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Determination of Polychlorinated Biphenyl (PCBs) and Dichlorodiphenyltrichloroethane (DDTs) in Sediments in Boeng Cheung Ek, Phnom Penh, Cambodia

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Abstract: In Cambodia, as in many other countries, PCBs and DDTs were used widely in many types of electrical equipment and in agriculture before being banned. They are, however, still being sold and used in Cambodia, and have been found at high levels in dead Irrawaddy dolphins from the Mekong river.

Boeng Cheung Ek is a wetland area south of Phnom Penh that receives all wastewater from the south central part of the city before it flows into the Bassac river. A rapid method was performed to measure PCBs and DDTs in sediments from Boeng Cheung Ek by High-Performance Liquid Chromatography. The results showed that *p,p'*-DDT and *p,p'*-DDE (up to 100 ppb) and possibly some PCBs, were present in the sediment from sites near where the wastewater entered the wetland, but were below detection limits at the site just before the water leaves the wetland and enters the Bassac river. The results show that the wetland is effectively trapping these toxic compounds and protecting local river systems but should cause concern for people who eat fish and shellfish caught in Boeng Cheung Ek.

Key words: PCBs, DDTs, HPLC, wastewater treatment wetlands, Phnom Penh, Cambodia.

Introduction

Polychlorinated Biphenyls (PCBs) and Dichlorodiphenyltrichloroethane (DDT) are synthetic organochlorine compounds that have been widely used around the world in industry and agriculture. Their most striking characteristics are their ability to persist for many years in the environment, transport over long distances and accumulate along food chains. PCBs and DDTs have been linked to chronic human health effects including endocrine disruption, developmental and reproductive problems, suppression of the immune system, neurotoxicity and cancer formation, although there still is debate on the level and mechanisms of impact (Ritter et al., 1995; Turusov et al., 2002; Safe, 2004; Spano et al., 2005; Beard, 2006; Carpenter, 2006). Evidence seems stronger that PCBs, DDT, and other organochlorine pollutants are related to reproductive failures in wildlife populations (Ludwig et al., 1993; Barron et al., 2000; Bowerman et al., 2000; Dykstra et al., 2001). In Cambodia, as in many other countries, PCBs and DDTs were used widely in many types of electrical equipment and in agriculture before being banned. They are, however, still being sold and used locally, and have been found at levels high enough in dead Irrawaddy dolphins from the Mekong river to possibly stress the animals and leave them susceptible to infections and other diseases (Dove, 2009; WWF, 2009). Significant levels of PCBs and DDTs have also been recorded in the breast milk of Cambodian women (Kunisue et al., 2002) and in fish

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and mussels (Monirith et al., 2000). PCBs and DDTs were measured in Mekong river sediments at Can Tho, Vietnam, as 0.9 and 1.9 ppb respectively (Minh et al., 2006).

Due to their lack of solubility in water, PCBs and DDTs usually attach to solid particles after they enter waterways and end up in sediments where they can persist for many years. The half life of DDT in soils is from 2 to 15 years where it is, under favourable conditions, metabolized by microorganisms to DDD (dichlorodiphenyldichloroethane) and DDE (dichlorodiphenyldichloroethylene). Due to their fat solubility both PCBs and DDTs tend to accumulate in fatty tissues of animals throughout food chains (ATSDR, 2002a, 2002b). The WHO recommends a maximum daily intake of DDT not exceeding 20 µg/kg body weight, and 20 ng/kg for PCBs (ATSDR, 1994; IPCS-WHO, 2003).

Boeng Cheung Ek is a wetland area south of Phnom Penh that receives the wastewater from the south central part of the city before it flows into the Bassac river. The city of Phnom Penh has no treatment plant so Boeng Cheung Ek provides the only remediation of wastewater before it enters the river system that eventually flows to Vietnam.

This study was conducted because of the importance of Boeng Cheung Ek in the natural remediation of effluent from Phnom Penh and also due to concerns for the surrounding population that uses the wetland for farming and collection of food (fish, frogs, snails, vegetables) and a lack of previous study of persistent organic pollutants (POPs) in this area. PCBs have been identified in storm water and wastewater throughout the world (Loganathan et al., 1997; Blanchard et al., 2004; Rossi et al., 2004) and if such organic contaminants are accumulating in the natural treatment wetlands sediment, new management options may need to be considered.

Materials and Methods

Collection of Samples

Four composite sediment samples were collected with a van Veen Grab sampler from different sections of the wetland as shown in Figure 1. Sites 1 and 2 were in areas where the wastewater from the city (Trabek and Meanchey sewer canals) enters the wetland. Site 3 was near the centre of the wetland about half-way between where the water enters and leaves the wetland, and site 4 was close to where the wastewater leaves the wetland and enters the Bassac river. Samples were collected on 9th March, 2009 (the dry season) and each was made by combining three grabs about 4-5 m apart and then combining this composite with two others about 60 m apart. Samples were stored in new, pre-rinsed (deionized water, acetone), polyethylene bottles at 4^{o}C until analysis.

