Songklanakarin J. Sci. Technol.
40 (5), 1219-1227, Sep. - Oct. 2018

http://www.sjst.psu.ac.th

Original Article

Occurrence and estrogenic risks of endocrine disrupting chemicals in wet and dry seasons of the Nan River, Phitsanulok, Thailand

Saowanee Deemoon[1*], Charoon Sarin[2], Kanokthip Juksu[3], Guang-GuoYing[3], Chanyud Kritsunankul[2], and Sarin Sriprang[4]

[1] *Office of Disease Prevention and Control Region 2, Mueang, Phitsanulok, 65000 Thailand*

[2] *Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Mueang, Phitsanulok, 65000 Thailand*

[3] *State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, GD 510640 China*

[4] *Faculty of Science, Naresuan University, Mueang, Phitsanulok, 65000 Thailand*

Received: 25 February 2017; Revised: 10 June 2017; Accepted: 16 July 2017

Abstract

The occurrence and estrogenic risks of endocrine disrupting chemicals (EDCs) in the Nan River, Phitsanulok were investigated for the first time. EDCs (octylphenol;OP, nonylphenol;NP, bisphenol A;BPA, and estrone;E1) as well as estrogenic activities (EEQs) were determined using gas chromatography-mass spectrometry (GC-MS) and Yeast Estrogen Screen (YES) bioassay. Concentrations of OP, NP, BPA, and E1in water, sediment, and fish were found up to 36.7, 1060, 1420, and 17.2 ng/L, 2.8, 97.3, 13.2, and 12.4 ng/g, and 812, 663000, 339, and 11700 ng/g, respectively. Wet season, EEQs in water, sediment, and fish were found with means of 0.36 ng/L, 0.1 ng/g, and 0.48 ng/g, respectively; while for dry season those were found with means of 0.79 ng/L, 0.87 ng/g, and 0.35 ng/g, respectively. Results of this study showed that the Nan River was contaminated with EDCs and can be harmful to aquatic organisms with high estrogenic risk.

Keywords: estrogenic risks, EDCs, chemical analysis, bioassay, river

1. Introduction

Endocrine disrupting chemicals (EDCs) as emerging pollutants have attracted great attention from scientific community and general public. Some EDCs such as octylphenol (OP), nonylphenol (NP) in detergents, bisphenol-A (BPA) in plastic materials and hormones such as estrone (E1),17 β-estradiol (E2), estriol (E3), and 17 α-ethynylestradiol (EE2) have been widely reported in the aquatic environment (Liu *et al*., 2011; Zhao *et al*., 2009). These chemicals enter waterways as domestic sewage, animal wastes, or industrial wastewater, which eventually reach the aquatic environments. The presence of these chemicals in the aquatic environment could cause adverse effects on normal functioning of the reproductive system of aquatic organisms (Esteban *et al*., 2013; Jackson & Sutton, 2008; Jobling & Tyler, 2003; Manickum & John, 2014; Nie *et al*., 2015; Quinn *et al*., 2004). Variations in concentration levels of EDCs have been reported in different rivers in different seasons due to different source inputs and flow conditions (Wang *et al*., 2011; Yu, Wu, & Chang, 2013; Zhao *et al*., 2011).

The Nan River is one of the lower northern parts of Thailand and Phitsanulok is the biggest city in the region. The

*Corresponding author
Email address: maewmong13@hotmail.com

Nan River is an important drinking water resource and it is also crucial for local fishery in this region. However, owing to discharge of untreated municipal wastewater from the city, the river may be contaminated with various chemicals including EDCs. Kanokthip (2015) first reported EDCs in wastewater that was later discharged into the Nan River in Phitsanulok. This work reported the concentrations of OP, NP, BPA, and E1 in wastewater were in the ranges of 1.73-384 ng/L, 864-1440 ng/L, 1440-2070 ng/L, and ND-20.0 ng/L, respectively. This report is evidence that the wastewater from municipal area is an important pollution source for the EDCs in the Nan River. However, so far there have been no reports of the EDCs in the Nan River in Thailand. To understand their potential risks to aquatic organisms, it is therefore essential to study the EDCs contamination in the Nan River in different seasons.

The objectives of this study were to determine the DCs and to test estrogenic activity of the Nan River in different seasons using both chemical analysis and Yeast estrogen screen (YES) bioassay and to assess their potential estrogenic risks. The findings from this study can assist further monitoring and control of EDCs in water resources.

2. Material and Methods

2.1 Materials

Purity standard of six target EDCs (OP, NP, BPA, E1, E2, and EE2) were purchased from Supelco, Dr Ehrenstorfer GmbH (Germany), and Riedel-de-Haën (Germany). The compounds such as 4-n-NP, BPA-d16, E1-d4, E2-d4 and EE2-c2 were used to internal standards which were purchased from Dr Ehrenstorfer GmbH (Germany), Supelco (USA), Cambridge Isotope Laboratories (USA) or Riedel-de-Haën (Germany). Pentafluorobenzoyl chloride (PFBOCl, purity >99%) as the derivatization agent was purchased from Aldrich. The reagents such as methanol, ethyl acetate, n-hexane, dichloromethane, toluene, pyridine, and trimethylamine (TEA) in HPLC grade used for sample processing and analysis which were obtained from Merck Corporation. The cartridges, Oasis HLB cartridges (6 cc, 500 mg of sorbent), used for solid phase extraction (SPE) which were purchased from Waters Corporation. Glass fiber filters (GF/F) with pore sizes 0.7 μm were purchased from Whatman (England).

