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Polychlorinated Biphenyl Levels and its Correlation to Size of Marine Organisms Harvested from a War-Induced Oil Spill Zone of the Eastern Mediterranean Sea

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Abstract

This is the first work establishing a base-line data of the level of total Polychlorinated Biphenyl (PCB) contaminants in selected marine organisms (Siganus rivulatus, Mullet spp., and oysters) and its relationship to organism size and the harvest distance from the oil spill source. Six locations across the Lebanese Mediterranean were included for sampling. Oysters and the two fish types were collected after 72 days of the spill. The length, maximum width, and whole weight of individual organisms were recorded. Methanol extracts of the samples were analyzed for total PCB using a Competitive Enzyme-linked Immunosorbent Assay (ELISA) based Spectrophotometry.

All means of PCB in the three selected marine organisms were below the guidance level set by US-FDA (2 ppm). A total of 6 significant regression equations were established between the total PCB level and certain size dimensions of specific selected marine species, with values of R^2 ranging between 0.719 – 0.909 and P values ranging from 0.038 – 0.099.

In addition, the total PCB level in Siganus rivulatus correlated with the harvest distance north of the oil spill source, signifying a drop in total PCB level with an increase in harvest distance from the oil spill source.

Keywords: Fish; Oysters; PCB; Harvest Distance.

Introduction

The war activity during July 13 and 15, 2006 resulted in a huge oil spill from a power plant located at 30.3 km south of the capital Beirut along a 225 km long Lebanese coastline (UNEP, 2007). A volume of 15,000 tons of number 6 heavy fuel oil spilled into the Mediterranean Sea, driven by south to north currents towards the northern parts of the shore. A previous search, accomplished three years before the oil spill, had already revealed a scarce presence of copepod density due to contamination of the Eastern Mediterranean artesian water zones (BARBOUR *et al.,* 2004). Spilled heavy fuel oil is among the most difficult to combat due to its highly viscous nature, low volatility, high specific gravity and tendency to sink to the seabed which could lead to prolonged persistence in the marine environment, affecting marine ecosystems and thus needing decades for rehabilitation to occur (UNEP, 2007).

Previous incidents of oil spills around the world, including the Exxon Valdez in Alaska in 1989, the ApexBarge in Galveston Bay in 1990, and the Erika off the Atlantic coast of France in 1999, have resulted in serious contamination of marine life (BUDZINSKI *et al.,* 2004); self- purification of major contaminants in marine and fresh water environment requires a long time to complete (BARBOUR *et al.,* 1986).

The major contaminants originating from the oil spill, and known to cause serious damage to the marine life, include many compounds in the Polychlorinated Biphenyls (PCB) family. The discharge of PCB into seas or oceans results in serious marine species contamination (USEPA, 2000).

PCB compounds are extremely persistent with a high affinity to lipids accumulating in the adipose tissue of various species and bio-accumulating via the food chain. Furthermore, this group of compounds biomagnifies, reaching higher concentrations with increasing trophic levels in aquatic environments (GREENFIELD *et al.,* 2003). The group has been classified by the United States Environmental Protection Agency (USEPA) as a B2 carcinogen group, with many members of the group characterized as animal and probably human carcinogens (USEPA, 2000).

These PCBs are able to affect the human health negatively through passage into human breast milk and from the placenta to the fetus, thus causing developmental delays, lower birth weight and decrease in IQ of infants and children. PCBs have other adverse health effects on different systems including the cardiovascular, hepatic, immune, musculoskeletal, endocrine, gastrointestinal, reproductive and integumentary systems (WHO, 2003).

PCB is no longer manufactured for inclusion in marketed products for the purpose of stability; however, it continues to enter the environment from many sources, including the oil spills (ATSDR, 2000).

The purpose of this work is to establish a base-line data of the total level of PCB in selected marine organisms following a war-induced spill of 15,000 tons of heavy fuel oil in the Eastern Mediterranean sea. The total level of PCB in oysters and in two predominant fish species namely, *Siganus rivulatus* and *Mullets* spp. of the Mugilidae family will be determined. Attempts will be made to establish regression equations to predict the total PCB level in a marine organism from its size, weight and its harvest distance from the oil source.

Materials and Methods

Location of the Study

Six locations across the Lebanese Eastern Mediterranean were included in the sampling design. Two of the six locations were expected control negatives located at greater than 24 km south of the bombarded Jiyeh plant (source of oil spill) namely, along the coast of the city of Tyre (47.8 km south of the Jiyeh plant), and the town of Sarafand (24.1 km South of the Jiyeh plant).

The other four locations were situated

at the following distances north of the Jiyeh plant, namely, the coast of the city of Beirut (30.3 km), the town of Tabarja (57.9 km), the town of Amchit (72.4 km) and the town of Barbara (78.3 km) (Fig.1). The direction of the sea currents at the time of the oil spill was south to north.