Chemicals and Standard Reference Materials

Stock solutions of the standard 1,7,8-trichlorodibenzo-*p*-dioxin (1,7,8-TriCDD) and PCB congener mix containing 41 congeners were purchased from AccuStandard (http://www.accustandard.com/). Potassium hydroxide, concentrated hydrochloric acid, concentrated sulfuric acid (95-97%), dichloromethane, HPLC-grade hexane, silica gel 60 (70-230 mesh), and acetone were obtained from Merck (Germany, http://www.merck.de/en/chemicals/chemicals.html). HPLC-grade pentane and copper turnings were obtained from Cica (Japan, http://www.kanto.co.jp/english/index.html). Basic silica gel (36% w/w potassium hydroxide–impregnated silica gel) and acidic silica gel (40% w/w sulfuric acid–impregnated silica gel) were prepared as described by Lebo et al. (1989); Smith et al. (1984); and Smith et al. (1990). Activated copper was prepared as described by Battelle Duxbury Operations SOP (2001).

Chemical Analysis

PCBs and DDTs were analysed in the Chemistry Department of the Royal University of Phnom Penh (RUPP) according to methods described by Buzitis et al. (2006), but with some slight modifications due to the limitations of local laboratory facilities in Cambodia. PCBs and DDTs were extracted from sediments by sonication with dichloromethane; separation from interfering compounds was done using activated copper and a gravity-flow cleanup column packed with acidic, basic and neutral silica gel eluted with 50:50 hexane:pentane (v/v). Subsequently, individual PCBs and DDTs were resolved by High-Performance Liquid Chromatography with UV-Visible detection (HPLC-UV/Vis). Recovery was tested with 1,7,8-TriCDD standard and found to be better than 95%. Detector response was tested with *p,p'*-DDT and found to be linear ($R^2 = 0.9998$) (see Figures 2 and 3).

The method detection limit was determined for each DDT isomer by repeated (×8) injection at the lowest concentration and found to be on average 3.3 ppb in dry sediment using the formula $MDL = t_{(n-1, a=0.01)} S$ where $t_{(n-1, a=0.01)}$ is the Student's *t* value appropriate for a 99% confidence level given the degrees of freedom $n - 1$ and S is the standard deviation (http://www.omafra.gov.on.ca/english/nm/regs/sampro/sampro03j07.htm#3).