2.2 Study area, sample collection, and preparation

The Nan River flows through the city in Phitsanulok and also receives domestic wastewater from the municipal area. The samples of water and sediment were collected from 12 sampling sites (Figure 1) in both wet and dry seasons. Among 12 sites, 2 sampling sites are located before river flowing through the municipal area (S1 and S2), 5 sampling sites within the municipal area (S3-S7), and 5 sampling sites after the municipal area (S8-S12). Fish samples were collected from 5 sites (S2, S3, S4, S6, and S8) in both wet season and dry season.

Water samples were collected from the river, using amber glass bottles (1 L). After collection, 50 mL of methanol was added into each bottle and 4 M H_2SO_4 were added immediately to adjust pH to 3. After transport to the laboratory, the water samples were filtered by 0.7 μm Whatman GF/F glass fiber membrane. Sediment samples were collected from the river at the same sites and same time using a stainless steel grab sampler, then freeze-dried and stored at -40 °C until analysis (Zhao *et al*., 2009). Fish samples were also collected from the Nan River at the same time. The samples were taken to laboratory for dissection with a scalpel blade. Fish muscles were then homogenized, freeze-dried, and stored at -40 °C until analysis (Yang, Li, Ran, & Chan, 2014).

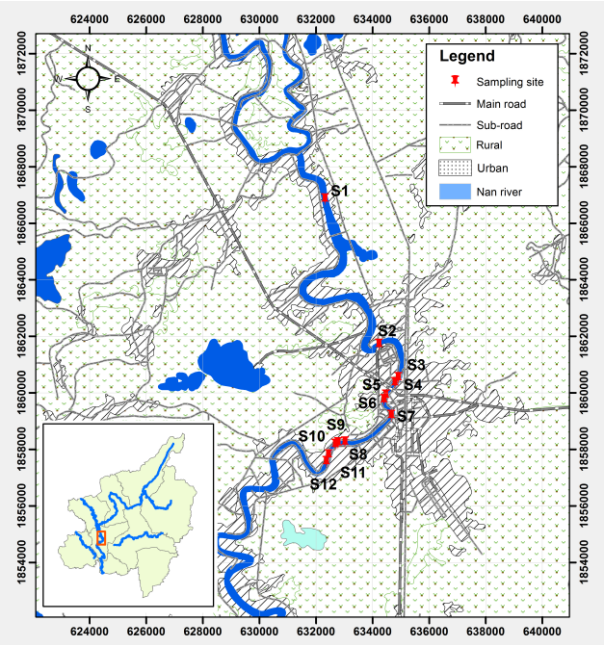


Figure 1. Sampling sites in the Nan River.

2.3 Sample extraction and purification

The procedures for sample extraction and purification based on the method were described by Zhao *et al.* (2009). Briefly, for water samples, three replicate water samples from each site were spiked with 100 μL of internal standards (IS) for chemical analysis (GC-MS), while another two replicates were unspiked for the Yeast Estrogen Screen (YES) bioassay. Solid phase extraction cartridges were conditioned using methanol (10 mL) follow by distilled water (10 mL), respectively. The filtered water samples were passed through the SPE cartridges under vacuum. After, the cartridges were dried. Then 7 mL of methanol and 5 mL of dichloromethane were added for EDCs elution. The extracted eluates were mixed and dried under gentle nitrogen stream and re-dissolved in 1 mL of methanol. The extracts were passed through 0.45 μm membrane filter into a 2 mL amber glass vial and were stored at -18 °C for until analysis.

For sediment samples, two grams of each sediment sample was put into a 20-mL glass vial with PTFE screw cap in five replications. Three replicate samples were spiked with 100 μL of IS for GC-MS analysis, while another two unspiked for YES bioassay. Sonication was used in sediment extraction. Briefly, 10 mL of ethyl acetate was added into each sample, then ultrasonicated for 15 min and centrifuged at 3,500 rpm for 10 min. The supernatant was collected in 100 mL pyriform flasks. The extraction was repeated twice by using ethyl acetate in 10 mL and 5 mL in sequence. SPE cartridges (200 mg of

sorbent) were conditioned by using 10 mL of methanol and 10 mL of distilled water, respectively. The extracts were purified by passing through the cartridges, then 6 mL of n-hexane, 6 mL of ethyl acetate, and 6 ml of methanol were added in sequence. The extracts were evaluated under a gentle nitrogen stream and reconstituted in 1 mL of methanol. The final extracts were filtered using membrane filter 0.45 μm into amber glass vial (2 mL) and were stored at -18 °C for later analysis.

For fish samples, five replicate of fish samples were weighed and each of them put into a 20-mL glass vial with PTFE screw cap, three replicate samples were spiked with 100 μL IS for chemical analysis, while another two unspiked for YES bioassay. 15 mL of CH_3COOH-CH_3COONa buffer was added into the vials, then sonicated for 10 min and centrifuged at 4,500 rpm for 15 min at 20 °C. Then supernatant was transferred into 250 mL flat bottom flask. The extraction was repeated twice. The extracted solution was purified by using tandem 500 mg/500 mg SAX/PSA cartridge with Water Oasis HBL cartridge (200 mg sorbent). The target EDCs were eluted using 2 mL of dichloromethane, 2 mL of ethyl acetate follow by 5 mL of methanol. The extracts were removed by a gentle nitrogen stream then re-dissolved in 1 mL of methanol.

2.4 Derivatization

The step of derivatizaton was based on the previous method (Zhao *et al*., 2009). Briefly, 100 μL of the purified sample was transferred into 10 mL glass tube with screw cap. After the sample was dried under a gentle nitrogen stream, 2 mL of 1 M $NaHCO_3$ solution and 1 mL of 1 M NaOH solution were added into the tube. The solutions were mixed for 10 s, then 2 mL of n-hexane, 50 μL of toluene containing 10% pyridine, and 50 μL of toluene containing 2 % PFBOCl were added respectively. The tube was caped and was shaken violently for 1 min. The supernatant was transferred to a 5 mL glass centrifugal tube after allowed at the room temperature. Then 2 mL of n-hexane was added into the tube again and manually shaken for 1 min. The supernatant was transferred and combined with the previous one in the tube. The solution was dried under a gentle nitrogen stream, next 100 μL of n-hexane was added. The final solution was transferred into a 250 μL flat bottom which put in 2 mL amber glass vial.

