

(77) DISTRIBUTION OF PCDDs/PCDFs IN SEDIMENTS FROM THE PONG RIVER, KHON KAEN, THAILAND

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Despite increasing concern over dioxins in water environments near pulp and paper mills built in Asian developing countries, little information was made available due to lack of technical and financial resources for HRGC/HRMS analysis. This study was conducted to determine a distribution of PCDDs/PCDFs in sediments from the Pong River using an immunoassay kit. The highest concentration of total PCDDs/PCDFs (130 pg-TEQ/g) was found in a sediment sample collected from the discharge point and the second highest (99 pg-TEQ/g) was found at the land treatment area. Principal congeners of PCDDs/PCDFs analyzed by HRGC/HRMS were 2, 3, 7, 8-TCDD and 2, 3, 7, 8-TCDF in a sediment sample collected from the holding pond of a pulp and paper mill, which indicated PCDDs/PCDFs formation from the bleaching process. Land treatment and the holding ponds of the pulp and paper mill showed effectiveness in reduction of PCDDs/PCDFs in the sediments of the Pong River. The levels of PCDDs/PCDFs detected in all samples was lower than the Japanese Environmental Quality Standard of 150 pg-TEQ/g sediment, but the results suggested that further efforts are necessary to reduce the levels of PCDDs/PCDFs in the Pong River.

Key Words : 2,3,7,8-TCDD, dioxins, ELISAs, immunoassay, pulp and paper industry

1. INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are commonly known as dioxins. They are considered highly toxic contaminant¹⁾ and can be found in soil²⁾, sediment^{3,4,5,6)}, air^{7,8)} and food such as fish and shellfish^{9,10)}, meat and milk¹¹⁾. Dioxins are formed as impurities in chemical manufacturing processes based on chlorophenols and chlorinated aromatic hydrocarbons¹²⁾. They are produced as by-products during incineration, chlorine bleaching in pulp and paper mills^{13, 14)}, metal industry, and wood burning¹⁵⁾. Among these processes, pulp and paper mill is one of dioxins sources that have been received much attention. The EPA's national dioxins source assessment reported that bleached pulp and paper production was ranked 4th overall as a source of dioxins contamination¹⁶⁾.

The Pong River is an important water resource for Khon Kaen Province and the upper northeastern region of Thailand. Not only the river water is used for community and municipal water supply, industry, agriculture and aquaculture but also the river itself is a recipient of wastewater from communities and industries and runoff from agriculture. The major industries situated along the river include a pulp and paper mill, a thermal power plant, a sugar factory, an alcohol distillery, a paper board factory, and tapioca mills.

Impact of pollution sources on water quality of the Pong River has been monitored by local researchers from Khon Kaen University^{17, 18)}. Monitoring results revealed that human activities had an impact on the river water quality¹⁸⁾. For example, heavy metals were found in fish livers collected from the Pong River, indicating heavy metal accumulation in the food chain of the Pong River¹⁹⁾. Phenols were detected in dry season with a

maximum concentration of 0.042 mg/L, suggesting a possibility of chlorophenol contamination from pulp and paper mill and also tapioca industry¹⁸⁾. However, there is no information in regard to PCDDs/PCDFs concentration in sediments from the Pong River though there is a potential input source of PCDDs/PCDFs along the River.

There are several analytical methods of dioxins in environmental matrices. High-resolution gas chromatography/ high resolution mass spectrometry (HRGC/HRMS) is considered as the most reliable and highly quantitative method used to determine dioxins concentration. However, it is time consuming²⁰⁾ due to a complicated sample cleanup. In addition, this method requires expensive equipment and highly trained staff to analyze the samples²¹⁾. To overcome those disadvantages, one of possible alternative methods for detecting dioxins in environmental matrices is enzyme-linked immunoassays (ELISAs). ELISAs were successfully used to detect dioxins in fish^{10, 21)}, human milk²²⁾, sediment and serum samples²³⁾, and fly ash²⁴⁾. Among the ELISAs kit available in the market, High-Performance Dioxin/Furan Immunoassay KitTM manufactured by CAPE Technologies, L.L.C. (South Portland, ME) is the only commercially available immunoassay product accredited by the US EPA for dioxins analysis^{25, 26)}. This kit allows us to screen great many samples for routine monitoring before sending in the samples for quantification by HRGC/HRMS and prior to undertaking of significant remedial measures.

