The effect of seasonal variation and lipid content on polychlorinated biphenyls and organochlorine accumulation in Spot Barb (*Puntius bimaculatus*)

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Abstract

After they reach an aquatic environment, polychlorinated biphenyls (PCBs) and organochlorine (OC) tends to accumulate in organisms, because of their high hydrophobic property which then causes adverse effects. However, there are some factors that have an influence on the accumulation. The aim of this study was to investigate the effect of seasonal variation and lipid content on PCBs and OC accumulation. Spot barb (*Puntius bimaculatus*) was selected as the representative of aquatic organisms because it is widely found across the country. Nine sampling stations located along the river receiving wastewater from surrounding farms, domestic, and industrial areas. Twenty fish (in each station) were collected by using floating nets in both dry and wet seasons for measurement of PCBs and OC in the samples. The results indicated that both PCBs and OC concentration in fish collected in the dry season was higher than that in the wet season. These findings could be explained by the characteristics of water channels in the dry season which are quite stagnant, resulting in toxicants being adsorbed onto organic matter surface and then becoming feed for the fish. For the effects of lipid content, the results showed that PCBs/OC concentrations and lipid content were of significant positive correlation ($p \leq 0.05$) in both dry and wet seasons. This phenomenon might be the result of hydrophobic property of toxicants which make it accumulate in lipid tissue.

Keywords: Polychlorinated biphenyls/Organochlorine/Pesticide/Spot barb(Puntius bimaculatus)

1.Introduction

Polychlorinated biphenyls (PCBs) and organochlorine (OC) are toxic chemicals which are widely found in the environment, especially in aquatic sources. Of these, PCBs and OC's have been banned in many countries because of their high toxicity. They are found persistently in many areas, such as developing countries like Thailand. PCBs are used in many industries such as the electrical industry because of their characteristics, which are both chemical and thermal stability, making them good insulators for use in capacitors etc.. For OC's, they are widely used as agro-chemicals for controlling and eliminating pests. Their high hydrophobic and lipophilic characteristic results in them being persistant which leads to accumulation in the environment, in both abiotic (Guo et al., 2008; Guo, 2008 and Carro, 2004) and biotic parts. The hydrophobic toxicants find their way into ponds, streams, and rivers. After being assimilated into aquatic organisms, they can disturb the development, reproduction and behavior of that organism. Moreover, they are passed and biomagnified through the food chain causing adverse effects on the ecosystem, and ultimately human health (Ribeiro et al., 2005).

There is a lot of evidence, to indicate that aquatic organism is being contaminated with PCBs and OC. In 1994, Adb-Allah found that fish samples collected from Aby-Quin bay and Idlen Lake in Alexandria of Egypt showed high levels of concentration of OC in their tissue. Menirith et al. (1999) also found that fresh water fish contained higher levels of OC than marine fish because of their habitat. They lived in the river nearby agricultural areas, where OC was being intensively used. For PCBs, there is a lot of evidence indicating that PCBs are being accumulated in commercial fish. Hansson et al. (2006) found that PCBs were accumulated in Atlantic salmon during the growth phase in open sea.

However, assimilation and accumulation of PCBs and OC in fish is influenced by a number of factors; both internally and externally, such as percentage of lipid content in fish (Bodin et al., 2014; Monosson et al., 2003) and seasonal variations (Greenfield et al., 2005; Rypel et al., 2007). Thus, the influences of these factors have to be studied for a better understanding of PCBs and OC accumulation. The aim of this study was to evaluate the effect of the percentage of lipid content in Spot Barb (*Puntius bimaculatus*) and the seasonal variation on PCBs and OC accumulation.

2. Materials and Methods

Fish sampling

In this study, Spot Barb (*Puntius bimaculatus*) was selected as the representative of

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local fish. It is commonly found in Thailand and could endure to PCBs and OC in the contaminated river. Twenty fishes in each station were collected using floating nets in both dry and wet season (20 fish per season). Nine sampling stations (fig 1 and table 1) located along the river, which receives organo-pesticides not only from surrounding farm area but also domestic and industrial sources. The river is in the area of Water Resource Development Program, Sakhon Nakhon province, located in Northeastern Thailand. In each station, the average length of fish sample was about 15 - 20 cm and weight was about 200 - 400 g. After collection, fish samples were placed in polyethylene, kept at 4°C and immediately transported to the laboratory. Their species were identified and 5 g. of flesh fish was taken. The flesh were freeze-dried for 3 days, ground into fine powder and kept in a desiccator until extraction.