2.5 Chemical analysis and YES bioassay

The step of EDCs quantification based on a previous method (Zhao *et al*., 2009). Gas chromatography-mass spectrometry (Agilent gas chromatograph 6890N (USA) joined to Agilent 5975B mass spectrometer with a chemical ionization source) was used. The carrier gas, helium was maintained at a constant flow rate of 1.0 mL/min for separation of the chemicals with HP-5MS GC capillary column (30 m × 0.25 mm, 0.25μm film thickness). For analysis target EDCs, 2 μL of sample was injected in the splitless mode at an inlet temperature of 300 °C. The column temperature was programmed as follows: from 80 °C for 1 min, 10 °C/min to 220 °C for first ramp, 4 °C/min to 260 °C for second ramp, 5 °C/min to 300 °C for third ramp, then to the temperature 310 °C at 20 °C/min and held at 310 °C for 15 min. The MS interface temperature was maintained at 310 °C.

Estrogenic activities of the samples were determined by using YES bioassay according to a previous method (Zhao *et al*., 2011). Briefly, the samples were diluted by 2-fold dilutions in a series on a row of 96-well plate. Then diluted samples (10 μL in ethanol) were transferred to a 96-well plate. After the solvent was dried, 200 μL of yeast solution in growth media and red-β-D-galactopyranoside (CPRG) were added to each well. The plates were packed using foil and were incubated at 32 °C. The plates were shaken for 2 min at 24 hr and 72 hr during the incubation. After incubating for 72 hrs, an absorbance at 540 nm and 620 nm were read by using a plate reader. The estrogenic activity of the samples was measured by YES and expressed as estradiol equivalent (EEQ).

2.6 Risk assessment

The estrogenic risk to aquatic organisms was judged by risk quotient (RQ), which was measured by the ratio between the EEQ and the predicted no effect concentration (PNEC). In this study calculated the PNEC of 1.5 ng/L was used. The risk assessment was followed by the ranking criteria: RQ lower than 0.1(RQ<0.1) means minimal risk, RQ higher or equal 0.1 but lower than 1 ($0.1 \leq RQ<1$) means median risk, and RQ higher than 1 (RQ > 1) means high risk (Zhao et al., 2011).

2.7 Statistical analysis

To analyze the quantity of EDCs detected at each sampling site, the data was collected using the mean. The statistic method of t-test was used to evaluate the seasonal variation of EDCs between wet season and dry seasons.

3. Results

3.1 Concentrations of EDCs in the Nan River

Among the six target compounds, only four chemicals (OP, NP, BPA, and E1) were detected in the Nan River. OP, NP, BPA, and E1 were found in water from 12 sampling sites in both wet and dry seasons. The concentrations of NP and BPA were in general higher than those for OP and E1. The concentrations of OP, NP, BPA, and E1 in wet season were found in ranges of 0.9-10.0, 244-1060, 43.4-1420, and 1.6-9.7 ng/L, respectively. For dry season, those were found in ranges of 0.8-36.7, 4.7-185, 2.8-150, and ND-17.2 ng/L, respectively (Figure 2).

In sediment, NP, BPA, and E1 were found in both wet and dry seasons while OP was only found in dry season. The concentrations of NP, BPA, and E1 in the wet season were found in ranges of 6.3-57.1, 5.1-7.6, and 1.3-11.3 ng/g, respectively. For dry season, the concentrations of OP, NP, BPA, and E1 were found in ranges of 0.3-2.7, 9.9-97.3 1.8-13.1, and 0.9-12.4 ng/g, respectively (Figure 3).

OP, NP, BPA, and E1 were also detected in the fish in both wet season and dry season. For wet season OP, NP, BPA, and E1 were found in ranges of 12.4-601, 5620-38900, 9.0-51.9, and 5.6-122ng/g, respectively. For dry season OP, NP, BPA, and E1 were found in ranges of 1.4-812, 1830-663000, and 3.3-11700 ng/g, respectively (Figure 4).

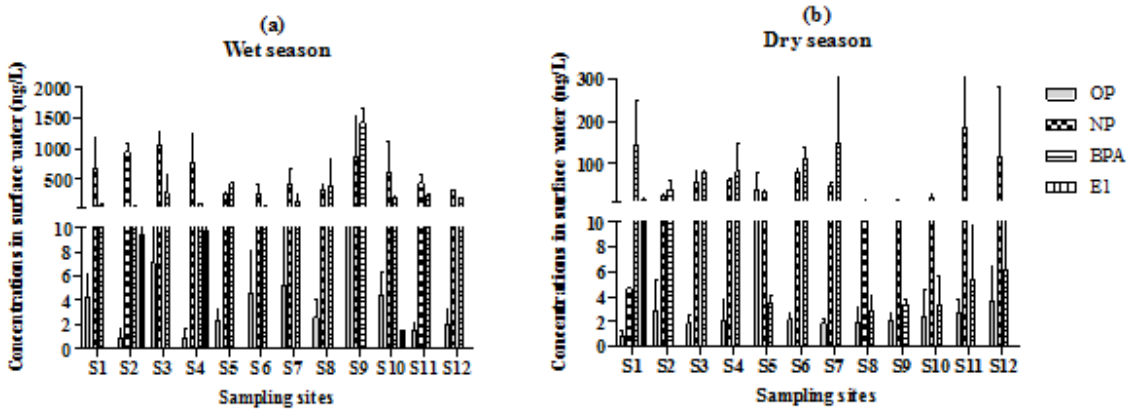


Figure 2. Concentrations of EDCs in water in wet season and dry season from the Nan River.