Thus, this study was conducted to determine a distribution of PCDDs/PCDFs in sediments from the Pong River by using an immunoassay as a screening method, and also to evaluate the effectiveness of the land treatment and holding pond systems that are currently used by the pulp and paper mill to reduce loadings of dioxins and other contaminants to the receiving water body.

2. MATERIALS AND METHODS

(1) Field sampling

a) Site description

The origin of the Pong River is at Phu Kradueng in Loei Province. It flows through Phu Kradeung District, Ubonrattana District and Muang District of Khon Kaen Province and then joins with the Chi River and flows through Mahasarakham Province. At Ubonrattana District, Ubonrattana Dam was built across the Pong River to generate electricity and to serve for irrigation in the area. The Pong River is divided into two sections; the first section starts at Ubonrattana Dam and runs 34 km downstream to Nong Wai irrigation weir, and the second section

runs from Nong Wai irrigation weir 180 km downstream to Mahasarakham Dam. Our sampling locations were on the first section where the land use comprises of a large pulp and paper mill, a thermal power plant, some rainfed agricultural areas and villages.

b) Sampling methods

Sediment and soil samples were collected between December 19 and 21, 2005. Sampling locations were chosen to cover potential dioxins source in the Pong River, i.e., the pulp and paper mill (Fig. 1). Treated wastewater of the mill is first stored in holding ponds at G1 and then sprayed to the Eucalyptus fields (G2) to reduce pollutant loadings into the Pong River. Seepage from the land treatment in the Eucalyptus fields is trapped and stored at another holding pond (G3) to prevent the movement of the seepage from the eucalyptus fields into Huay Chod Creek. In rainy season, there is an overflow from this G3 pond down through Huay Chod Creek to the natural holding pond, i.e., Chod Lagoon, which is connected to the Pong River at the discharge point.

At each location, the three individual grab sediment samples were pooled and further treated as one sample except at G2 where 3 soil cores were collected at 0-15 cm depth using soil probe with a diameter of 2.5 inches, pooled and further treated as one sample. Samples were thoroughly mixed with a stainless steel spatula and dried overnight in a fume hood and kept at room temperature prior to analysis.

Sediment samples (D1-D4) were taken from the Chod Lagoon which receives the water from Huay Chod Creek and is a point where the wastewater enters the Pong River. Samples from an upstream of a discharge point (U1) were taken as a control. Samples were also taken from the downstream of a discharge point (DH1-DH2, DN1, and DK1) to evaluate the influence of discharge from pulp and paper mill on dioxins contamination in the Pong River.

(2) Immunoassay of PCDDs/PCDFs

a) Reagents and sample preparation materials

Hexane, acetone, toluene (Kanto Chemical Co. Inc., Tokyo, Japan) and methanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were dioxin-analysis grade. Tetradecane and anhydrous sodium sulfate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sample preparation materials, including acid silica columns, activated carbon mini-columns and hardware for rapid extraction and rapid clean-up, were purchased from CAPE Technologies, L.L.C. (South Portland, ME) and was performed as described in CAPE Technologies Application Note AN-008²⁵⁾.

