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Occurrence of endocrine disrupting chemicals (EDCs) and estrogenic activity in the Nan River, Phitsanulok, Thailand

Saowanee Deemoon [a], Charoon Sarin [a], Guang-GuoYing [b], Chanyud Kritsunankul [a] and Sarin Sriprang [c]

[a] *Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand*
[b] *State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China*
[c] *Faculty of Chemistry, Naresuan University, Phitsanulok, Thailand*

Abstract

The occurrence of EDCs (OP, NP, BPA, and E1) and estrogenic activities were investigated in the water, suspended solids, sediments, and fish from the Nan River, Phitsanulok, Thailand. The samples were collected from 12 sites along the Nan River, which was divided into 3 zones as upstream, midstream (municipality area) and downstream. Gas chromatography-mass spectrometry (GC-MS) was used to determine EDCs concentrations and yeast estrogen screen (YES) bioassay was used to test for estrogenic activity. The results from this study showed that in water samples, OP, NP, BPA, and E1 were found in ranges of 0.87-10.09 ng/L, 179.63-1,164.41 ng/L, 34.97-1,554.14 ng/L, and ND-9.73 ng/L, respectively. In sediment samples, NP, BPA, and E1 were found in ranges of 5.55-35.60 ng/g, 5.15-7.62 ng/g, and 1.32-11.31 ng/g, respectively, while OP not detected at all sites. In suspended solids samples, OP, NP, BPA, and E1 were found in ranges of 4.04-18.03 ng/L, 225.29-554.90 ng/L, 10.80-22.61 ng/L, and 6.48-22.28 ng/L, respectively. In addition, in fish samples, OP, NP, BPA, and E1 were found in ranges of 62.40-600.98 ng/g, 5,623.75-5,7281.48 ng/g, 9.04-44.12 ng/g, and 5.62-133.82 ng/g, respectively. For the results of estrogenic activities in terms of the EEQ for water, sediment, suspended solids, and fish samples were found in ranges of 0-0.985 ngEEQ/L, 0-0.168 ngEEQ/g, 0-0.391 ngEEQ/L, and 0.087-0.873 ngEEQ/g, respectively. Risk assessment of EDCs present in water, sediments, suspended solids, and fish indicated low to medium risk.

Keywords: occurrence; endocrine disrupting chemicals (EDCs); yeast estrogen screen (YES); estrogenic activity

1. Introduction

Endocrine disrupting chemicals (EDCs) have had considerable interest recently from several researchers (Ying *et al*., 2009; Zhao *et al*., 2011). These compounds can affect the reproductive systems of human and wildlife. EDCs such as steroid hormones, either natural or synthetic (estrone (E1), estradial (E2), and 17-βestradial (EE2)) and alkylphenol (octylphenol (OP), nonylphenol (NP), and bisphenol-A (BPA)) were used in industrial, agriculture and household products (Jeannot *et al*., 2002). The consumption of these chemicals leads to EDCs contamination of the environment. The main source of these compounds is municipal effluents of treated and untreated domestic or industrial wastewater discharged into the natural water (Wang *et al*., 2012; Gong *et al*., 2011).

Owing to the awareness of the effect of EDCs on aquatic organisms, investigation of these chemicals in surface water, sediments and fish have been followed out in many regions of the world. Detection of EDCs has revealed both quantity and their estrogenic activities. Gas chromatography–mass spectrometry (GC–MS) is widely used to quantify EDCs and the yeast estrogen screen (YES) is used to measure estrogenic activity in the environment (Zhao *et al*., 2011; Yu *et al*., 2011; Wang *et al*., 2012).

The studies of ECDs in the Nan River were various which have displayed different concentration and the effects of these chemicals. However, little is known about EDCs contamination and estrogenic activity in Thailand.

The Nan River is one of the main rivers in the lower northern part of Thailand which flows through several provinces. Phitsanulok is the biggest city in this zone and the Nan River flows through the city and receives domestic wastewater from the community. Therefore, the investigation of EDCs concentration, their estrogenic activities, and risk assessment in the Nan River are important.