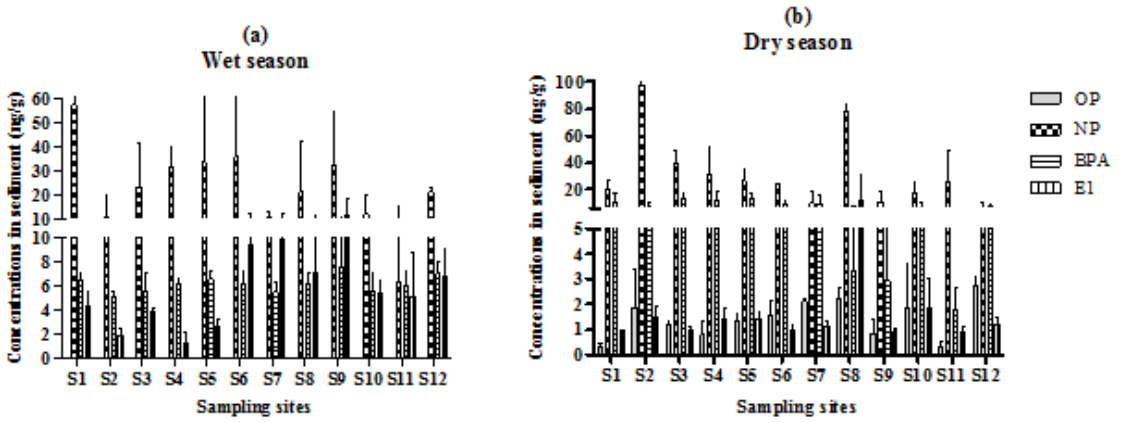
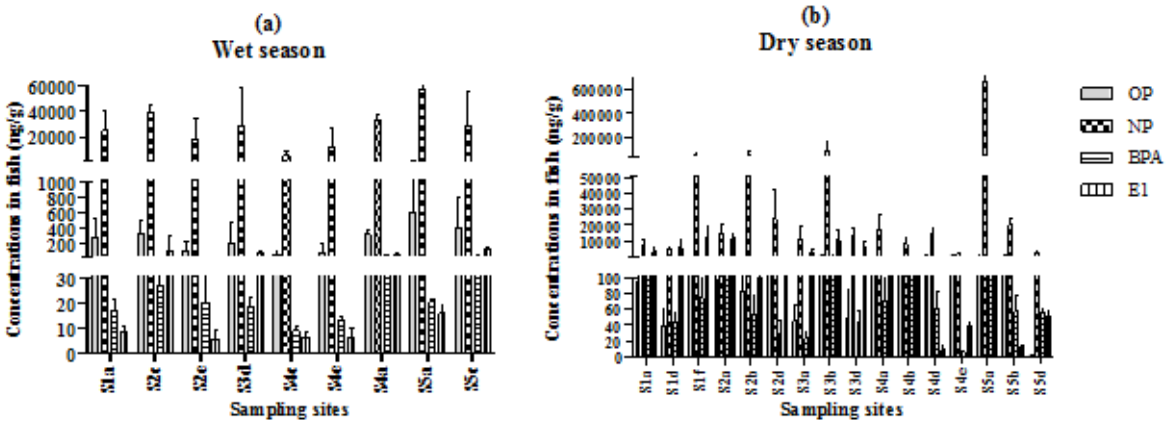


Figure 3. Concentrations of EDCs in sediment in wet season (a) and dry season (b) from the Nan River.



a= *Parachela siamensis*, b= *Parachela oxygastroides*, c= *Barbonymus schwanenfeldii*, d= *Kryptopterus cryptopterus*, e= *Henicorhynchus siamensis*, and f= *Barbonymus altus*.

Figure 4. Concentrations of EDCs in fish in wet season (a) and dry season (b).

Seasonal variation of EDCs in the Nan River, the results showed that in water most of EDCs were higher in wet season than dry season. While in sediment and fish, most of EDCs were higher in dry season than wet season (Figure 5 to 7). The results indicated that for water NP were found significant difference between wet and dry seasons with p value

equal 0.000. For sediment, OP and E1 were found significant difference between two seasons with a p value of 0.000 and 0.012, respectively. For fish, BPA and E1 were found significant difference with a p value of 0.024 and 0.044, respectively.

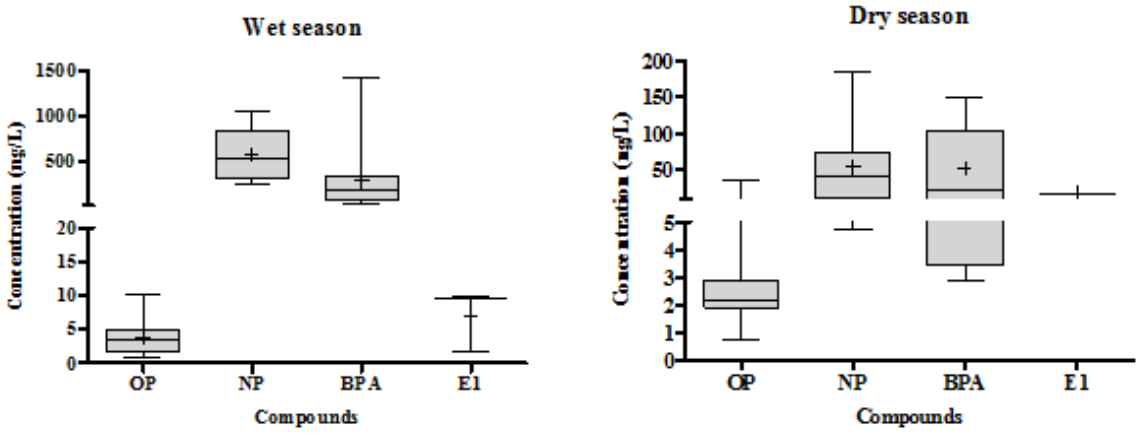


Figure 5. Box plots showing concentrations of EDCs in water in wet season and dry season of the Nan River. The boxes represent 25th and 75th percentiles and horizontal lines represent 5th, 50th, median and 95th percentiles. Median concentrations are displayed as solid horizontal lines. Mean concentrations are displayed as plus symbol.