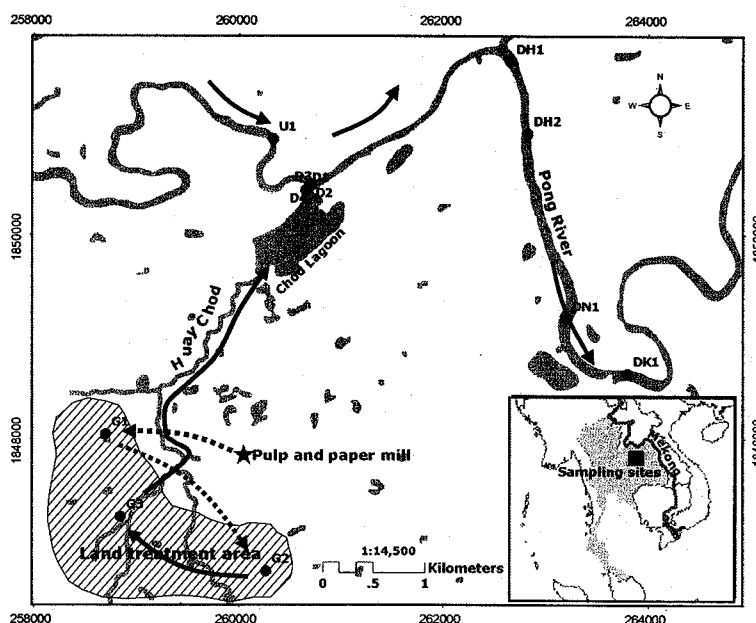


Fig. 1. Sampling locations in the Pong Watershed. Inset: map of Thailand.

b) ELISAs

High-Performance Dioxin/Furan Immunoassay Kit™ was purchased from CAPE Technologies, L.L.C. (South Portland, ME) and used according to the manufacturers recommendations described in CAPE Technologies DF1-Kit Insert IN-DF1²⁵⁾. A sample size for extraction and cleanup in this study was 150 pg/g, which is the Environmental Quality Standard in sediments set by the Ministry of the Environment, Government of Japan²⁷⁾. The evaporation protocol option used was Option B in which 50 uL of methanol was added to residue to dilute for EIA addition.

Absorbances were read with a Pocket Colorimeter II (Hach, Loveland, CO) at 450 nm. QA samples used in this study included spiked and unspiked matrix duplicates, spiked and unspiked evaporation blanks, and spiked and unspiked method blanks. Data from standards and samples obtained from the ELISA were entered into a specially built Microsoft Excel spreadsheet, which performs sigmoid curve fitting and calculates sample concentrations based on the standard curve²⁵⁾.

c) GC/HRMS Analysis

Sample extraction, cleanup and GC/HRMS analysis were conducted by TOWA Sciences Company Ltd., Hiroshima, Japan, following the manual for the analysis of dioxins in sediments²⁸⁾. Briefly, for 4–6 chlorines, GC/HRMS analysis was carried out using a JMS-700 MStation mass spectrometer coupled to a HP-6890 HR-GC with a capillary column of SP-2331

(60 m x 0.25 mm i.d., film thickness 0.2 um) for the isomer specific separation. The GC program was as follows: 100 °C for 1 min hold, raised 20 °C/min to 150 °C and subsequently 3 °C/min to 261 °C, maintained for 12 min, and then raised 3 °C/min to 273 °C, maintained for 1.5 min. Helium was used as carrier gas at the rate of 1 mL/min (constant flow). The injector temperature was 270 °C. The injector volume was 2.0 uL with a split-less injection.

For 8 chlorines, a capillary column of DB-5MS (30 m x 0.25 mm i.d., film thickness 0.25 um) was used. The GC program was as follows: 100 °C for 1 min hold, raised 15 °C/min to 250 °C and subsequently 5 °C/min to 290 °C, maintained for 6 min. Helium was used as carrier gas at the rate of 1 mL/min (constant flow). The injector temperature was 270 °C. The injector volume was 2.0 uL with a split-less injection.