The objectives of this study are to investigate EDCs concentrations and their estrogenic activities in the Nan River by using chemical analysis and YES bioassay. Octylphenol (OP), Nonylphenol (NP), bisphenol A (BPA), and estrone (E1) in water, sediments, suspended solids, and fish were investigated by using GC-MS, while their estrogenic activities were measured by YES.

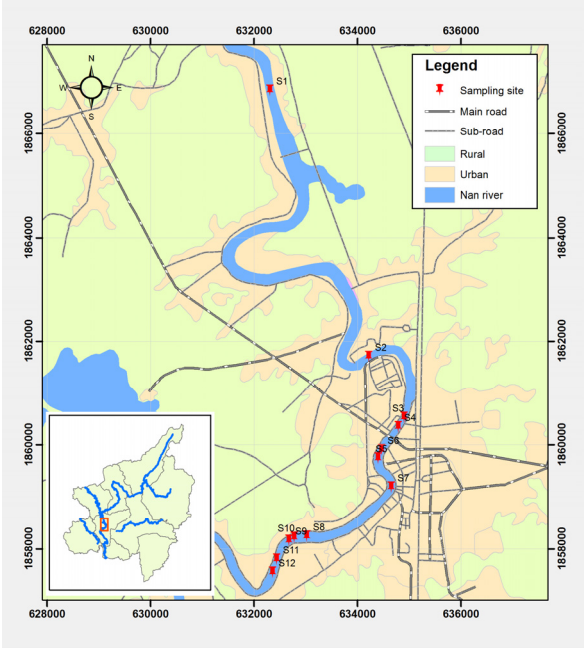

Figure 1. Map of water, sediment, and suspended solids sampling in the Nan River

2. Materials and Methods

2.1. Sampling sites

Water, sediment, suspended solid and fish samples were collected from the Nan River at 12 sites along the river: 2 sampling sites of before flowing through the city (S1 and S2), 5 sampling sites of flowing through the city (S3-S7), and 5 sampling sites of after flowing through city (S8-S12) (Fig. 1).

Fish samples were collected from 5 sites; 1 sampling site before flowing through the city (S1), 3 sampling sites of flowing through the city (S2-S4) and 1 sampling site after flowing through the city (S5) (Fig. 2).

2.2. Sample collection and preparation

The water, sediment, suspended solids, and fish samples were collected in August 2014. Water samples, collected in amber glass bottles (1 L), were taken from 0.5 m below the water surface. Fifty mL of methanol was added into each bottle and the pH was adjusted to 3 by using 4 M H_2SO_4 to preserve the samples. Water samples were filtered with 47 mm Whatman GF/F glass fiber membrane after that 1 g of sodium azide was added into each water sample. The samples were kept at 4 °C until extraction (Zhao *et al*., 2009).

