Therefore, this experiment focuses on the ERK, JNK and P38 signal pathways in MAPK signals.

As shown in Fig. 4, BPA increased the expression of ERK protein in Sertoli cells of testis, induced ERK protein to a high degree, and induced p-ERK protein to a stronger degree, which significantly increased its expression and activated ERK signal pathway. The expression of JNK protein and p-JNK protein could not be induced in the control group, but BPA could induce the expression of JNK protein and p-JNK protein. BPA has a strong induction ability and plays a major role in the induction process. BPA significantly increased the expression of P38 protein and compared with the control group, the induction of P38 protein was enhanced considerably. C-myc protein was slightly expressed in the control group, while BPA increased the expression of C-myc protein. BPA can induce cyclin D1 expression, but the amount induced by BPA is less.

To sum up, BPA activates the expression of nuclear transcription factor C-myc protein, induces the expression of cyclinD1, and promotes cell proliferation through ERK and JNK signaling pathway.

(2) Effect of nonylphenol (NP) on male cells

NP can seriously affect the male reproductive system and produce a series of reproductive toxicity to male animals, but the specific mechanism remains unclear. Therefore, rat testicular Sertoli cells was selected to experiment on the effects of NP on males and explore its effect through the study of MAPK signaling pathway.

It can be seen from Fig. 5 that NP reduces the expression of ERK protein and has no difference in inducing p-ERK in Sertoli cells compared with the control group. The induction of JNK protein by NP is like that by BPA, which means it has high

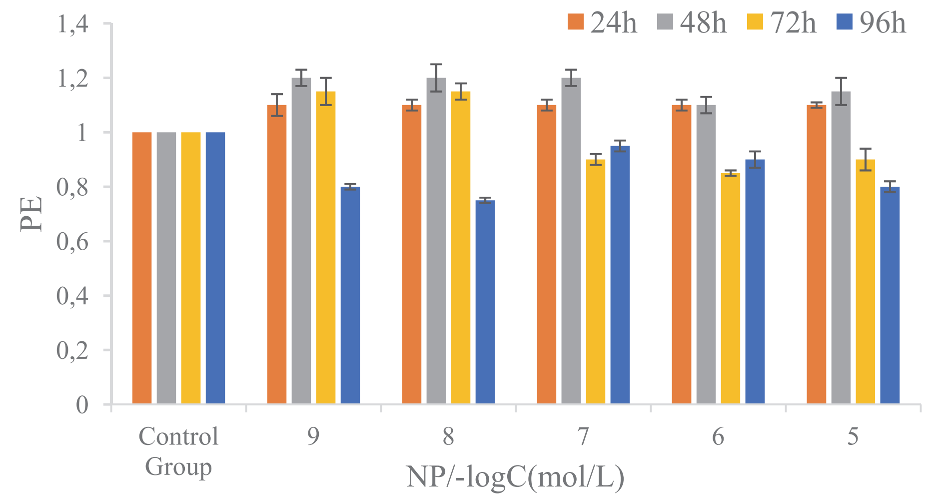

Fig. 3. Effects of different concentrations of NP on proliferation of MCF-7 cells