Tabel 1: The position of sampling stations

Station	Latitude	Longitude
A	17.880947115251306	103.90605085556186
B	17.80874255697901	103.85593147432401
C	17.159538129015072	104.37029687712942
D	17.20715266617645	104.30074795771846
E	17.112841829760796	104.32142781673865
F	17.103024961344495	104.36113515482201
G	17.144708321656264	104.27309397392001
H	17.13811854602241	104.18348849046020
I	17.788672433608987	103.99963961982765

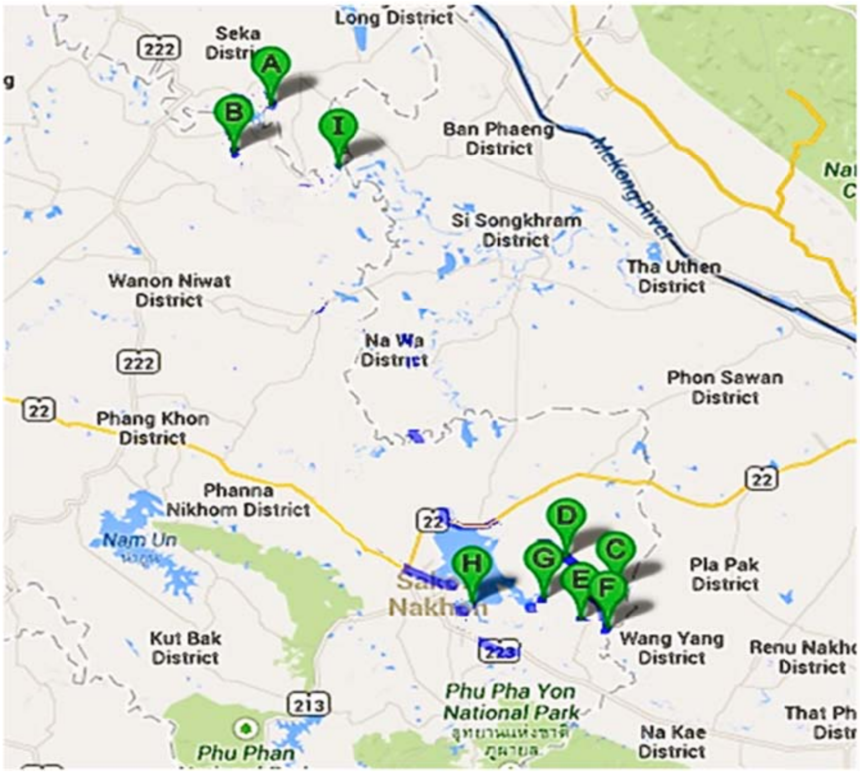

Figure 1: Sampling stations

Soxhlet extraction

The Soxhlet extraction method was modified from Guo (2008). In brief, 3 g. of dried flesh powder was put in an extraction thimble. The surrogate standard used was 5 ng. of 4,4'- dibromooctafluorobiphenyl (DBOFB). Then, the thimble was inserted into a Soxhlet extractor filled with 150 ml solvent mixture of n-hexane –

acetone (4:1, v/v). The extraction process was run continuously for 7 hours and the temperature was kept at 50°C. The remaining extract was concentrated to 6 ml by using a rotary evaporator. Then, it was cleaned by using the column packed with 20 g of silica gel and 10 g of alumina. The remaining solution was eluted by 100 ml of dichloromethane. The recovery percentage of this measurement method was 69.51 ± 11.08% and 87.96 ± 10.66 for PCB and OC, respectively. Finally, the solution was kept at 4°C until analysis.