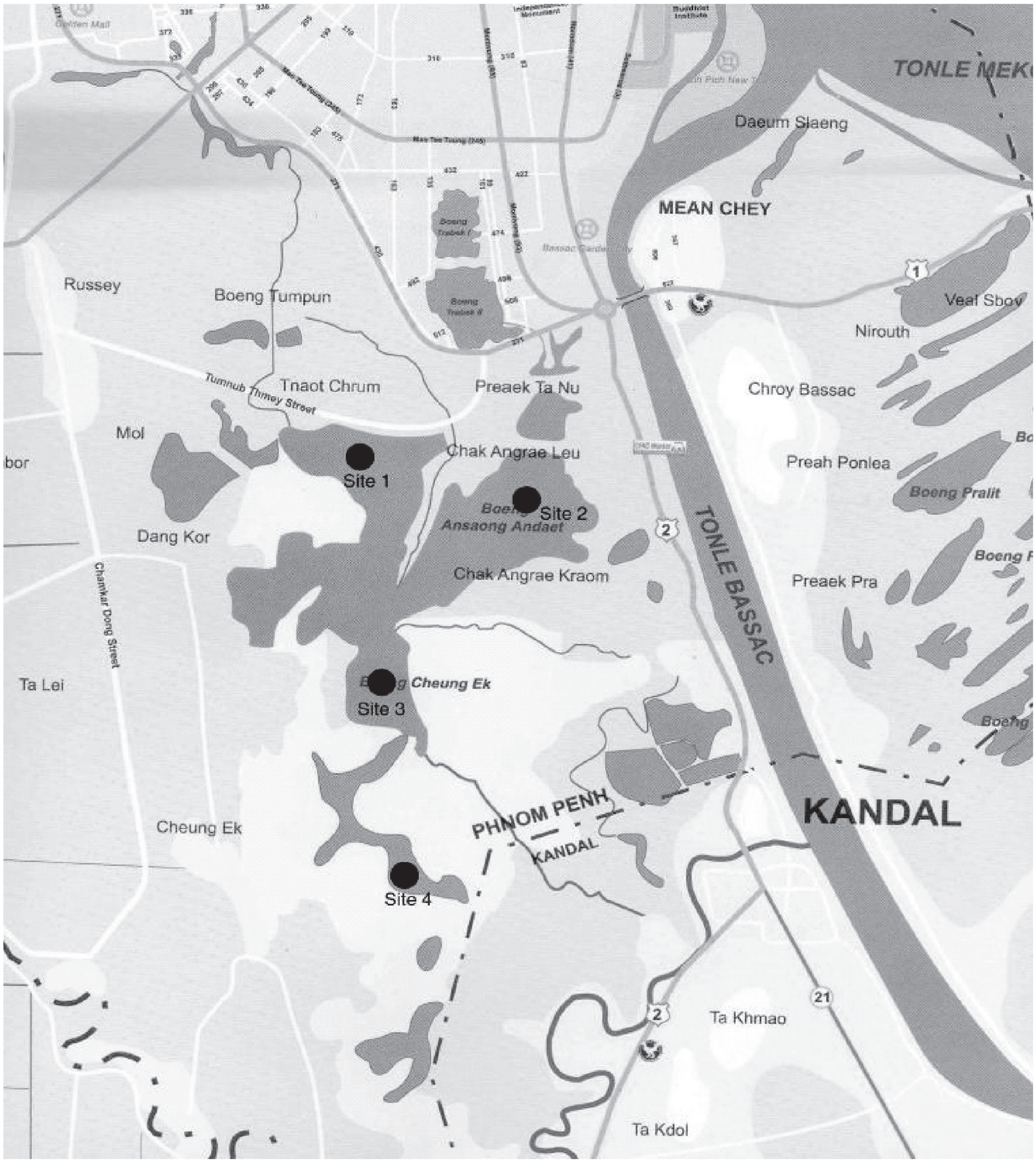

Figure 1: Location map of Boeng Cheung Ek and sample sites.

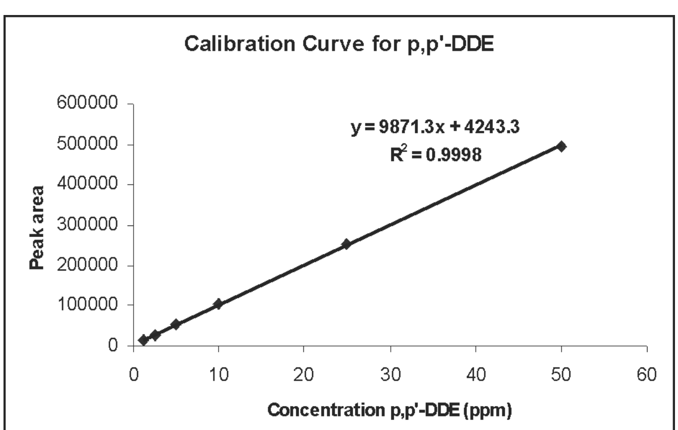

Figure 2: Calibration curve for *p,p'*-DDT.

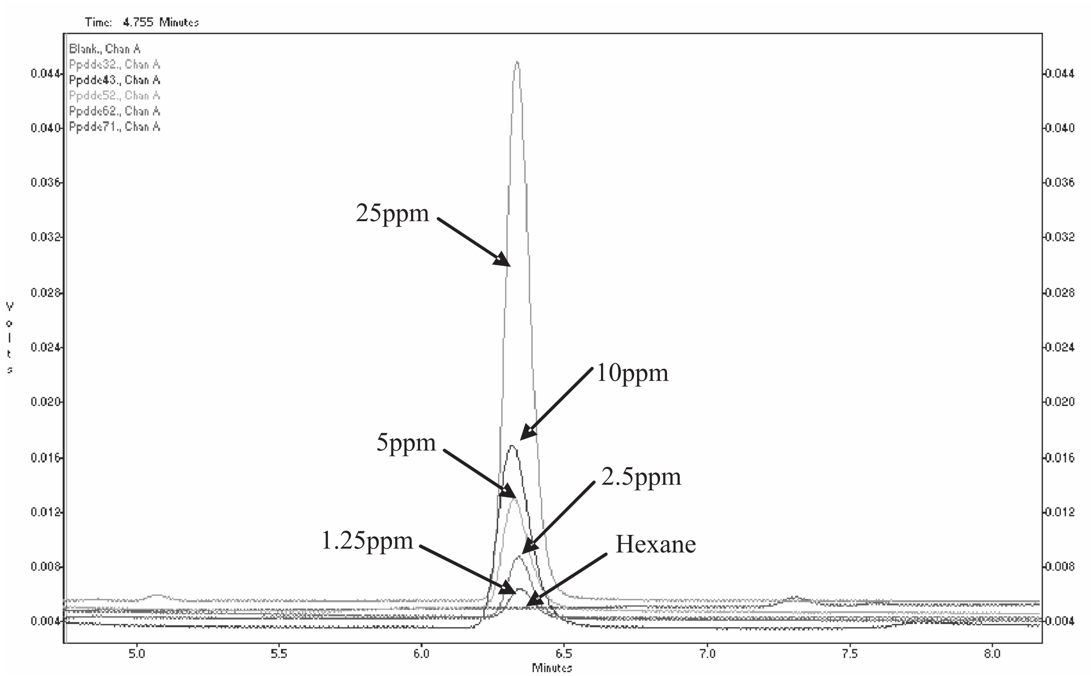

Figure 3: Detector response for *p,p'*-DDE.

Extraction by Sonication

Briefly, 4 grams of dried sediment were transferred to a centrifuge tube and mixed with 20 mL of dichloromethane, 25 grams of anhydrous sodium sulfate and 20 grams of activated copper. The sample was spiked with 20 µl of surrogate standard [1,7,8-TriCDD; 100 ng/µL]. The sample was sonicated for 15 minutes in a water bath and centrifuged for two minutes at 1500 rpm. The dichloromethane layer was transferred to a clean glass concentrator tube. The extraction step was repeated two times, respectively, with 20 and 10 mL of dichloromethane. After combining, the extracts were concentrated to ~1 mL by gentle heating.