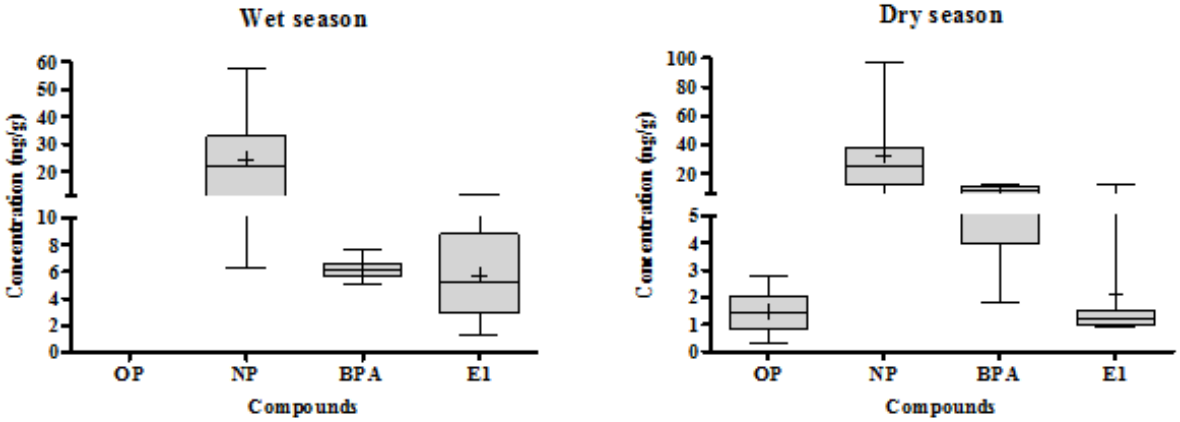


Figure 6. Box plots showing concentrations of EDCs in sediment in wet season and dry season of the Nan River.

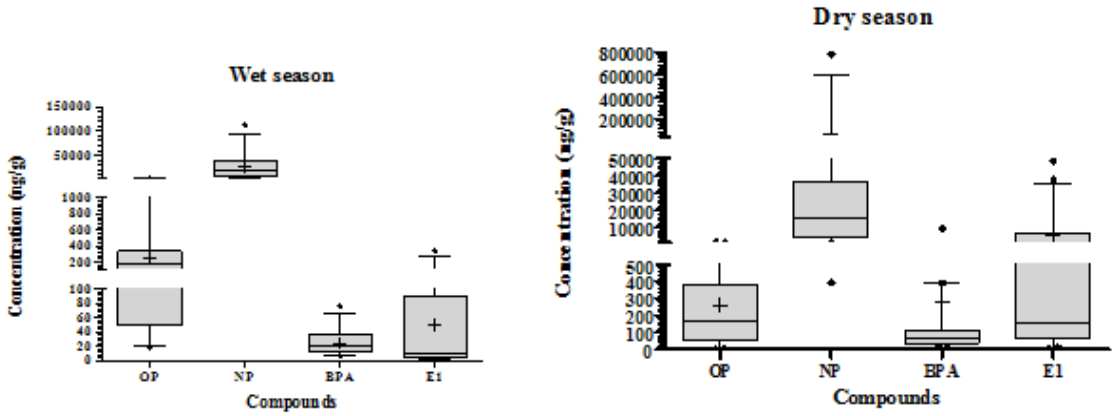


Figure 7. Box plots showing concentrations of EDCs in fish in wet season and dry season of the Nan River.

3.2 Estrogenic activity in the Nan River

The EEQs were measured using YES (Figure 8). In wet season, the mean EEQs in water, sediment, and fish were 0.36 ng/L, 0.1 ng/g, and 0.48 ng/g, respectively and 0.79 ng/L, 0.87 ng/g, and 0.35 ng/g, respectively in dry season. Most of high EEQs were found in sites of municipal area, where they received direct discharge of domestic wastewater from municipal area.