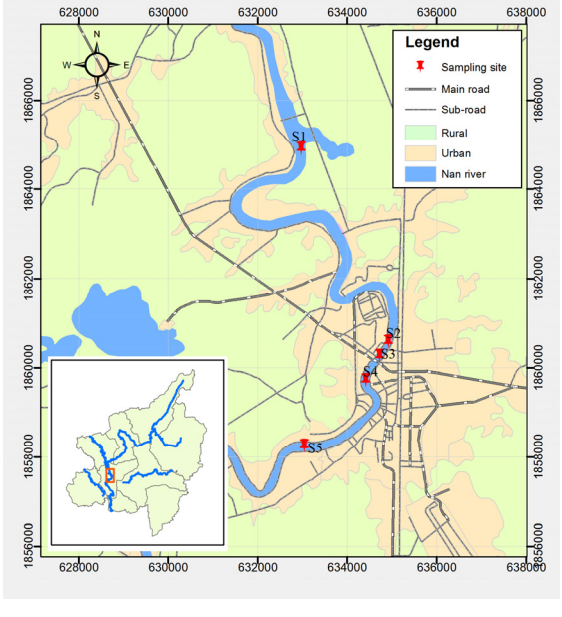

Figure 2. Map of water, sediment, and suspended solids sampling in the Nan River

Sediments samples at a depth of 0 to 20 cm were collected by using a stainless steel grab sampler (Gong *et al*., 2011). The sediment samples were freeze-dried and kept at 4 °C until extraction. Suspended solids samples obtained from filtered water samples then were freeze-dried and kept at 4 °C until extraction. The Cyprinidae fish and Kryptopterus fish were collected from the Nan River. Fish were dissected with a clean scalpel blade to separate the tissue. Muscles were separated for homogenization and freeze drying (Yang *et al*., 2014).

2.3. Samples extraction and purification

Water samples were extracted by using solid phase extraction (SPE). Five replicates of filtered water bottles were prepared for SPE. Three bottles were spiked with 100 µL of the internal standards (IS) for chemical analysis and another two bottles un-spiked for bioassay. Five EDCs, 4-n-NP, BPA-d16, E1-d4, E2-d4 and EE2-d4 were used as internal standards. The water samples were passed through HLB cartridges (Oasis HLB cartridge 6 cc, 500 mg of sorbent) under vacuum. The target chemicals were eluted with 7 mL methanol, and 5 mL dichloromethane, respectively. The mixed solutions were dried under a gentle nitrogen stream and reconstituted in 1 mL of methanol (Zhao *et al*., 2011).

Sediment samples were extracted by ultrasonication. Two grams of each sediment sample was weighed into a 30 mL glass centrifuge tube for five replicates. Three tubes were spiked with 100 µL of IS for chemical analysis while another un-spiked for bioassay. The samples were extracted with 10 mL of ethyl acetate (EA), ultrasonicated for 15 min and centrifuged at 3,500 rpm for 10 min. Supernatants were transferred in 100 mL pyriform flasks. The sediment samples were repeated twice using 10 mL and 5 mL of ethyl acetate (EA). The extracts were purified by passing through HLB cartridges, 200 mg of sorbent and eluted with 6 mL of n-hexane, 6 mL of ethyl acetate, and 6 ml of methanol in sequence. The ethyl acetate phase was dried under gentle nitrogen and reconstituted by using 1 mL of methanol (Gong *et al*., 2011).

Suspended solids samples were extracted and purified the same as sediment samples.

Fish samples were weighed and recorded, three replicate muscle samples were spiked with 100 µL IS for chemical analysis while other two un-spiked replicate samples were used for bioassay. The samples were extracted with 15 mL CH_3COOH-CH_3COONa buffer, ultrasonicated for 10 min and centrifuged at 4500 rpm for 15 min at 20 °C. Supernatant was transferred into a 250 mL flat bottom flask. The samples were extracted twice. The extracts were purified by using tandem 500 mg/500 mg SAX/PSA cartridge with Water Oasis HBL cartridge (200 mg sorbents). The solutions were eluted with 2 mL of dichloromethane, 2 mL of EA and 5 mL of methanol (Merck) in sequence. The combined elutes were dried under a gentle nitrogen stream and reconstituted in 1 mL of methanol.

2.4. Derivatization and chemical analysis

EDCs were derivatized prior GC-MS analysis. The purified samples were derivatized with 2 mL of n-hexane, 50 µL of 10% pyridine in toluene and 50 µL of 2 % PFBOCl in toluene and mixed by manually shaken for 1 min. The supernatants were transferred to the 5 mL glass centrifugal tube and dried under a gentle nitrogen stream. The final extracts were re-dissolved in 100 µL of n-hexane, which transferred to a 2 mL amber glass vial with a 250 µL flat bottomed insert (Zhao *et al*., 2011).

The samples were determined by using an Agilent gas chromatograph 6890N (Agilent, USA) connected to an Agilent 5975B mass spectrometer with a chemical ionization source that supported by the State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Guangzhou, China.