Cleanup of Sediment Extracts

The cleanup column was rinsed three times with 50:50 hexane:pentane (v/v) and air-dried before use. The following were added sequentially to each column: a plug of glass wool, neutral silica gel (2.0 g), basic silica gel (2.5 g), and acidic silica gel (15 g). The packed column was washed with 30 mL 50:50 hexane:pentane (v/v) to condition the silica. Each sample extract was quantitatively loaded onto the cleanup column by transferring the extract with a glass Pasteur pipette, rinsing the 100 mL concentrator tube with 1 to 2 mL dichloromethane, and adding the rinsate to the cleanup column. After the entire sample extract was loaded into the acidic silica, 1 to 2 mL 50:50 hexane:pentane (v/v) was used to rinse the inside wall of the cleanup column and then allowed to stand for 15 minutes (stop-flow technique).

The PCB congeners were eluted from the cleanup column with 45 mL 50:50 hexane:pentane (v/v) and collected into a solvent-rinsed 100 mL glass concentrator tube. Activated copper (1 g) and solvent rinsed boiling chips were added to the concentrator tube, and the extract was concentrated to ~1 mL by gentle heating. An HPLC recovery standard (1,7,8-TriCDD; 20 µL; 100 ng/µL) was added to the sample. The sample extract was transferred to a 10 mL glass tube, and the extract was concentrated on a tube heater to ~150 µL and analyzed by HPLC-PDA (High-Performance Liquid Chromatography Photo Diode Array).

HPLC/PDA Analyses

The HPLC system consists of a Shimadzu LC-10AD head pump, a Shimadzu SPD-10A UV/Vis detector, PE Nelson 900 Series Interface, and EzChrom software operating on a Pentium (II) computer. A Cosmosil 5-PYE guard column (4.6 mm × 10 mm; 5 µm particles; Nacalai Tesque, Inc., Kyoto, Japan, http://www.nacalai.co.jp/en/) and two Cosmosil 5-PYE analytical columns (4.6 mm × 250 mm; 5 µm particles) were coupled in series and connected to the PDA detector. The flow rate of the hexane eluent was 1.5 mL/min. throughout the analysis. A column heater/chiller (Jones Chromatography; Model 7955) maintained the temperature of the analytical columns at 9°C. A portion of the sample extract (50 µL;

~1/3 of the extract) was injected onto the columns and repeated in triplicate. Individual standards and mixtures of standards were injected to test for coelution and to determine retention times.

Results and Discussion

While all six DDT congeners were easily identifiable, only 23 of the 41 PCB congeners contained in the standard mixture could be seen (Figures 4 and 5). The other 18 were assumed to coelute.

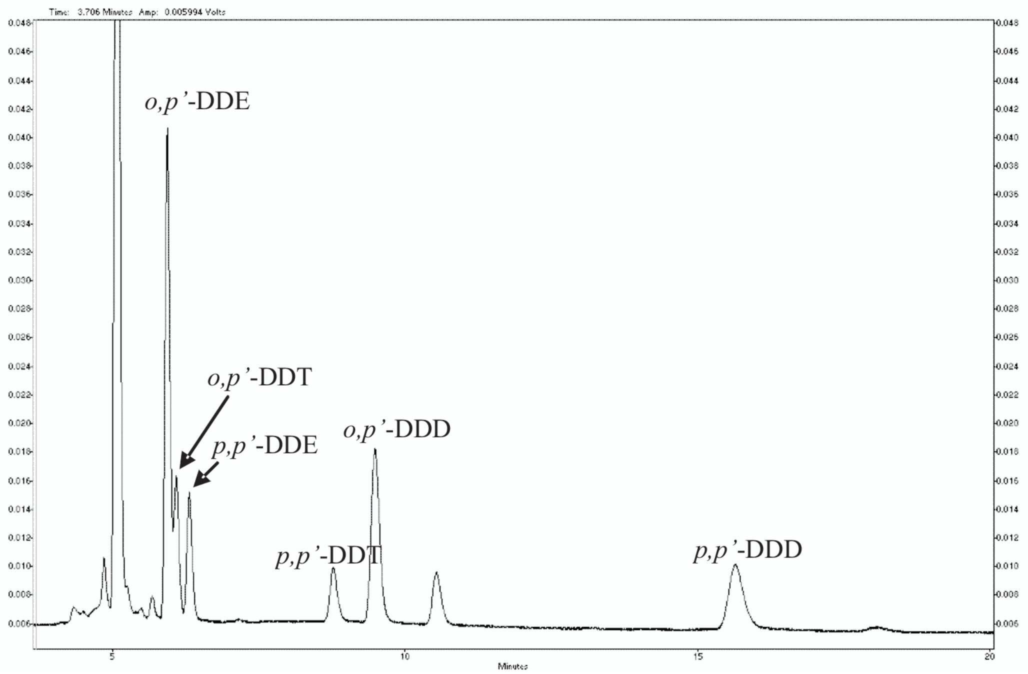

Figure 4: Chromatogram of mixture of standard DDTs.