3. RESULTS AND DISCUSSION

(1) Sediment/soil properties

Organic carbon contents ranged between 0.6 and 49.33% (Table 1). Sample G1 was taken from the holding pond used to store treated effluent from the pulp and paper mill before discharging over the land treatment area. Therefore, G1 has extremely high organic carbon content, i.e., 49.33%, due to lignin in the effluent from the pulp and paper mill.

Sediment/soil textures were similar for all sediments/soils (Table 1), which are classified as silty loam, except DH2 is classified as loam.

Table 1. Organic carbon content and soil texture of sediment samples.

Sampling point	Sample ID	%OC	Texture	Total PCDDs/PCDFs Concentration (pg-TEQ/g)
Upstream	U1	0.60	silty loam	36
Land treatment area	G1	49.3	silty loam	99
	G2	2.40	silty loam	94
	G3	2.50	N.A.	77
	D1	0.94	silty loam	80
Discharge point	D2	N.A.	N.A.	89
	D3	1.43	silty loam	130
	D4	4.21	silty loam	82
	DH1	1.25	silty loam	68
Downstream	DH2	1.72	loam	44
	DN1	0.76	silty loam	74
	DK1	0.95	silty loam	45

N.A. = Not Applicable

(2) Concentrations and distributions of total PCDDs/PCDFs in the Pong River

Total PCDDs/PCDFs concentrations for the Pong River ranged from 36 to 130 pg-TEQ/g (Table 1) with a mean value of 76.5 pg-TEQ/g and a median value of 78.5 pg-TEQ/g. Agency for Toxic Substances and Diseases Registry (ATSDR) recommends that if samples are determined to have dioxins TEQ levels between 50 and 1000 pg-TEQ/g, the site should be further evaluated and the action is recommended for levels of greater than 1,000 pg-TEQ/g²⁹⁾. The background level of PCDDs/PCDFs at this site may be affected by pesticide from agricultural areas and villages along the Pong River, and by localized wood burning. However, there is no report on baseline PCDDs/PCDFs levels in the Pong River. Hence, it is worth noting that concentrations of the total PCDDs/PCDFs in sediments from the Pong River were higher than the mean baseline PCDDs/PCDFs levels in Japan³⁰⁾, the US³¹⁾, and in Europe³¹⁾, which are 9.6, 8 and 9 pg-TEQ/g dry mass, respectively.

The environmental quality standards of dioxins in soils and sediments vary from county to country and depend on the criterion involved. For instance, the Canadian Council of Ministers of the Environment (CCME) set a soil quality guideline for residents/parkland of 4 pg-TEQ/g³²⁾. Finland has proposed a guideline of 2 pg I-TEQ/g³³⁾. However, there is no regulatory level of PCDDs/PCDFs in the environment in Thailand. Although PCDDs/PCDFs levels in the Pong River exceeded the regulatory levels in Canada or Finland, no sample in the Pong River exceeded the Japanese Environmental Quality Standard of 150 pg-TEQ/g in sediment²⁷⁾.

The highest concentration of total PCDDs/PCDFs at 130 pg-TEQ/g was collected at D3 near the

discharge point of the Chod Lagoon. The lagoon receives water from Huay Chod Creek and is a point where the water enters the Pong River. Huay Chod Creek is a small stream located close to holding ponds used by the pulp and paper mill to store the effluent (G1) before discharging over the eucalyptus plantation areas (G2). Therefore, there is a possibility that the effluent from holding ponds will seep to Huay Chod Creek or seepage from G3 (a seepage holding pond) will overflow into Huay Chod Creek in rainy season, hence Chod Lagoon plays an important role for the removal of re-suspended sediments before they enter the Pong River via this stream.

Despite a close proximity of sampling points D1 to D4, D3 contained the highest concentration of the total PCDDs/PCDFs among others. Although the exact reason of such variation is not clear at the moment, it was estimated that the complex flow path and sediment matrices might have affected the difference in PCDDs/PCDFs contents.