Lipid content

The Lipid content measurement was performed by the method modified from Oh. (2000). In brief, Two milliliters of concentrated solution was dried for about 2 hours at 60°C. The Lipid content was calculated following the equation:

$$\% \text{ Lipid} = \frac{(W2 - W1) \times FV}{10SW}$$

where, W_1 is the weight of aluminum foil cup (g), W_2 the weight of aluminum foil cup and lipid content after dried (g), *FV* is solvent volume after extracted (ml), and *SW* is sample weight (g).

PCBs and OC measurement

Four milliliters of concentrated solution was purified by using florisil as described in Hyung (1997). Briefly, the concentrated solution was applied to the head of florisil packed column. The first fraction was eluted with 150 ml of dichloromethane/hexane (1/4 v/v) and discarded.

The second fraction was eluted with 70 ml of hexane and retained. The extract was concentrated to 4 ml. Next, the extract was applied into HPLC cleanup column, and then concentrated to 1 ml. Finally, 5 ng. of trichloro-m-xylene (TCMX) which used as the internal standard solution was spiked in the extracts before instrumental analysis by using gas chromatography (GC).

The instrument used in this study was a gas chromatograph (HP 5890 Hewlett–Packard). For PCBs, the column used was DB-XLB capillary column (30 m x 0.25 mm i.d. x 0.25 µm thickness, Agilent technologies, Inc., Santa Clara, CA, USA). The carrier gas was helium. Oven temperature was set at 100°C for 1 min after injection then raised 10°C min^{-1} to 300°C, and remained for 15 min. For OC measurement, the column was DB-5 (30m x 0.25 mm i.d. x 0.25 µm thickness). The carrier gas was helium. Oven temperature was set at 100°C for 1 min after injection then raised 5°C min^{-1} to 140°C, and held for 1 min, then ramped to 300°C at a rate of 5°C. The detection limit of this measurement procedure was 0.5 – 2.0 µg/ml for both PCBs and OC.

3. Results

After PCBs became concentrated in Spot Barb, it was collected in both dry and wet seasons and then being measured. The results indicated that the concentration in the dry season was higher than that in the wet season, as expressed in Fig 2. The highest concentration was found in station 3 which was 53.78 ng/g dry weight. In the wet season the concentration was 44.32 ng/g dry weight, at the same station. In general, PCBs concentration in fish decreased with an increasing in the distance from the point source of PCBs.

After OC concentration in Spot Barb collected in both dry and wet seasons was measured, the results clearly showed that the