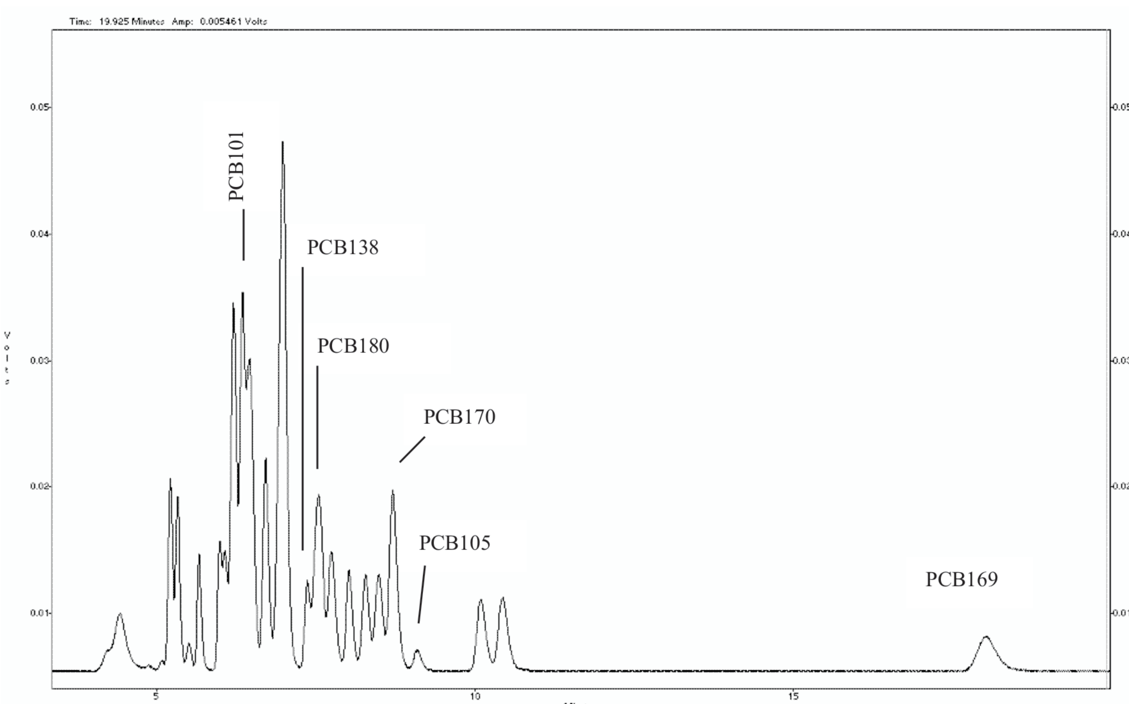

Figure 5: Chromatogram of mixture of PCB standards.

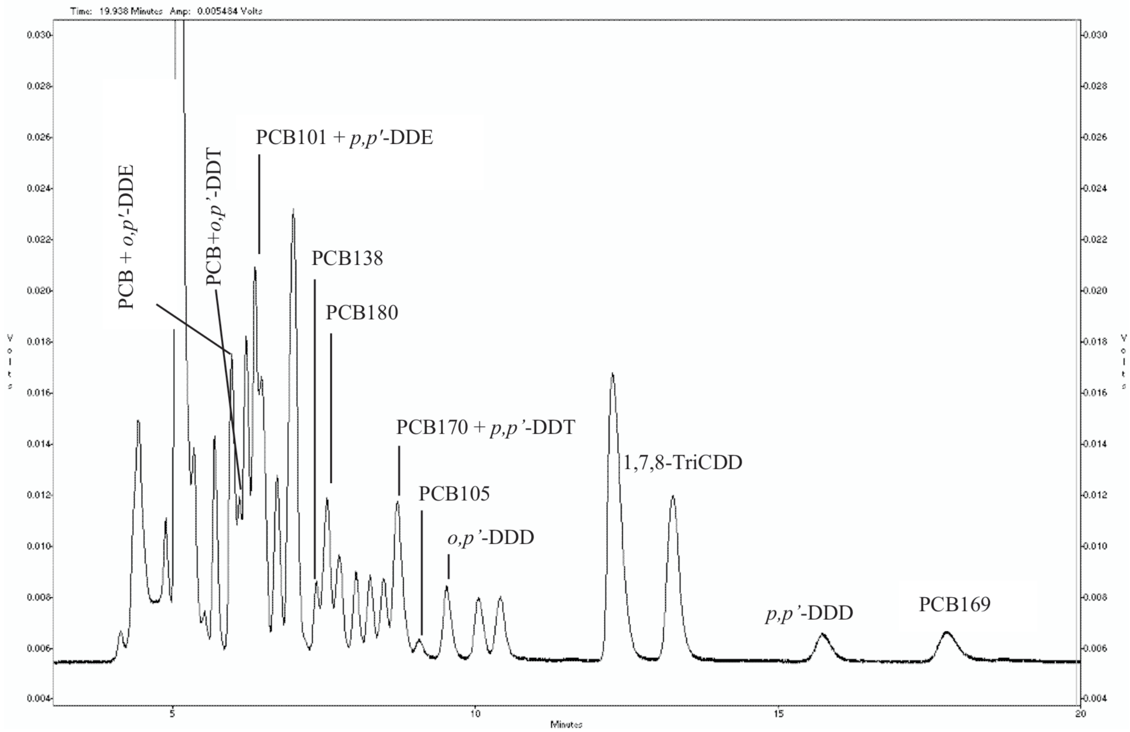

Figure 6: Chromatogram of PCB, DDT and internal standard, 1,7,8-TriCDD.