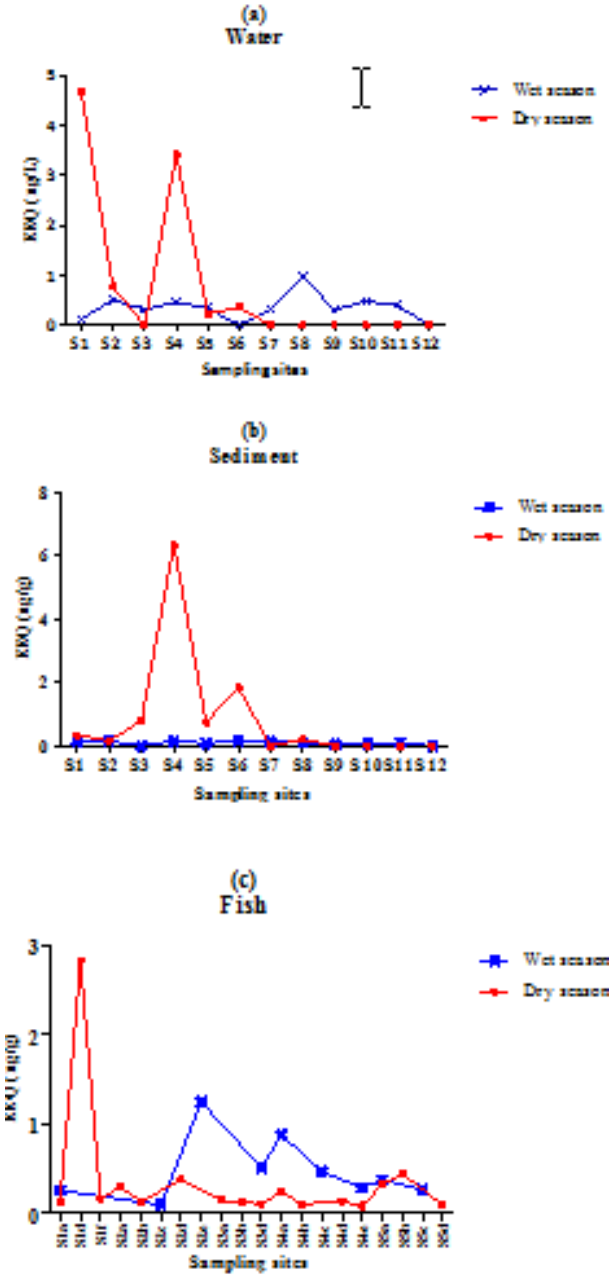


Figure 8. Estrogenic activity (EEQ) by YES bioassay in water (a), sediment (b), and fish (c) in wet season and dry season of the Nan River.

4. Discussion

4.1 EDCs in the Nan River

The presence of EDCs in the Nan River indicates that the river was contaminated with EDCs with major source from municipal wastewater discharged into the river. The detection of OP and NP maybe originated from the use of surfactants in personal care products, detergents, and cleaners from domestic activities. While, BPA is found in the coating of canned foods, water bottles, food storage containers, and baby bottles which can contaminate in wastewater (Jackson & Sutton, 2008). E1 is excreted from animal and human then ending in municipality wastewater. In this study, the levels of EDCs in water were higher than in sediment in both seasons probably due to these chemicals are dissolved in the river (Zhang, Meng, & Zhang, 2014) and water flow affected low sedimentation of EDCs. Moreover, these chemicals were contaminated in low concentrations because of temporary wastewater discharged.

In comparison with other regions (Table 1), we found that most of water samples contaminated with high levels of OP, NP, and E1 especially in USA and China. This is because the Mississippi river and Pearl River (the study sites of USA and China) flow through big cities and receive wastewater treatment plant effluents and other untreated wastewater sources. While EDCs concentrations in Italy were lower than this study, although Italy has many scenery towns consisting of many activities, probably due to the fact that the study site was stagnant water in lagoon which affected EDCs settling onto sediment.

For the sediment samples when compared with other countries, we found that EDCs concentrations in this study were lower than those found in China and Italy because the Pearl River in China flows through the big cities and industrial zones. Therefore Pearl River contaminated with a high level of EDCs in wastewater and contaminated in long time. The surface water in Italy was standing water which affected pollutants in wastewater to settle onto sediment.

For the fish samples, the EDCs concentrations in this study were higher than those found in Netherland, USA, and China. This may due to the different kind of fish and the levels of EDCs concentration in the river.

The different levels of EDCs concentrations were found in fish higher than water and sediment probably due to the chemicals could be taken up from the diet, by inhalation or by absorption through the skin or gills. In addition, they stay in the aquatic environment for a long time and can accumulate these compounds in their life (Ingre-Khans et al., 2017). For EDCs concentrations in water were higher than those in sediment because the water flow resulted in EDCs sedimentation.

The concentrations of each compound were found at different levels of certain media. This results were similar to that of other countries which may due to the physiochemical properties of each chemical. For example, NP is a dominant substance in several media as it is more commonly used in a wide range of household and industrial applications. NP is persistently found in the aquatic environment with half-life at 150 days in water (ECB, 2002) and is of low volatility (Vapor pressures 7-10 Pa). In contrast E1 was found at minimal concentration as the compound is estrogen which has short half-life of 2-3 days in water and sediment and it can be degraded by microorganisms in water.

Table 1. Comparison of the EDCs in water, suspended solid, sediment, and fish samples from different regions of the world.

Location	OP	NP	BPA	E1	Reference
Water (ng/L)					
Pearl River Delta, China	1-12.8	28.1-8890	2.2-1030	ND-75.0	Zhao et al.,2009
Venice Lagoon, Italy	-	< 0.5-211	< 1.0-145.0	<1.2-10	Pojana et al., 2007
Mississippi River, USA.	<10-1200*	<50-14000*	<10-900*	ND-112**	Barberei al., 2015*; Stuart et al., 2005**
The Nan River, Thailand	0.87-6.22 [a] 0.78-11.9 [b]	180-1160 [a] 4.7-185 [b]	43.4-1550 [a] 2.85-111 [b]	ND-9.73 [a] ND-17.2 [b]	This study
Sediment (ng/g)					
Pearl River Delta, China	<LOQ-210.1	107-16198	<LOQ-376.2	ND-7.1	Gong et al.,2011
Venice Lagoon, Italy	-	53000-101000	<2000-99000	-	Pojana et al., 2007
The Nan River, Thailand	ND[a] 0.30-2.25 [b]	8.81-53.6[a] 5.71-97.3[b]	3.61-7.62[a] 1.78-13.2[b]	1.32-11.3[a] 0.91-12.4[b]	This study
Fish (ng/g)					
Scheldt river delta, Netherland	-	-	1-5	-	Belfroid et al., 2002
Kalamazoo River, Michigan, USA	-	<3.3	-	-	Kannan et al., 2003
Dongjiang river, China	15–39	2388–4648	300–1020	17–23	Yang et al.,2014
The Nan River, Thailand	62.4-601[a] 1.41-812[b]	5620-57300[a] 1830-663000[b]	9.04- 51.9[a] 5.97-339[b]	5.62-122[a] 8.71-11700[b]	This study

4.2 Risk assessment

The estrogenic activities of EDCs in aquatic systems indicating they could be harmful to aquatic organisms. The risk of estrogenic activities was displayed by the risk quotient (RQ) and then assessed according to the rank of RQs that can indicate the risk levels to aquatic organisms.