2.5. Yeast estrogen screen bioassay (YES)

The estrogenicity of the samples was determined by using yeast estrogen screen (YES) bioassay. The recombinant Yeast estrogen screen test was supported by the State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Guangzhou, China. The samples were diluted 2-fold in a series on a row of 96 well plate for YES assay analysis. Then diluted samples using 10 µL ethanol were transferred to a 96-well plate. After solvent was dried 200 µL of a mixture of red-β-D-galactopyranoside (CPRG) and yeast solution in growth media were added to each well. The plates were sealed and packed with foil before incubated at 32 °C. After incubating for 72 h, the absorbance at 540 nm and 620 nm were measured using a plate reader. The plates were shaken vigorously for 2 min at 24 and 72 hours incubating. The estrogenic activity of the samples determined by YES assay was expressed as estradiol (E2) equivalent (EEQ) (Zhao *et al*., 2011).

2.6. Risk assessment

The risk of estrogenic activity to aquatic organisms was assessed according to the rank of risk quotient (RQ) which was ratio of measured environmental concentration (MEC) of estrogenic activity in a certain site and the Predicted No Effect Concentration (PNEC) of E2. In this study EEQ was used as the MEC of estrogenic activity and 1.5 ng/L of PNEC was used (Zhao *et al*., 2011). The risk assessment was followed by ranking criteria: RQ lower than 0.1(RQ<0.1) means minimal risk, RQ higher than or equal to 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).

3. Results and Discussion

3.1. Levels of endocrine disrupting chemicals

3.1.1. Water

OP, NP, and BPA were detected in water samples from all sampling sites with concentration ranges of 0.87-10.09 ng/L, 179.63-1,164.41 ng/L, and 34.97-1,554.14 ng/L, respectively, while E1 was detected in water samples from S2 and S3 with a concentration range of non-detected 9.73 ng/L (Fig. 3).

The EDCs were detected at all sampling sites indicating that the contamination of EDCs may have sources from the wastewater of hotels and residential buildings nearby which discharge directly into the Nan River because of the unavailability of wastewater treatment systems. Contaminations of EDCs in water ecosystem have reported in several countries such as South Korea, Australia, China, and USA. In this study, when compared with those countries, the levels of OP were lower than those found in surface water in Australia, China, and USA. In addition, the levels of NP also were lower than those in surface water in USA and China. In other hand, the levels of BPA were higher than those in countries mentioned above while the levels of E1 were lower than those found in those reported (Esteban *et al*., 2014; Boyd *et al*., 2004; Kuch and Ballschmiter, 2001; Zhao *et al*., 2011; Duong *et al*., 2010; Ko *et al*., 2007; Ying *et al.*, 2009).

3.1.2. Sediment

NP, BPA, and E1 were found in sediment samples from the Nan River (Fig. 4). NP was detected with the highest concentration range of 5.55-35.60 ng/g. BPA was found at all sampling sites with a concentration range of 5.15-7.62 ng/g. In addition, E1 was detected in range of 1.32-11.31 ng/g, while OP not detected at any sampling sites.

In this study, EDCs were found at all sampling sites indicating EDCs contaminated into water and then precipitated with organic matter and accumulated in sediment in the river. Based on the analytical data of water and sediment, it was indicated that these chemicals have tendency to adsorb onto sediment due to their moderate hydrophobic character (Wang *et al*., 2012). When compared with other reports, the results from this study showed that the levels of EDCs in sediments were lower than those from China and UK (Zhao *et al*., 2011) probably due to those study area had heavier contaminated than in this study area.