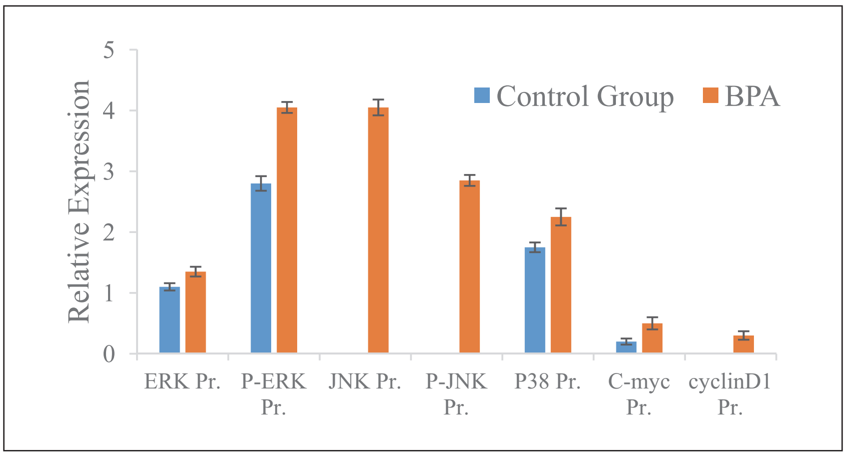

Fig. 4. Effect of BPA on different proteins in sertoli cells of testis

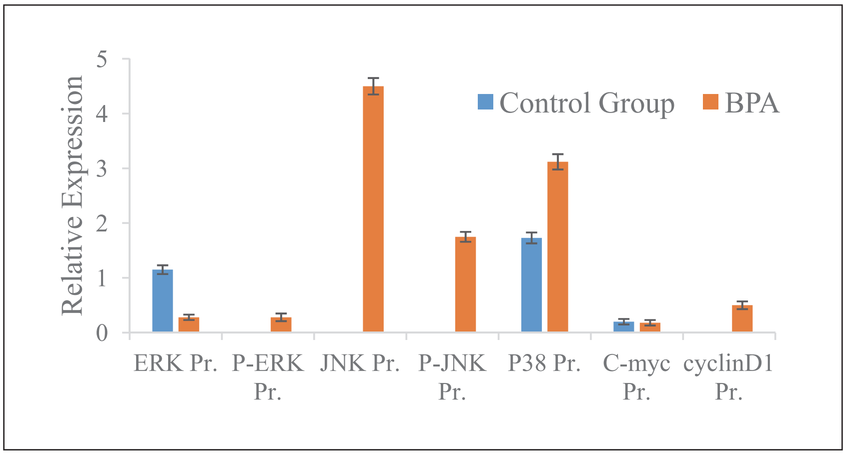

Fig. 5. Effect of NP on different proteins in sertoli cells of testis

induction ability and can significantly induce the expression of p-JNK protein and play a role in activating JNK pathway. NP can induce the expression of P38 protein, which is much higher than that of the control group. There is no significant difference between NP and the control group in inducing C-myc protein. The expression of cyclinD1 protein was decreased by NP.

To sum up, NP can induce the proliferation of Sertoli cells in testis. The effect of NP on testicular Sertoli cells is exerted through the JNK signaling pathway, which can induce the expression of p-JNK protein and inhibit apoptosis, then causing the expression of C-myc protein and inducing the expression of cyclinD1 protein and promoting the proliferation of cells.

Conclusion

This study investigated the types and contents of phenolic pollutants in four typical urban lakes in Xi'an city. Based on these findings, the study assessed the ecological risks and reproductive toxicity associated with the targeted contaminants. This study provides a reference for urban lake management and ecological security. Results showed that:

1. There are five kinds of phenolic compounds in the water of four lakes in Xi'an, with the concentration ranging from ND to 100.32 ng/L, among which BPA and NP were the main pollution types of phenolic compounds in the lakes. BPA, 4-t-OP and NP in the four urban lakes are at medium risk level, while E1 is at high risk level.
2. There is a significant positive relationship between BPA concentration and COD value, between 17-beta-E2 and E1 concentrations and TP and TN values, between 4-t-OP concentration and pH value in the four lakes in Xi'an, indicating that TN, TP and pH are important factors affecting phenolic compounds in surface water.
3. Both BPA and NP can induce the proliferation of Sertoli cells. BPA activates the expression of nuclear transcription factor C-myc protein, induces the expression of cyclinD1 and promotes cell proliferation through ERK and JNK signaling pathway. The effect of NP on Sertoli cells is exerted through JNK signaling pathway, which can induce the expression of p-JNK protein and inhibit apoptosis, thus causing the expression of C-myc protein and inducing the expression of cyclinD1 protein, and promoting the proliferation of cells.

Acknowledgements

This work has been supported by the National Natural Science Foundation of China (51809211), the China Postdoctoral Science Foundation (2018M633548, the Natural Science Foundation of Shaanxi Province (2019JQ-745), the Scientific Research Program Funded by Shaanxi Provincial Education Department (Program No.20JY045) and Key Research and Development Program of Shaanxi Province (2017ZDXM-SF-081).

Disclosure statement

No potential conflict of interest was by the author(s).