The RQs of water samples from 12 sampling sites in wet season showed minimal risks at 3 sites (S1, S6, and S12) and medium risks at 9 sites (S2-S5 and S7-S11), while in dry season, 7 sampling sites (S3 and S7-S12) showed minimal risks, 3 sites (S2, S5, and S6) showed medium risks, and 2 sites (S1 and S4) showed high risk. The RQs of sediment samples from 12 sampling sites showed medium risks at 8 sites (S3, S5, and S8-S12) and minimal risks at 4 sites (S1, S2, S4, S6, and S7), while in dry season minimal risks were shown at 5 sites (S7 and S9-S12), 5 sites (S1-S3, S5 and S8) were showed medium risks and 2 sites (S4 and S6) were showed high risks (Figure S1). Higher risks were found in wet season than dry season for water phase indicating that the runoff might carry EDCs from the municipal area into the river. However, the highest risks from some sampling sites were found in dry season probably due to the directly contamination from community wastewater.

4.3 Bioaccumulation factors for EDCs in fish samples of the Nan River

EDCs contamination in the river was not only found in water and sediments but in fish also. These chemicals can accumulate in aquatic organisms, which is expressed by the field bioconcentration factors (BCF) (Crookes & Brooke, 2011). The U.S. EPA uses BCF >1000 as the threshold for high concern to bioaccumulation effect. In this study, BCF values of OP, NP, and BPA, and E1 in wet season were in the ranges of 7.69-179, 9.76-70.7, 0.02-0.66, and NA-5.40, respectively. For dry season, BCF values of OP, NP, and BPA, and E1 were in the ranges of 0.28-207, 11.3-36700, 0.00-33.5, and NA, respectively (Figure S2-S3).

BCF values had been reported for OP, NP, BPA, and E1 in carp of Pearl River, China with reported ranges of 482-12960, 131-11137, 1137-14178, and 13280-39623 respectively (Yang, Ran, & Chan, 2014). The study in UK found BCF values of OP in rainbow fish to be 94-68800 (Ferreira-Leach & Hill, 2001) and NP in roach 13-34100 (Smith & Hill, 2004). These data showed that the BCF found in China and UK were higher than in this study probably due to the different fish habitat and species as well as environmental contamination of EDCs (Yang, Ran, & Chan, 2014).

5. Conclusions

This study determined EDCs (OP, NP, BPA, and E1) concentrations and their estrogenic activities (EEQs) in water, sediment, and fish from the Nan River using chemical analysis and YES bioassay. For water, the EDCs concentrations in the wet season were higher than dry season with the concentrations of NP and BPA higher than those for OP and E1. NP was dominant component among these compounds. In sediment, the concentrations of OP, NP, and BPA in dry season were higher than those in wet season, while OP not detected in wet season. Minimal to medium estrogenic risks to aquatic organisms were mostly found in the Nan River, while high risks were found in dry season. The bioaccumulation of NP in fish was the highest among all chemicals but lower than those in China and UK. To protect the aquatic environment, onsite treatment system for

commercial accommodations and central wastewater treatment plant for community is necessary before discharge into the aquatic environments. The monitoring of the level of EDCs in water sources should be taken to surveillance levels of contaminants in source waters. Further studies should be carried out for investigating the adverse effects of these EDCs in aquatic organisms.

Acknowledgements

The authors would like to thank the National Research Council of Thailand (NRCT) and Faculty of Agriculture Natural Resources and Environment, Naresuan University for the financial support. We also appreciate State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, for laboratory supporting, including materials, chemicals, chemical analyses, and bioassays.

References

Barber, L. B., Loyo-Rosales, J. E., Rice, C. P., Minarik, T. A., & Oskouie, A. K. (2015). Endocrine disrupting alkylphenolic chemicals and other contaminants in wastewater treatment plant effluents, urban streams, and fish in the Great Lakes and Upper Mississippi River Regions. *Science of Total Environment, 517*, 195-206. doi:10.1016/j.scitotenv.2015.02.035

Belfroid, A., van Velzen, M., van der Horst, B., & Vethaak, D. (2002). Occurrence of bisphenol A in surface water and uptake in fish: evaluation of field measurements. *Chemosphere, 49*, 97-103. doi:10.1016/S0045-6535(02)00157-1

Esteban, S., Gorga, M., Petrovic, M., González-Alonso, S., Barceló, D., & Valcárcel, Y. (2014). Analysis and occurrence of endocrine-disrupting compounds and estrogenic activity in the surface waters of Central Spain. *Science of Total Environment*, 939-951. doi:10.1016/j.scitotenv.2013.07.101

Ferreira-Leach, A. M. R., & Hill, E. M. (2001). Bioconcentration and distribution of 4-tert-octylphenol residues in tissues of the rainbow trout (*Oncorhynchus mykiss*). *Marine Environmental Research, 51*, 75-89. doi:10.1016/S0141-1136(00)00256-7

Gong, J., Ran, Y., Chen, D. Y., & Yang, Y. (2011). Occurrence of endocrine-disrupting chemicals in riverine sediments from the Pearl River Delta, China. *Marine Pollution* Bulletin, 63, 556-563. doi:10.1016/j.marpolbul.2011.01.026