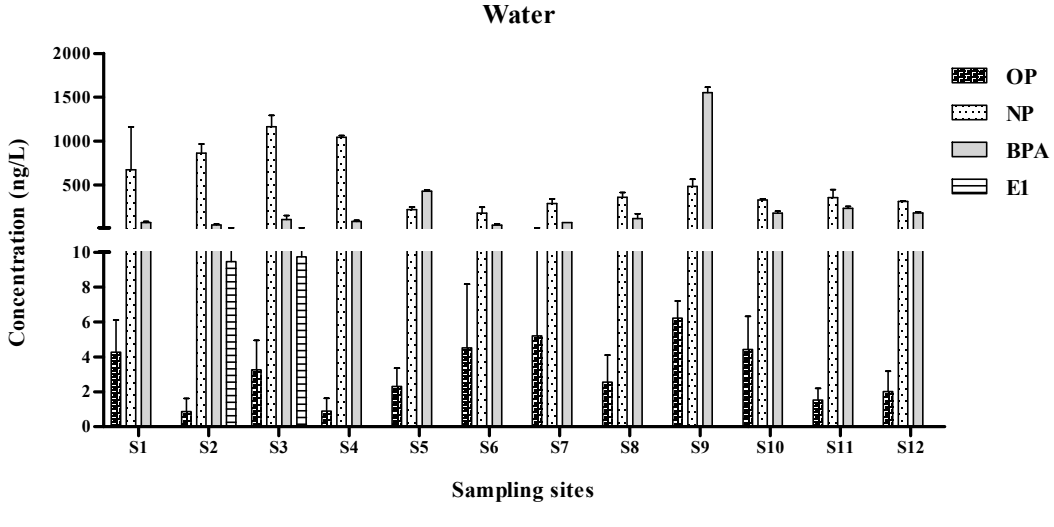

Figure 3. Endocrine disrupting chemical concentrations in water samples from the Nan River

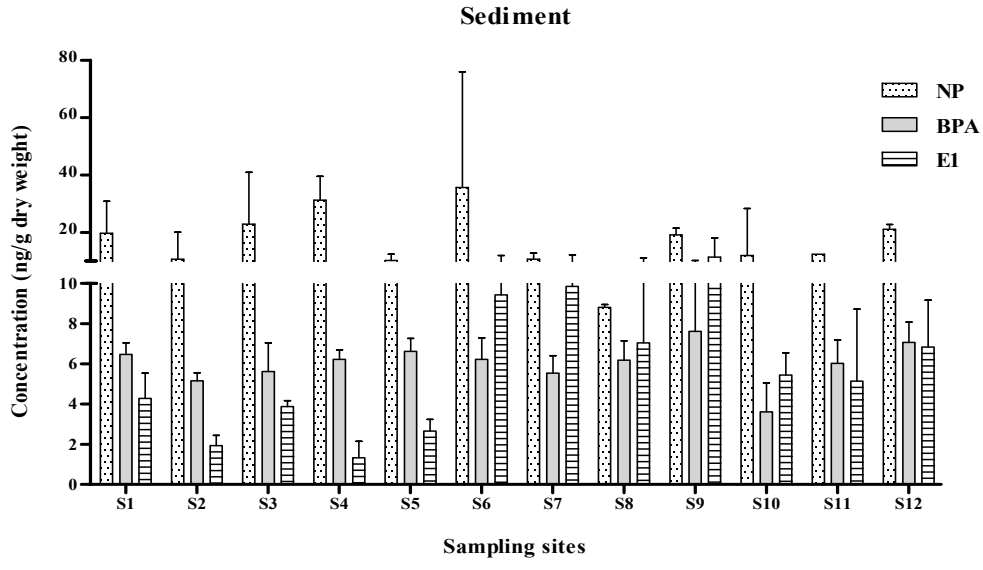

Figure 4. Endocrine disrupting chemical concentrations in sediment samples of the Nan River

3.1.3. Suspended solids

OP, NP, BPA, and E1 were detected at all sampling sites and NP was detected with the highest concentration range of 225.29-554.90 ng/L. OP was found in a range of 4.04-18.03 ng/L, BPA was detected in a range of 10.80-22.61 ng/L, and E1 was found in a range of 6.48-22.28 ng/L, respectively (Fig. 5).