References

Atieh, Y., Anis, E. & Kiarash, G. (2022). Quantitative evaluation senx3-regx3 gene of Mycobacterium tuberculosis by real-time RT-PCR assays for monitoring the response to anti-TB therapy. *Gene Reports*, 28, 101642. DOI:10.1016/j.genrep.2022.101642

Biam, R.S., Robichaud, P.P. & Mbarik M. (2022). Loss of detection of fatty acid-metabolizing proteins in Western blot analyses – Impact of sample heating. *Biochemical and Biophysical Research Communications*, 607, pp. 110–116. DOI:10.1016/j.bbrc.2022.03.130

Chen, M.H., Guo, M. & Liu, D. (2017). Occurrence and distribution of typical endocrine disruptors in surface water and sediments from Taihu Lake and its tributaries. *China Environmental Science*, 37(11), pp. 4323–4332. (in Chinese)

Diao, P.P., Chen, Q. & Wang R. (2017). Phenolic endocrine-disrupting compounds in the Pearl River Estuary: Occurrence, bioaccumulation and risk assessment. *Science of The Total Environment*, 584–585, pp. 1100–1107. DOI:10.1016/j.scitotenv.2017.01.169.

Dong, J., Sun, L.N. & Chen, R.H. (2009). A study on the pollution of chlorophenol compounds in the surface water of the pearl river estuary area. *Environmental Science & Technology*, 32(07), pp. 82–85. (in Chinese)

Duan, X.Y., Li, Y.X. & Li, X.G. (2014). Alkylphenols in surface sediments of the Yellow Sea and East China Sea inner shelf: Occurrence, distribution and fate. *Chemosphere*, 107, pp. 265–273. DOI:10.1016/j.chemosphere.2013.12.054

Fan, Z.L., Hu, J. & An, W. (2013). Detection and occurrence of chlorinated byproducts of bisphenol A, nonylphenol, and estrogens in drinking water of China: Comparison to the parent compounds. *Environmental Science&Technology*, 47(19), pp. 10841–10850. DOI:10.1021/es401504a

Hernando, M.D., Mezcua, M. & Fernández-Alba, A.R. (2006). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta*, 69(2), pp. 334–342. DOI:10.1016/j.talanta.2005.09.037

Hodaka, K., Hidekazu, O. & Mayuri I. (2004). Endocrine disrupter nonylphenol and bisphenol A contamination in Okinawa and Ishigaki Islands, Japan – within coral reefs and adjacent river mouths. *Chemosphere*, 55(11), pp. 1519–1527. DOI:10.1016/j.chemosphere.2004.01.032

Hu, S.L., Ji, J.Y. & Chen, R. (2016). Analysis of eutrophication status and causes of urban landscape water body in xi'an. *Environmental Monitoring Management and Technology*, 28(05), pp. 62–65. (in Chinese)

\Kong, M., Bu Y.Q. & Zhang Q. (2021). Distribution, abundance, and risk assessment of selected antibiotics in a shallow freshwater body used for drinking water. *China Journal of Environmental Management*, 280, pp. 111738. DOI:10.1016/j.jenvman.2020.111738

Legler, J., Zeinstra, L.M. & Schuitemaker, F. (2002). Comparison of in vivo and in vitro reporter gene assays for short-term screening of estrogenic activity. *Environ Sci Technol*, 36(20), pp. 4410–4415. DOI:10.1021/es010323a

Li, H.J., Li, H.X. & Shi, X.M. (2019). Pollution charasteristics of heavy metals and ecological risk assessment for the surface sediments of the lakes in Xi'an. *Resources And Environment In Arid Areas*, 33(02), pp. 122–126. DOI:10.13448/j.cnki.jalre.2019.051. (in Chinese)

Liang, J.J. & Gu, A.H. (2021). Multigenerational and cross--generational effect of environmental endocrine disruptors on reproductive system in male animals. *Chinese Journal of Public Health*, 37(02), pp. 375–380. (in Chinese)

Liu, Q.,Wang, S. & Xu, J.J. (2017). Analysis of phytoplankton community structure and water quality status in Hancheng Lake, Xi'an. *Safety and Environmental Engineering*, 24(03), pp. 48–56. DOI:10.13578/j.cnki.issn.1671-1556.2017.03.009. (in Chinese)

Liu, Y.H., Zhang, S.H. & Ji, G.X. (2017). Occurrence, distribution and risk assessment of suspected endocrine-disrupting chemicals in surface water and suspended particulate matter of Yangtze River (Nanjing section). *Ecotoxicology and Environmental Safety* 135, pp. 90–97. DOI:10.1016/j.ecoenv.2016.09.035

Lv, Y.Z., Zhao, J.L. & Yao, L. (2019). Bioaccumulation of phenolic endocrine disrupting chemicals in the plasma of wild fish from Yangtze River, China. *Environmental chemistry*, 38(03), pp. 443–453. (in Chinese)