The sample with the second highest concentration of total PCDDs/PCDFs, 99 TEQ pg/g, was taken at G1 (Table 1), which is the first holding pond used to store treated effluent from the pulp and paper mill before discharging over the eucalyptus plots. It was lower than the sample (D3, 130 pg-TEQ/g) collected at the discharge point of Chod Lagoon. This might be because G1 had a very high organic carbon content (49.3%, Table 1), thus dioxins were tightly bound to the sediment. This result suggested that the extraction method²⁵⁾ we employed in this study might not be strong enough to extract dioxins out from the samples with very high organic carbon contents.

The pulp and paper mill has two kraft pulp lines; one uses elemental chlorine and the other uses

chlorine dioxide to bleach its pulp³⁴). Effluents from the two lines are treated together by activated sludge¹⁷) and then dumped into holding ponds before released over the land treatment area, i.e., eucalyptus plantation plots. Forty percent of paper effluent is reused and 60% is discharged over the land treatment area and to the Pong River¹⁷). The information of the bleaching process of the pulp and paper mill suggested that PCDDs/PCDFs in G1 sample were from a bleaching process of the mill. To further confirm our estimation, we investigated the congener pattern and concentration of each congener by using HRGC/HRMS. Total PCDDs/PCDFs of G1 sample analyzed by HRGC/HRMS was 85 pg-TEQ/g. Congener analysis indicated that concentration of 2, 3, 7, 8-TCDD and 2, 3, 7, 8-TCDF in G1 sample were 27 and 42 pg-TEQ/g, respectively, which were approximately 32% and 50% of the total PCDDs/PCDFs. The rest of the 15 congeners had the concentrations between 0.0002 and 10.5 pg-TEQ/g, which ranged from 0.0002% to 13%. Congeners of dioxins are varied according to their sources e.g., octachlorodibenzo-*p*-dioxin (OCDD) is a principal component of PCDDs/PCDFs from a formulation of pentachlorophenol (PCP)-based wood preservative formulations, diesel emissions, coal combustion, municipal solid waste, or other incineration stack emissions (fly ash, in particular), chimney soot from oil central heating, black-liquor recovery furnace flue gas, scrap wire and car incineration³⁵). Fielder et al. (1994)³⁶) and Rappe et al. (1994)³⁷) reported that 2, 3, 7, 8-TCDD and 2, 3, 7, 8-TCDF were associated with pulp bleaching with elemental chlorine. Another study on the occurrence and fate of PCDDs and PCDFs in five bleached kraft pulp and paper mills by Ammendola et al. (1989)¹⁴) indicated that principal PCDDs/PCDFs found in bleached kraft pulp and paper mill were 2, 3, 7, 8-TCDD and 2, 3, 7, 8-TCDF. Our results were consistent with this report though their percent composition of 2, 3, 7, 8-TCDD and 2, 3, 7, 8-TCDF ranged from 93% to greater than 99%, which were higher than what we found in G1. Based on literature review, it can be concluded that the bleaching process of the mill using elemental chlorine or chlorine derivatives contributed to the increase of PCDDs/PCDFs in the sediments.

Concentrations of the total PCDDs/PCDFs for the sites along the Pong River, above (U1) and below discharge points (DH1-DH2, DN1, DK1), discharge points (D1-D4) and land treatment area (G1-G3) were depicted in Fig. 2. The Shapiro-Wilk test of those data indicated significant fitness to a normal distribution ($p < 0.01$). Statistical analysis using Student's *t*-test was performed to examine the

difference in the mean dioxins concentrations of land treatment areas (G1-G3) and discharge points (D1-D4), and the downstream region, (DH1-DH2, DN1 and DK1). Results indicate that the mean TEQ PCDDs/PCDFs concentration in the downstream section was significantly ($p < 0.01$) less than the mean concentration of discharge and land treatment areas, which suggested that the land treatment combined with holding pond system worked well to retain dioxin-laden re-suspended sediments from entering the Pong River. The TEQ PCDDs/PCDFs concentration at the upstream of the discharge point (U1) was also lower than the land treatment and discharge areas ($p < 0.01$).