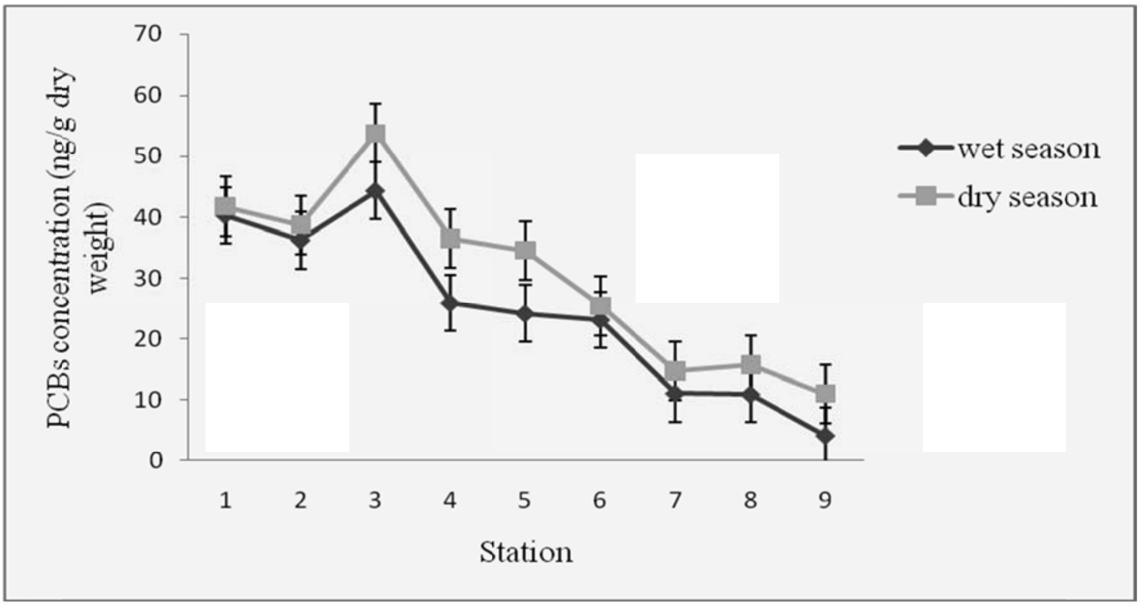

Figure 2: PCBs concentration in spot barb in dry and wet seasons

concentration in the dry season was higher than that in the wet season as shown in Fig. 3. The highest concentration was found in station 1 which was 88.57 ng/g dry weight while in the wet season it was 82.17 ng/g dry weight (at the same station). OC concentration in fishes decreased quite sharply with an increase in the distance from its point source. From station 1 to station 9, the reduction of OC concentration in the dry season and the wet season was 41.70% and 21.47%, respectively.

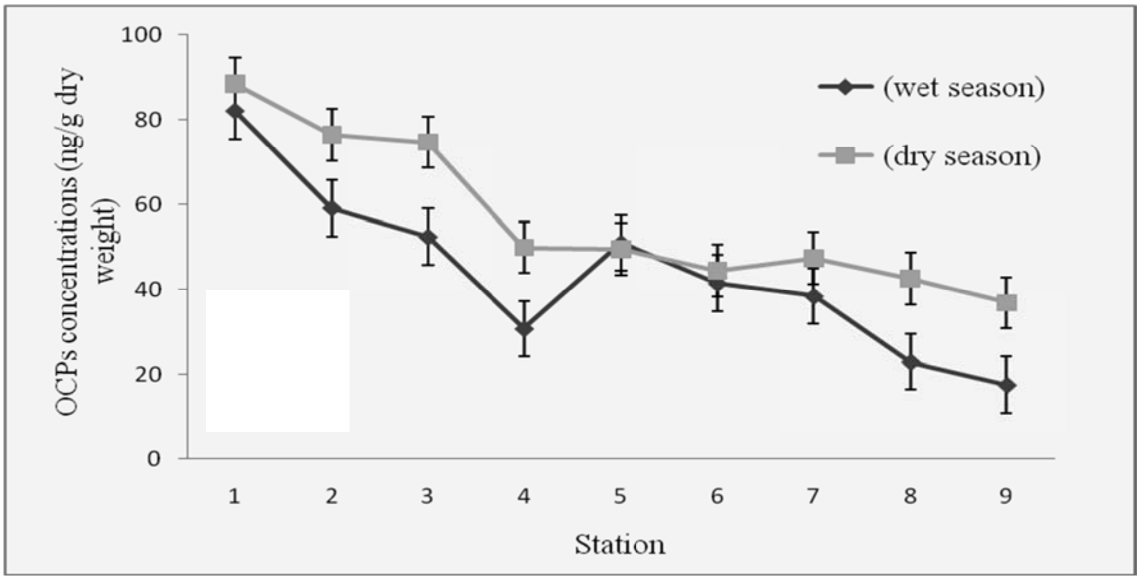

Figure 3: OC concentration in Spot Barb in the dry and wet seasons

In addition, a significant positive correlation ($p \leq 0.05$) between PCBs/OC concentration and percentage of lipid content in fish (Fig 4) was found. Overall, it was the highest in station 1 and then decreased along an increasing distance from the point source.