The coordinates of the 6 sites, according to the Global Positioning System (GPS) data provided by the Greenpeace divers from the Rainbow Warrior ship, were: Tyre (N 33° 17.548', E 035° 12.741'), Sarafand (N 33° 28.388', E 035° 16.749'), Beirut (N 33° 53.579', E 035° 27.855'), Tabarja (N 34° 01.089', E 035° 37.264'), Amchit (N 34° 07.729', E 035° 38.209') and Barbara (N 34° 12.181', E 035° 38.060').

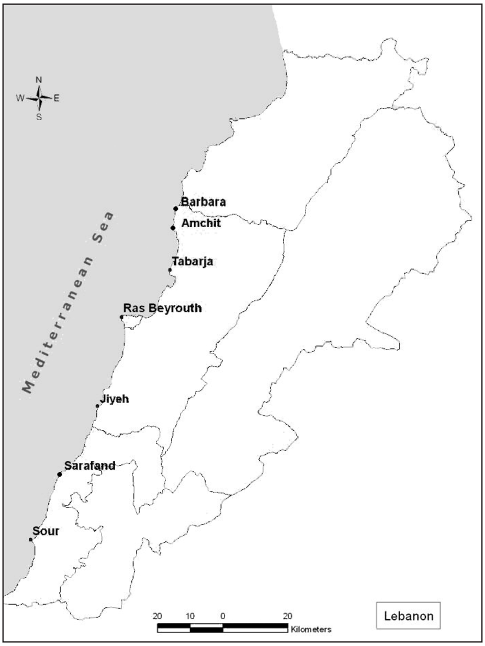

Fig. 1: Map of the locations of the 6 sites across the Lebanese Eastern Mediterranean shore.

Sampling Nature and Size

Two major fish species (*Siganus rivulatus* and *Mullets* spp. of the Mugilidae family) and oysters, predominant in artisanal fishing activity of Lebanon, were sampled from the six locations mentioned above. The fish and oysters were harvested at 100-200 meters from the shore, and at depths of 3-5 meters. The total number of collected samples from the six locations, at 72 days post the oil spill, was 360 individuals, including 20 individual samples of each species per location. Sampling of oysters from the six designated sites was accomplished by divers from the Greenpeace Rainbow Warrior. The sampling of fish was accomplished at 72 days post the oil spill, by a designated fisherman. The oysters were transported and kept at -80°C at the Animal and Veterinary Sciences Laboratory of the American University of Beirut. Individual organism dimensions of fish were recorded, namely, length, maximum width, and weight and oyster shell length, shell width, and whole weight. Dorsal muscles of freshly caught fish were collected and kept at -80°C. The sampling, cleaning and storage were carried out according to previously reported standard methods (LASRADO *et al.,* 2003).

Sample Preparation and Methanol Extraction

Thawed dorsal muscle parts of the two fish species and whole oysters were individually homogenized, and lyophilized at -50°C and 0.22 mBar pressure for 48 hours (NIST, 2001) and then rehomogenized. The twenty freeze dried and homogenized individual samples of each specie, collected in a specific location, and at a specific time, were pooled in equal weights into 4 replicates for fish and 5 replicates for oysters. The average of each measured size parameter was obtained in each pooled sample. Amounts of 0.5 g of each pooled sample and 2.5 ml of HPLC grade methanol (1/5 w/v ratio) were mixed in each tube. The tubes were then vortexed for three minutes and centrifuged for 10 min at 2000 rpm to extract the PCB compounds into the Methanol phase (YUSÀ *et. al,* 2005).

Analysis of Total PCB

The level of the total PCB organic contaminant in each methanol extract was determined, using a Rapid Assay PCB Test Kit (Strategic Diagnostic Inc., Newark, USA). The Rapid Enzymatic Assay is a kit especially designed to detect the Aroclor 1254 compounds. The minimum detection limit (MDL), known as the sensitivity of the kit, is 0.001 $\mu g\ g^{-1}$. dry weight.