The spatial pattern shown in Fig. 2 showed some PCDDs/PCDFs contamination in samples collected from downstream of discharge points were higher than the upstream point (U1). In addition to the current PCDDs/PCDFs loading, the effluent from this mill was released directly into the Pong River³⁸) before 1993, which may be moving downward resulting in some PCDDs/PCDFs detected at the downstream sampling sites.

The results of our study indicated that the immunoassay used in this study was responsive enough to detect a low level contamination of PCDDs/PCDFs in sediments. We found that this bioassay can be an alternative to HRGC/HRMS, and that it is very useful in such developing nations where expensive HRGC/HRMS analysis is not accessible and not economically feasible. However, a confirmation of results from the immunoassay should be conducted by a conventional method such as HRGC/HRMS to precisely quantify the concentration of the total PCDDs/PCDFs.

4. CONCLUSION

Concentrations of PCDDs/PCDFs in the sediment from the Pong River, Khon Kaen Province, Thailand were reported for the first time using a commercial immunoassay method. The highest concentration of total PCDDs/PCDFs were found in sediment samples collected from discharge point (130 pg-TEQ/g) and the second highest (99 pg-TEQ/g) was found at the land treatment area for wastewater from the pulp and paper mill. Principal congeners of PCDDs/PCDFs analyzed by GC/MS were 2, 3, 7, 8-TCDD and 2, 3, 7, 8-TCDF in the sediment samples collected from the effluent holding pond of the pulp and paper mill, which indicated PCDDs/OCDFs formation from the bleaching process. Land treatment and the holding ponds of pulp and paper mill showed effectiveness in reduction of PCDDs/PCDFs in sediments of the Pong River. PCDDs/PCDFs detected in all samples

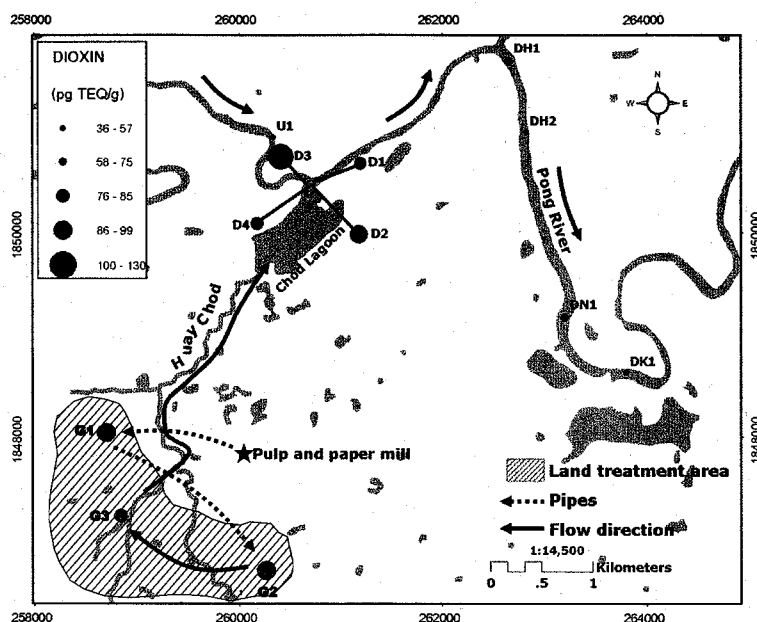


Fig. 2. Spatial pattern of total PCDDs/PCDFs in sediments from the Pong River.

were lower than the Japanese Environmental Quality Standard of 150 pg-TEQ/g in sediment, but samples taken in the land treatment area near the pulp and paper factory contained significantly higher concentration of PCDDs/PCDFs than the sediments in Pong River, which suggested that further efforts to bring them down are necessary.

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