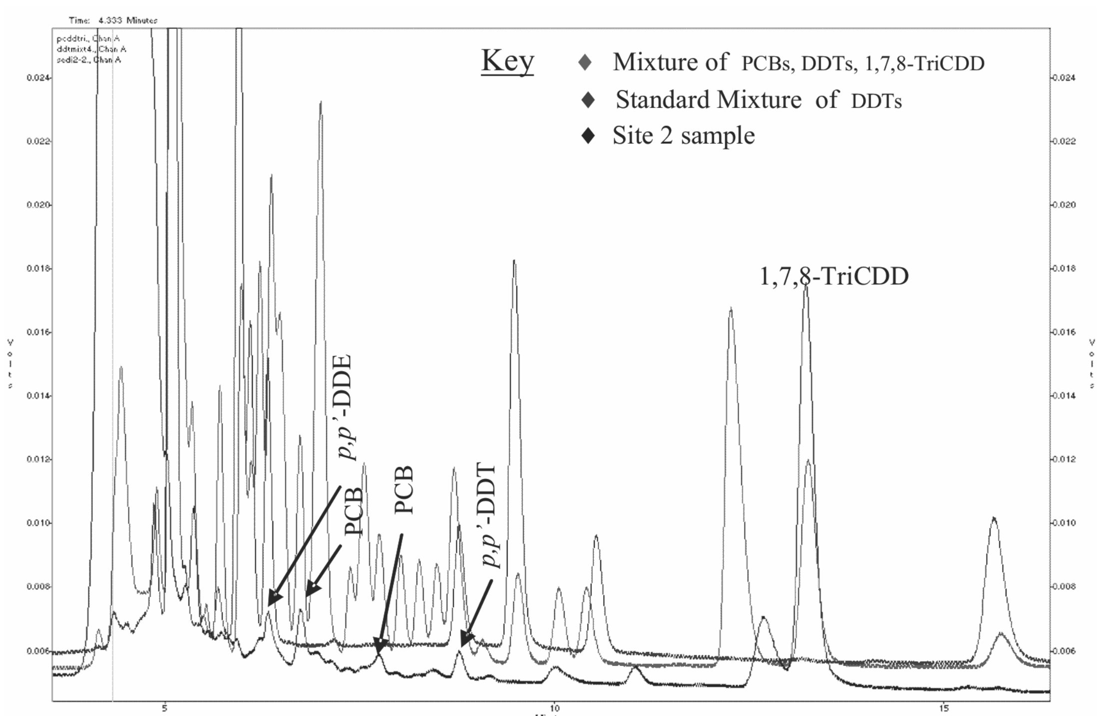

Figure 7: Overlay of chromatograms of PCB, DDT and internal standards, with site 2 sample.

Table 1: Concentrations of *p,p'*-DDT (ng g^{-1} dry wt.) in sediments

Sample	*Injection 1*	*Injection 2*	*Injection 3*	*Average ± SD*
Site 1	48.901	50.153	53.461	51 ± 2
Site 2	25.959	25.900	31.465	28 ± 3
Site 3	63.452	54.069	46.993	55 ± 8
Site 4	ND	ND	ND	ND

ND: Not Detected (<MDL of 3.3 ng g^{-1})

Table 2: Concentrations of total DDTs (ng g^{-1} dry wt.) at each sample site

	Site 1	*Site 2*	*Site 3*	*Site 4*
ΣDDTs ± SD	51 ± 2	100 ± 9	55 ± 8	ND

ND: Not Detected (<MDL of 3.3 ng g^{-1}); ΣDDTs = *p,p'*-DDT and *p,p'*-DDE

A mix of PCB and DDT standards together with the internal standard 1,7,8-TriCDD was also injected and showed that several DDT and PCB congeners coeluted, e.g. *p,p'*-DDE with PCB 101 and *p,p'*-DDT with PCB 170. However some PCB congeners were clearly identifiable; PCBs 101, 138, 180, 170, 105 and 169 (Figure 6).

Samples from each of the four collection sites were prepared together with internal standard and injected into the system three times (Table 1). The chromatogram from site 2 is shown as an example in Figure 7.

Concentrations of *p,p'*-DDE were determined similarly and the total DDT i.e. *p,p'*-DDT and *p,p'*-DDE for each site is shown in Table 2.