Ministry of Ecology and Environment of the People's Republic of China. (2017). Water quality – determinnation of the chemical oxygen demand-dichromate method. http://www.mee.gov.cn/ywgz/fgbz/bz/bzwb/jcffbz/201704/t20170410_409547.shtml (in Chinese)

Ministry of Ecology and Environment of the People's Republic of China. (2013). Water quality – determination of total phosphorus – Flow injection analysis (FIA) and ammonium molybdate spectrophotometry. http://www.mee.gov.cn/ywgz/fgbz/bz/bzwb/jcffbz/201311/t20131106_262959.shtml. (in Chinese)

Ministry of Ecology and Environment of the People's Republic of China. (2012). Water quality – determination of total nitrogen--Alkaline potassium persulfate digestion UV spectrophotometric method. http://www.mee.gov.cn/ywgz/fgbz/bz/bzwb/jcffbz/201203/t20120307_224383.shtml. (in Chinese)

Ministry of Ecology and Environment of the People's Republic of China. (2009). Water quality – determination of ammonia nitrogen-Nessler's reagent spectrophotometry. http://www.mee.gov.cn/ywgz/fgbz/bz/bzwb/jcffbz/201001/t20100112_184155.shtml. (in Chinese)

Ministry of Ecology and Environment of the People's Republic of China (2009). Water quality – determination of dissolved oxgen – Electrochemical probe method. http://www.mee.gov.cn/ywgz/fgbz/bz/bzwb/jcffbz/200911/t20091106_181278.shtml. (in Chinese)

Ministry of Ecology and Environment of the People's Republic of China. (2020). Water quality – determination of pH-Electrode method. http://www.mee.gov.cn/ywgz/fgbz/bz/bzwb/shjbh/xgbzh/202011/t20201127_810274.shtml. (in Chinese)

Ministry of Ecology and Environment of the People's Republic of China. (2009). Water quality sampling – technical regulation of the preservation and handling of samples. http://www.mee.gov.cn/ywgz/fgbz/bz/bzwb/jcffbz/200910/t20091010_162157.shtml (in Chinese)

Namita, P., Ankita, P. & Mitali, M.S. (2022). A comprehensive review on eco-toxicity and biodegradation of phenolics: Recent progress and future outlook. *Environmental Technology & Innovation*, 27, 102423. DOI:10.1016/j.eti.2022.102423

Peranandam, T., Kulanthaivel, L. & Shanmugam, V. (2014). Efficiency of lycopene against reproductive and developmental toxicity of Bisphenol A in male Sprague Dawley rats. *Biomedicine & Preventive Nutrition*, 4(4), pp. 491–498. DOI:10.1016/j.bionut.2014.07.008

Qiu, L.N., Yun, X. & Na, G.S. (2015). On the bioaccumulation and biomagnification of phenols endocrine disruptors in the organisms in the coast of Northern Yellow Sea. *Journal of Safety and Environment*, 15(04), pp. 353–357. DOI:10.13637/j.issn.1009-6094.2015.04.074.(in Chinese)

Schultis T. & Metzger J.W. (2004). Determination of estrogenic activity by LYES-assay (yeast estrogen screen-assay assisted by enzymatic digestion with lyticase). *Chemosphere*, 57(11), pp. 1649–1655. DOI:10.1016/j.chemosphere.2004.06.027

Standnicka, J., Schirmer, K. & Ashauer, R. (2012). Predicting concentrations of organic chemicals in fish by using toxicokinetic models. *Environmental Science &Technology*, 46(6), pp. 3273–3280. DOI:10.1021/es2043728

Sui, Q., Huang, J. & Yu, G. (2009). Priority Analysis for Controlling Endocrine Disrupting Chemicals in Municipal Wastewater Treatment Plants of China. *Environmental Science*, 30(02), pp. 384–390. DOI:10.13227/j.hjkx.2009.02.013. (in Chinese)

Sun, Y., Huang, H. & Hu, H.Y. (2010). Concentration and Ecological Risk Level of Estrogenic Endocrine-Disrupting Chemicals in the Effluents from Wastewater Treatment Plants. *Environmental Science Research*, 23(12), pp. 1488–1493. DOI:10.13198/j.res.2010.12.46.suny.005. (in Chinese)