The presence of EDCs in suspended solids at all sampling sites indicated that the Nan River was contaminated with these chemicals as EDCs are hydrophobic matters and become associated with organic matter within suspended solids in the river (Auriol *et al*., 2006). In this study, NP was higher than those found in China, while BPA was lower than those detected in China (Zhang *et al*., 2014).

3.1.4. Fish

OP, NP, BPA, and E1 were detected at all sampling sites with concentration ranges of 62.40-600.98 ng/g, 5,623.75-57,281.48 ng/g, 9.04-51.86 ng/g, and 5.62-133.82 ng/g, respectively (Fig. 6).

OP, NP, BPA, and E1 were detected in fish samples at all sampling sites indicated that these chemicals have entered to aquatic ecosystem of the Nan River and accumulated in fish. Fish in aquatic system exposed with EDCs through their skin and gills or through contaminated food and these fish can be use as bioindicator (Pinto *et al*., 2014) for monitoring of EDCs. In comparison with other regions, the levels of NP in the Nan River were found in same levels in Dongjiang River and Zhujiang River, China. The levels

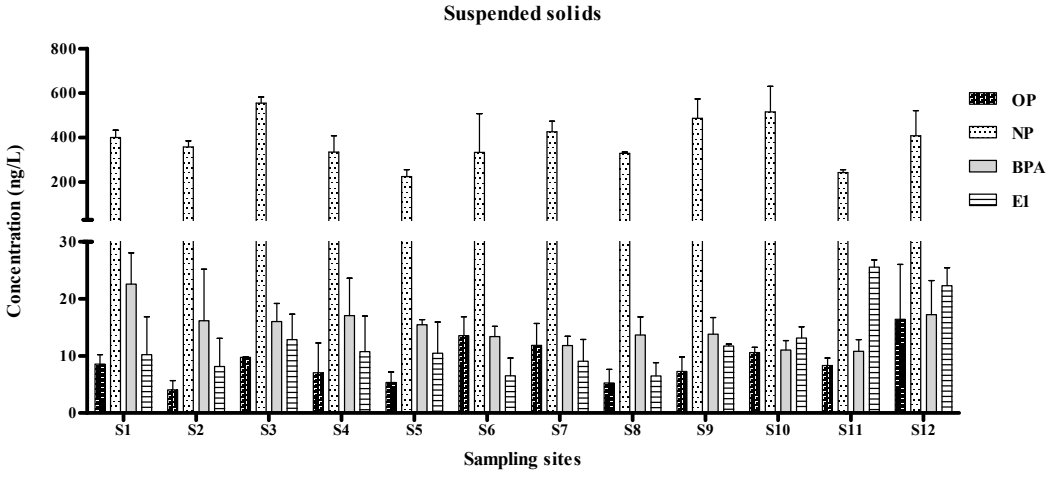

Figure 5. Endocrine disrupting chemical concentrations in suspended solids samples of the Nan River