The Florida Department of Environmental Protection (USA) (1994) established sediment quality guidelines of 1.2 and 4.8 ppb as threshold effect levels (TELs) and probable effect levels (PELs) for *p,p'*-DDT, respectively. Ingersoll et al. (2000) identified a consensus-based probable effect concentration (PEC) of 62.9 ppb for the sum of *p,p'*-DDT and *o,p'*-DDT in sediment. Samples from sites 1, 2 and 3 exceed Florida's PEL guideline (Table 1); depending on the level of *o,p'*-DDT in the sample, sites 1, 2 and 3 approach, but are less than the PEC suggested by Ingersoll et al. (2000). The *p,p'*-DDT levels at site 4 appear lower than the TEL for Florida or the PEC suggested by Ingersoll et al. (2000).

Conclusion

The present research was an investigation into an important wetland that receives almost all of the wastewater from south central Phnom Penh, the capital city of Cambodia with a population of upto two million people. PCBs and DDTs were imported and used widely throughout Cambodia in the past few decades and so it is likely that significant residues still remain in the environment.

The HPLC method chosen for analysis proved adequate but numerous PCB congeners coeluted with each other and some coeluted with DDT isomers. It was however possible to identify *p,p'*-DDT, *p,p'*-DDE and some PCBs in the sediment samples from Boeng Cheung Ek.

p,p'-DDT was recorded at sites 1, 2 and 3 as 51 ± 2 ng g^{-1}, 28 ± 3 ng g^{-1} and 55 ± 8 ng g^{-1} respectively. *p,p'*-DDE was found to be 72 ± 6 ng g^{-1} at site 2, but both *p,p'*-DDT and *p,p'*- DDE at site 4 were below detection limits. This pattern of occurrence is consistent with the flow and treatment of the wastewater through the wetland as discussed by Irvine et al. (2008) and Visoth et al. (this issue). Sites 1 and 2 are near where the wastewater enters Boeng Cheung Ek, site 3 is in the middle and site 4 is near where the water exits during the dry season. The absence of both POPs at site 4 would appear to indicate that they have been removed from the water column almost completely before the water enters the Bassac river and then the wider Mekong river system. Given the levels of *p,p'*-DDT identified in this preliminary study, additional sampling and analysis of fish, frogs, snails, and vegetables collected from Boeng Cheung Ek should be done.

Acknowledgements

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References

ATSDR (Agency for Toxic Substances and Disease Registry) (2002a). Toxicological profile for DDT, DDE and DDD. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. http://www.atdsr.cdc.gov/ toxprofiles/ tp35.html

ATSDR (Agency for Toxic Substances and Disease Registry) (2000b). Toxicological profile for Polychlorinated Biphenyls (PCBs). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. http:// www.atdsr.cdc.gov/ toxprofiles/tp17.html

ATSDR (Agency for Toxic Substances and Diseases Registry) (1994). Toxicological Profile for 4,4'-DDT, 4,4'-DDE, and 4,4'-DDD. US Public Health Service, Atlanta, GA.

Barron, M.G., Anderson, M.J., Cacela, D., Lipton, J., The, S.J., Hinton, D.E., Zelikoff, J.T., Dikkeboom, A.L., Tillitt, D.E., Holey, M. and N. Denslow (2000). PCBs, liver lesions, and biomarker responses in adult walleye (*Stizostedium vitreum vitreum*) collected from Green Bay, Wisconsin. *Journal of Great Lakes Research,* **26(3):** 250-271.

Battelle Duxbury Operations SOP (2001). Soil/sediment extraction for trace metal semi-volatile organic contaminant analysis. SOP No. 5-192-05, 1-10.

Beard, J. (2006). DDT and human health. *Science of the Total Environment,* **355:** 78-89.

Blanchard, M., Teil, M.J., Ollivon, D., Legenti, L. and M. Chevreuil (2004). Polycyclic aromatic hydrocarbons and polychlorobiphenyls in wastewaters and sewage sludges from the Paris area (France). *Environmental Research,* **95:** 184-197.

Bowerman, W.W., Best, D.A., Grubb, T.G., Sikarskie, J.G. and J.P. Giesy (2000). Assessment of environmental endocrine disruptors in bald eagles of the Great Lakes. *Chemosphere,* **41:** 1569-1574.

Buzitis, J., Ylitalo, G.M. and M.M. Krahn (2006). Rapid method for determination of dioxin-like polychlorinated biphenyls and other congeners in marine sediment using sonic extraction and photodiode array detection. *Arch. Environ. Contam. Toxicol.*, **51:** 337-346.

Carpenter, D.O. (2006). Polychlorinated biphenyls (PCBs): Routes of exposure and effects on human health. *Reviews on Environmental Health*, **21(1):** 1-23.

Dove, V. (2009). Mortality Investigation of the Mekong River Irrawaddy River Dolphin (Orcaella Brevirostris) in Cambodia based on Necropsy Sample Analysis. WWF Technical Report, 72 p.

Dykstra, C.R., Meyer, M.W., Stromborg, K.L., Warnke, D.K., Bowerman, W.W. and D.A. Best (2001). Association of low reproductive rates and high contaminant levels in bald eagles on Green Bay, Lake Michigan. *Journal of Great Lakes Research,* **27(2):** 239-251.