Takuo, K. & Kunio, K. (1996). Studies on the mechanism of toxicity of chlorophenols found in fish through quantitative structure--activity relationships. *Water Research*, 30(2), pp. 393–399. DOI:10.1016/0043-1354(95)00152-2

Tan, R.J., Li, Z.S. & Liu, R.X. (2015). PContamination Level of Endocrine Disrupting Compounds in Natural Aquatic Environment. *Anhui Agricultural Sciences*, 43(23), 167-169+288. DOI:10.13989/j.cnki.0517-6611.2015.23.067. (in Chinese)

Tanaka, H., Yakou, Y. & Takahashi, A. (2001). Comparison between estrogenicities estimated from DNA recombinant yeast assay and from chemical analyses of endocrine disruptors during sewage treatment. *Water Sci Technol*, 43 (2), pp. 125–132. DOI:10.2166/wst.2001.0081

Tao, S.Y., Wang, L.H. & Zhu, Z.L. (2019). Adverse effects of bisphenol A on Sertoli cell blood-testis barrier in rare minnow *Gobiocypris rarus*. *Ecotoxicology and Environmental Safety*, 171, pp. 475–483. DOI:10.1016/j.ecoenv.2019.01.007

Tülay, A.Ö., Önder, H.Ö. & Songül, Z.B. (2002). Removal of phenolic compounds from rubber–textile wastewaters by physico--chemical methods. *Chemical Engineering and Processing. Process Intensification*, 41(8), pp. 719–730. DOI:10.1016/S0255-2701(01)00189-1

Wang, W. & Kurunthachalam, K. (2018). Inventory, loading and discharge of synthetic phenolic antioxidants and their metabolites in wastewater treatment plants. *Water Research*, 129, pp. 413–418. DOI:10.1016/j.watres.2017.11.028

Wang, Z, Yang, XH, Fan, D.L. (2017). Ecological Risk Assessment of Triclocarban in Fresh Water of China by Species Sensitivity Distribution. *Journal of Ecology and Rural Environment*, 33(10), pp. 921–927. (in Chinese)

Wei, H., Wang, J.W. & Yang, X.Y. (2017). Contamination characteristic and ecological risk of antibiotics in surface water of the Weihe Guanzhong section. *China Environmental Science*, 37(6), pp. 2255–5562. (in Chinese)

Yang, M.F., Zou, Y.Q. & Wang, X. (2022). Synthesis of intracellular polyhydroxyalkanoates (PHA) from mixed phenolic substrates in an acclimated consortium and the mechanisms of toxicity. *Journal of Environmental Chemical Engineering*, 10, (3), 107944. DOI:10.1016/j.jece.2022.107944

Yin, W., Fan, D.L. & Wang, Z. (2020). Pollution Characteristics and Ecological Risks of 7 Phenolic Compounds of High Concern in the Surface Water and Sediments of Tianjin, China. *Asian Journal of Ecotoxicology*, 15(01), pp. 230–241. (in Chinese)

Yoel, S., Ann, S. & Monica, S. (2018). The influence of in vivo exposure to nonylphenol ethoxylate 10 (NP-10) on the ovarian reserve in a mouse model. *Reproductive Toxicology*, 81, pp. 246–252. DOI:10.1016/j.reprotox.2018.08.020

Yousefi, H., Yahyazadeh, A. & Moradi Rufchahi, E.O. (2013). Spectral properties, biological activity and application of new 4-(benzyloxy)phenol derived azo dyes for polyester fiber dyeing. *Journal of Molecular Liquids*, 180, pp. 51–58. DOI:10.1016/j.molliq.2012.12.030

Zhang, F., Lu, X. & Yang, X.H. (2017). Investigation Report on Water Environment of Hancheng Lake in Xi'an City. *Journal of Xi'an University (Natural Science Edition)*, 20(05), 109–112, 117. (in Chinese)

Zhang, Y.B. (2016). Application of fuzzy comprehensive evaluation method to the assessment of surface water environment quality with the example of surface water environment in Xi'an Qujiang Pool. *Journal of Xi'an Shiyou University (Social Science Edition)*, 25(04), pp. 1–6. (in Chinese)

Zhou, L.J., Ying, G.G. & Liu, S. (2012). Simultaneous determination of human and veterinary antibiotics in various environmental matrices by rapid resolution liquid chromatography electrospray ionization tandem mass spectrometry. *Journal of Chromatography A*, 1244, pp. 123–138. DOI:10.1016/j.chroma.2012.04.076