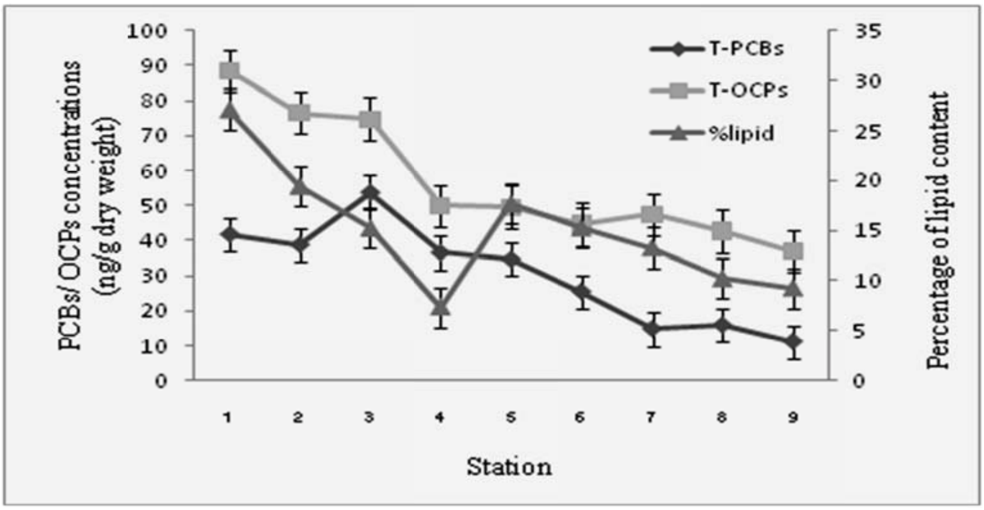

Figure 4:The relationship between PCBs/OC concentration and the percentage of lipid content in Spot Barb

4. Discussion

The highest concentration of PCBs and OC was found in the dry season, this might be explained by the characteristic of water channels. Except lack of water input, the river in the dry season is quite stagnant, causing the contaminants to settle down and being adsorbed onto organic matter surface and then eventually being eaten by the fish. Our result is in agreement with the study of Das et al. (2002) which found that hydrophobic toxicant residuals in fish collected in the dry season was higher than those in the wet season.

The positive correlation between the percentage of lipid content and PCBs/OC concentration in fish could be caused by the hydrophobic character of both hydrophobic toxicants. As is generally known, hydrophobic substances tend to be accumulated in lipid tissues of the organism. In 2004, Verweij et al. assessed the bioavailability of PCBs and OC in caged carp. They found that the chemicals tend to be accumulated in fish, rather than remain in the aquatic environment because of their high K_{OW}. This finding was in agreement with the study of Guo et al. (2008) which compared the effect of biomagnification and bioaccumulation by using δ^{15} N isotope. This isotope was used to assess biomagnification of hydrophobic organic compound passing from prey organisms to the predator. They found the influence of the higher lipid content (bioaccumulation), overwhelmed the effect of biomagnification. The hydrophobic substance concentration in planktonivorous fish was higher than that in carnivorous fish, which is in the upper trophic level. This explanation was also supported by the study of Das et al. (2002) which measured OC residual concentration in catfish, *Tachysurus thalassinus*, from the South Patches of the Bay of Bengal. They found a positive correlation and linear relationship between OC and lipid content in fish. Moreover, Ribeiro et al. (2004) studied bioaccumulation and the effects of organochlorine pesticides in the Eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France and found the greatest OC concentration in organs which high lipid content such as gills, livers, and spleens. In 2009, Hansson et al. studied the accumulation of PCBs in migrating Atlantic salmon from the River Morrum, Sweden and found that the migrating salmon had lower lipid levels in muscles, compared to their own previous study conducted in 2006. For PCBs concentration, they found significant positive correlation with lipid levels in salmon.

5. Acknowledgements

In this study, we found that the concentration of PCBs and OC decreased with an increase in the distance from the point source. This phenomenon might be caused by the dilution effect of water.

The results showed that there was a significant difference ($p \leq 0.05$) between PCBs and OC concentration in Spot Barb (*Puntius bimaculatus*) collected in the wet and dry seasons. It can be explained by the difference of water characteristics. In the dry season, the water is quite stagnant and less dilution capacity,therefore the lack of water pouring into the river causing the contaminants to settle down to the bottom. They could be adsorbed by organic matter and then eventually being taken in by the fish. For the influence of lipid content in fish tissue, the results strongly indicated that there was a positive correlation ($p \leq 0.05$) between PCBs and OC concentration and lipid levels in fish.

Our findings can be used as the fundamental knowledge to develop an effective environmental monitoring program. In order to receive the best results, the program should be performed in the dry season and nearby the point source.

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