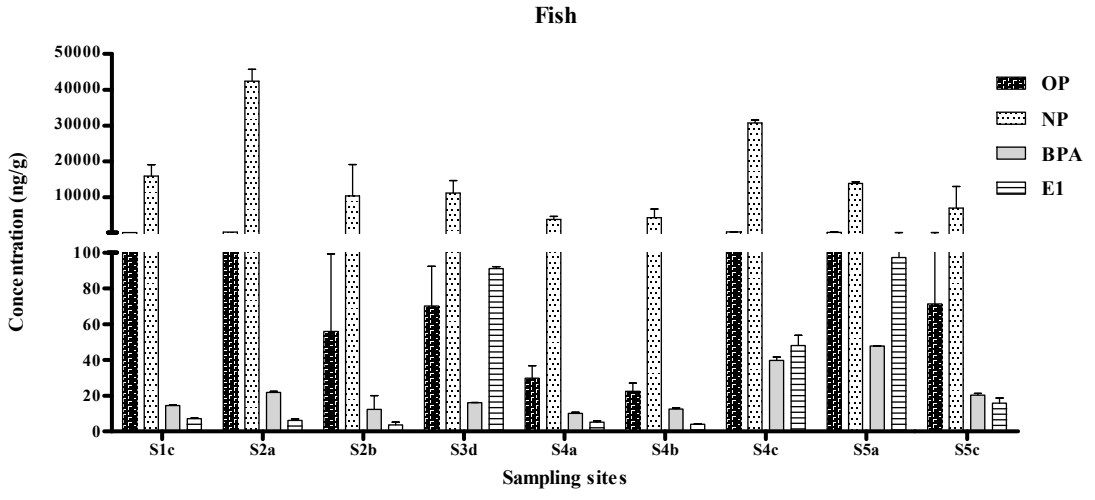

Figure 6. Endocrine disrupting chemical concentrations in fish samples of the Nan River (Fish: a=*Barbonymus schwanenfeldii*; b=*Henicorhynchus siamensis*; c=*Oxygaster anomalura*; d= *Kryptopterus cryptopterus*)

of OP were lower than those found in Spain, and BPA were lower than those detected in China. In addition, E1 in the Nan River were found in low concentration, but higher than those found in China (Yang *et al*., 2014; Navarro *et al*., 2010).

3.2. Estrogenic activity

The total estrogenic activities in the environmental samples were measured by comparing the activity of estrogen (E2) and expressed as estradiol equivalent (EEQ). In this study the results of estrogenic activities showed that EEQ values of water, sediment, suspended solids, and fish samples were ranges of 0-0.98 ng EEQ/L, 0-0.17 ng EEQ/g, 0-0.52 ng EEQ/L, and 0-1.24 ng EEQ/g, respectively (Fig. 7).

The potential of estrogenic activity of the Nan River depends on EDCs concentration levels in studied samples. In this study, the results indicated that weak estrogenic activities were found in water, sediment, suspended solids, and fish samples. However, when compared with the other samples, potential of estrogenic activity in terms of EEQ value was found at highest level in fish samples. This indicates that fish may accumulate substantial concentrations of contaminants in their lifelong. In this study, the EEQ values in the water samples were lower than those found in China, South Korea, Vietnam, Laos, France, and the UK (Zhao *et al*., 2011; Duong *et al*., 2010). The EEQ values in sediment samples were lower than those found from the river in China, UK, and South Korea (Zhao *et al*., 2011; Peck *et al*., 2004; Oh *et al*., 2000). The EEQ values in fish samples in this study were lower than those found in aquatic systems in Norway, Scotland, Portugal, Poland, and the UK (Garcia-Reyero *et al*., 2007; Gibson *et al*., 2005). The low estrogenic activities of the Nan River indicating low contamination of these pollutant sources probably due to the study area is a small city with low population and there is no industrial activity in this area. Moreover, the pollutants were diluted by a large water flow from upstream because the samples were collected during the rainy season.

3.3. Risk assessment

The risk of estrogenic activities to aquatic organism was assessed according to rank of RQs. Based on RQs criteria, RQ lower than 0.1(RQ<0.1) means minimal risk, RQ higher than or equal 0.1 but lower than 1 (0.1 ≤ RQ<1) means medium risk, and RQ higher than 1 (RQ > 1) means high risk. The result from this study indicated that the RQ values of water samples mostly lower than 1 and higher than 0.1 that showed a medium risk to aquatic organism whereas the samples from S1, S6, and S12 were found RQs values lower than 0.1 that showed a low risk. In sediment samples, the RQ values mostly lower than 1 and higher than 0.1 that showed a medium risk to aquatic organisms, while the samples from S3, S9, and S10 were lower than 0.1 that showed a low risk. In suspended solids samples, the RQ values mostly lower than 1 and higher than 0.1 indicated a medium risk to aquatic organisms, while RQ values of samples from S3, S4, and S7 were lower than 0.1 indicated a low risk. For fish samples, the results indicated a medium risk with RQ values between 0.1-1. In this study the estrogenic risks in water and sediment samples were lower than those in the Shijing River, China (Zhao *et al*., 2011).