Florida Department of Environmental Protection (1994). Approach to the Assessment of Sediment Quality in Florida Coastal Waters. Volume 1: Development and Evaluation of Sediment Quality Assessment Guidelines. Prepared by MacDonald Environmental Sciences Ltd., accessed at http://www.dep.state.fl.us/waste/quick_topics/publications/pages/default.htm

Ingersoll, C.G., MacDonald, D.D., Wang, N., Crane, J.L., Field, L.J., Haverland, P.S., Kemble, N.E., Lindskoog, R.A., Severn, C. and D.E. Smorong (2000). Prediction of Sediment Toxicity Using Consensus-based Freshwater Sediment Quality Guidelines. EPA Report 905/R-00/007.

IPCS-WHO (2003). Polychlorinated biphenyl: Human health aspects. Concise international chemical assessment document 55.

Irvine, K.N., Sampson, M., Visoth, T., Yim, M., Veasna, K., Koottatep, T. and J. Rupp (2008). Spatial patterns of E. coli and detergents in the Boeng Cheung Ek treatment wetland, Phnom Penh, Cambodia. The 6th International Symposium on Southeast Asia Water Environment, Bandung, Indonesia, pp. 78-81.

Kunisue, T., Watanabe, M., Someya, M., Monirith, I., Minh, T.B., Subramanian, A., Tana, T.S., Viet, P.H., Prudente, M. and S. Tanabe (2002). PCDDs, PCDFs, PCBs and organochlorine insecticides in human breast milk collected from Asian developing countries: Risk assessment for infants. *Organohalogen Compounds,* **58:** 285-287.

Lebo, J.A., Zajicek, J.L. and T.W. May (1989). Large-scale preparation of potassium hydroxide-modified silica gel adsorbent. *Assoc. Off. Anal. Chem.,* **72:** 371-373.

Loganathan, B.G., Irvine, K.N., Kannan, K., Pragatheeswaran, V. and K.S. Sajwan (1997). Distribution of selected PCB congeners in the Babcock street sewer district: A multimedia approach to identify PCB sources in combined sewer overflows (CSOs) discharging to the Buffalo River, New York. *Arch Environ Contam Toxicol*, **33:** 133-140.

Ludwig, J.P., Giesy, J.P., Summer, C.L., Bowerman, W., Aulerich, R., Bursian, S., Auman, H.J., Jones, P.D., Williams, L.L., Tillitt, D.E. and M. Gilbertson (1993). A comparison of water quality criteria for the Great Lakes based on human and wildlife health. *Journal of Great Lakes Research,* **19(4):** 789-807.

Minh, N.H., Minh, T.B., Kajiwara, N., Kunisue, T., Iwata, H., Viet, P.H., Tu, N.P.C., Tuyen, B.C. and S. Tanabe (2006). Contamination by polychlorinated biphenyl ethers and persistent organochlorines in catfish and feed from Mekong River Delta, Vietnam. *Environmental Toxicology and Chemistry,* **25(10):** 2700-2709.

Monirith, I., Nakata, H., Watanabe, M., Takahashi, S., Tanabe, S. and T.S. Tana (2000). Organochlorine contamination in fish and mussels from Cambodia and other Asian countries. *Water and Science Technology,* **42(7-8):** 241-252.

Ritter L, Solomon, K.R., Forget, J. and M. Stemeroff (1995). Persistent Organic Pollutants. United Nations Environment Programme. http://www.chem.unep.ch/POPS/ritter/en/ritteren.pdf. Retrieved on 16 September 2007.

Rossi, L., de Alencastro, L., Kupper, T. and J. Tarradellas (2004). Urban stormwater contamination by polychlorinated biphenyls (PCBs) and its importance for urban water systems in Switzerland. *Science of the Total Environment,* **322:** 179-189.

Safe, S. (2004). Endocrine disruptors and human health: Is there a problem. *Toxicology*, **205:** 3-10.

Smith, L.M., Stalling, D.L. and J.L. Johnson (1984). Determination of part-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. *Analytical Chemistry,* **56:** 1830-1842.

Smith, L.M., Schwartz, T.R., Feltz, K. and T.J. Kubiak (1990). Determination and occurrence of AHH-active polychlorinated biphenyls, 2,3,7,8-tetrachloro-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran in Lake Michigan sediment and biota: The question of their relative toxicological significance. *Chemosphere,* **21:** 1063–1085.

Spano, M., Toft, G., Hagmar, L., Eleuteri, P., Rescia, M., Rignell-Hydbom, A., Tyrkiel, E., Zvyezday, V., Bonde, J.P. and INUENDO (2005). Exposure to PCB and *p,p'*-DDE in European and Inuit populations: Impact on human sperm chromatin integrity. *Human Reproduction,* **20(12):** 3488-3499.

Turusov, V., Rakitsky, V. and L. Tomatis (2002). Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence, and risks. *Environmental Health Perspectives,* **110(2):** 125-128.

Visoth, T., Yim, M., Vathna, S., Irvine, K. and T. Koottatep (2010). Efficiency of Phnom Penh's natural wetlands in treating wastewater discharges. *Asian Journal of Water, Environment and Pollution,* **7(3):** 39-48.

WWF internal report (2009). Diagnostic summary report on samples collected from dead Irrawaddy dolphins along the Mekong River, Phnom Penh, Cambodia.

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