Ingre-Khans, E., Ågerstrand, M., & Rudén, C. (2017, March 31). Endocrine disrupting chemicals in the marine environment. Retrieved from http://balticeye.org/globalassets/fokusomraden/farliga-amnen/edcs-in-the-marine-environment-report.pdf

Jackson, J., & Sutton, R. (2008). Sources of endocrine-disrupting chemicals in urban wastewater, Oakland, CA. *Science of Total Environment*, 153-160. doi:10.1016/j.scitotenv.2008.06.033

Jobling, S., & Tyler, C. R. (2003). Endocrine disruption in wild freshwater fish. *Pure and Applied Chemistry, 75*(11–12), 2219-2234. doi:10.1351/ pac200375112219

Juksu, K. (2015). *Contamination of endocrine disrupting chemicals (EDCs) in wastewater from hotels and the Nan River, Mueang district, Phitsanulok, Thailand* (Master thesis, Naresuan University, Phitsanulok, Thailand).

Kannan, K., Keith, T. L., Naylor, C. G., Staples, C. A., Snyder, S. A., & Giesy, J. P. (2003). Nonylphenol and Nonylphenol ethoxylates in fish, sediment, and water from the Kalamazoo River, Michigan. *Archives of Environmental Contaminant and Toxicology, 44*, 77-82. doi:10.1007/s00244-002-1267-3

Liu, J., Wang, R., Huang, B., Lin, C., Wang, Y., & Pan, X. (2011). Distribution and bioaccumulation of steroidal and phenolic endocrine disrupting chemicals in wild fish species from Dianchi Lake, China. *Environmental Pollution, 159*, 2815-2822. doi:10.1016/j.envpol.2011.05.013

Manickum, T., & John, W. (2014). Occurrence, fate, and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). *Science of Total Environment, 468–469*, 584–597. doi:10.1016/j.scitotenv.2013.08.041

Nie, M., Yan, C., Dong, W., Liu, M., Zhou, J., & Yang, Y. (2015). Occurrence, distribution and risk assessment of estrogens in surface water, suspended particulate matter, and sediments of the Yangtze Estuary. *Chemosphere, 127*, 109-116. doi:10.1016/j.chemosphere.2015.01.021

Pojana, G., Gomiero, A., Jonkers, N., & Marcomini, A. (2007). Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. *Environment International, 33*, 929-936. doi:10.1016/ j.envint.2007.05.003

Quinn, B., Gagné, F., Costello, M., McKenzie, C., Wilson, J., & Mothersill, C. (2004). The endocrine disrupting effect of municipal effluent on the zebra mussel (*Dreissena polymorpha*). *Aquatic Toxicology, 66*(3), 279-292. doi:10.1016/j.aquatox.2003.10.007

Smith, M. D., & Hill, E. M. (2004). Uptake and metabolism of technical nonylphenol and its brominated analogues in the roach (*Rutilus rutilus*). *Aquatic Toxicology, 69*, 359-370. doi:10.1016/j.aquatox.2004.06.006

Stuart, J. D., Capulong, C. P., Launer, K. D., & Pan, X. (2005). Analyses of phenolic endocrine disrupting chemicals in marine samples by both gas and liquid chromatography-mass spectrometry. *Journal of Chromatography A, 1079*, 136-145. doi:10.1016/j.chroma.2005.03.075

Wang, L., Ying, G. G., Chen, F., Zhang, L. J., Zhao, J. L., Lai, H. J., . . . Tao, R. (2012). Monitoring of selected estrogenic compounds and estrogenic activity in surface water and sediment of the Yellow River in China using combined chemical and biological tools. *Environmental Pollution, 165*, 241-249. doi:10.1016/j.envpol.2011.10.005

Yang, J., Li, H., Ran, Y., & Chan, K. (2014). Distribution and bioconcentration of endocrine disrupting chemicals in surface water and fish bile of the Pearl River Delta, South China. *Chemosphere, 107*, 439-446. doi:10.1016/j.chemosphere.2014.01.048

Ying, G. G., Kookana, R. S., & Ru, Y. J. (2002) Occurrence and fate of hormone steroids in the environment. *Environment International, 28*, 545- 551.

Yu, Y., Wu, L., & Chang, A. C. (2013). Seasonal variation of endocrine disrupting compounds, pharmaceuticals and personal care products in wastewater treatment plants. *Science of Total Environment, 442*, 310-316. doi:10.1016/j.scitotenv.2012.10.001

Zhang, Y. Z., Meng, W., & Zhang, Y. (2014). Occurrence and partitioning of phenolic Endocrine-Disrupting Chemicals (EDCs) between surface water and sus-pended particulate matter in the North Tai Lake Basin, Eastern China. *Bulletin of Environmental Contaminant Toxicology, 92*, 148-153. doi:10.1007/s00 128-013-1136-y

Zhao, J. L., Ying, G. G., Chen, F., Liu, Y. S., Wang, L., Yang, B., . . . Tao, R. (2011). Estrogenic activity profiles and risks in surface waters and sediments of the Pearl River system in South China assessed by chemical analysis and in vitro bioassay. *Journal of Environmental Monitoring, 13*, 813-821. doi:10.1039/C0EM 00473A

Zhao, J. L., Ying, G. G., Wang, L., Yang, J. F., Yang, X. B., Yang, L. H., & Li, X. (2009). Determination of phenolic endocrine disrupting chemicals and acidic pharmaceuticals in surface water of the Pearl Rivers in South China by gas chromatography–negative chemical ionization–mass spectrometry. *Science of Total Environment, 407*, 962-974. doi:10.1016/j.scitotenv. 2008.09.048