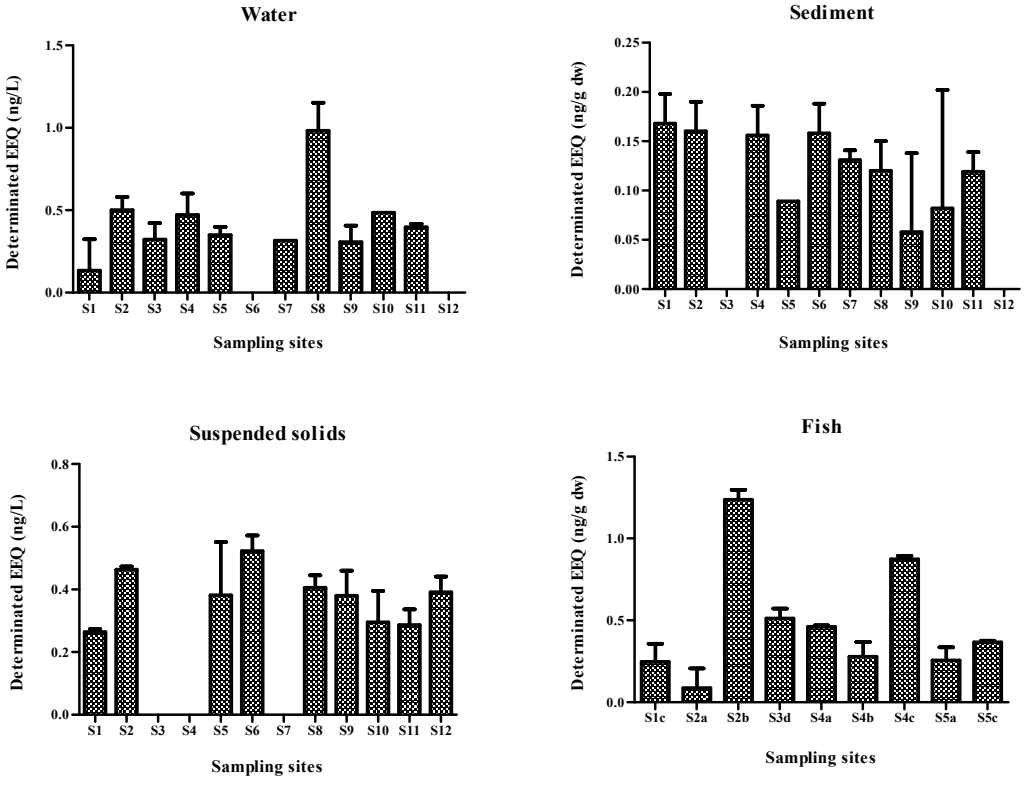

Figure 7. EEQs (estradiol equivalents) of water, sediment, suspended solids, and fish samples in the Nan River

4. Conclusions

The study of occurrence and their estrogenic activity of the four EDCs (OP, NP, BPA, and E1) in the Nan River revealed that NP and BPA were frequently detected at all sampling sites of surface water, sediment, suspended solids and fish samples whereas OP was not detected at all sampling sites of sediment samples. E1, the estrogen was detected at all sampling sites of sediment, suspended solids and fish samples while it was detected at a few sampling sites of water samples. The highest concentration levels of these compounds mostly found in samples from the study site located in municipality area. These indicated that ECDs in the Nan River mainly came from wastewater from municipality area as there is no central wastewater treatment plant in this area. Although EDCs concentration in the Nan River were lower than other regions, but the risk level in terms of RQ of samples in the Nan River were found in medium risk indicating these chemicals could harmful to aquatic organisms in the river system. However, for clear understanding of EDCs, further work need to be performed on determination of the occurrence and estrogenic activity of EDCs in different season to compare with this study.

Acknowledgments

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Correspondence to
Dr. Saowanee Deemoon
Faculty of Agriculture,
Natural Resources and Environment,
Naresuan University, Phitsanulok
Thailand
E-mail: maewmong13@hotmail.com