The procedure is briefly described by the addition of a volume of 250 µl of either standards (Aroclor 1254 at 0.25, 1.0, 5.0 ppb), kit controls (Aroclor 1254 at 3.0 ppb), or methanol extract of marine organisms to a polystyrene test tube, followed by an addition of 250 µl of Enzyme PCB conjugate. After a thorough mixing, a volume of 500 µl of magnetic particles coated with antibodies specific to the common antigenic determinant of PCB compounds was added. This coated antibody competed for the PCB carried by the Enzyme PCB conjugate, and for the total PCB in either standards, controls, or in the methanol extract of the marine samples. The mixture was incubated for 30 minutes at room temperature. An opposite charged magnet was placed at the bottom of the tube rack to attract the reactants bound to magnetic particles to the bottom of the tubes. The supernatants were discarded. Magnetically attracted reactants were washed twice using 1 ml of a washing solution provided by the commercial kit, followed by the addition of 500 µl of substrate (the enzyme substrate is hydrogen peroxide and the chromogen is 3,3',5,5'-tetramethylbenzidine), and an incubation for 20 minutes. An amount of 500 µl of a Stop Solution (2M sulfuric acid) was then added to terminate the enzymatic reaction, and the optical densities of the developed colors in the tubes were read at a wavelength of 450 nm, using a spectrophotometer (Turner Spectrophotometer SP-890, UV-VIS, Kerper Boulevard Dubuque, Iowa).

A regression equation for total PCB standards was established correlating the different concentrations of total PCB (ppb) to optical density values. The level of the total PCB in marine organisms was deduced from the regression equation of the standards, changing units from ppb to ppm ($\mu g\ g^{-1}$ dry weight). Each point on the standard curve was a triplicate of certain specific concentration in which the OD values of the triplicates were tightly clustered around their mean.

Statistical Analysis

Statistical analysis for comparisons of the mean of total PCB was done by one-way ANOVA followed by Duncan's Test (SPSS 15.0, SPSS Inc., Chicago, USA). Statistical differences in total PCB levels of the same species were compared across the six different locations at $0.05 \le P < 0.1$ (STEEL *et al,* 1997). In addition, the means of total PCB level were compared among the three marine organisms collected from each site at $0.05 \le P < 0.1$. Linear regression equations were established relating the PCB level (y) to size parameters (x) or to the distance of harvested organisms from the oil spill source (x) at significance levels of $0.05 \le P < 0.1$ and $P < 0.05$.

Results

The total PCB analysis of the collected oysters and the two fish species in the year

2006 was deduced from a linear standard curve that was established based on different concentrations of pure Aroclor 1254. The linearity of this standard curve for total PCB concentrations (y-axis) versus optical density values (x-axis) is shown in Fig. 2, revealing a regression equation of $y = -0.0237x + 0.1805$, with $R^2 = 0.978$, and a highly significant correlation at $P < 0.05$.

The mean of the total PCB of each organism at each of the six sites, deduced from the standard curve of Figure 2, is presented in Table 1. Four out of the six sites had higher levels of mean PCB in *Mullets* spp. compared to that in the *Siganus rivulatus* fish species and in oysters with variable levels of significance indicated by alphabet superscripts in the Table ($0.05 \leq P < 0.1$); however, there was only one site in the north with a higher level of mean PCB in oysters compared to the other two organisms namely, in Amchit (72.4 km north of the oil spill source). In comparing the means of PCB level in the same marine organism, at the six different sites (Table 1), the level of PCB did not differ significantly in oysters collected from the 4 sites in the north compared to the level in oysters at the two reference sites in the south ($P > 0.1$). In addition, only one site in the north (Amchit,

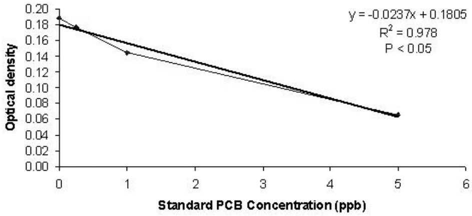

Fig. 2: Line of best fit correlating the Standard PCB concentration (Aroclor 1254 in ppb units) to optical density for assessing the total PCB in organisms.

Table 1
Comparison of the mean total PCB in μg g^{-1} of dry weight among he three marine organisms* in the six selected sites of the Eastern Mediterranean at 72 days post the oil spill.

Marine organisms	Mean total PCB level at different coast sites (μg g^{-1} dry weight)					
	Cities or towns south of oil spill source (kms South)		Cities or towns north of oil spill source (kms north)			
	Tyre (47.8)	Sarafand (24.1)	Beirut (30.3)	Tabarja (57.9)	Amchit (72.4)	Barbara (78.3)
Siganus rivulatus	$0.017^{a,b;1}$	$0.040^{b,1}$	$0.029^{a,b;1}$	$0.018^{a,b;1}$	$0.017^{a,b;1}$	$0.006^{a;1}$
***Mullets* spp.**	$0.033^{a,b;1}$	$0.044^{b;1}$	$0.036^{a,b;1}$	$0.027^{a,b;1}$	$0.014^{a;1}$	$0.044^{b;2}$
Oysters	$0.025^{a;1}$	$0.030^{a;1}$	$0.027^{a;1}$	$0.033^{a;1}$	$0.045^{a;2}$	$0.025^{a;1,2}$

* The harvest of oysters and the two fish species was accomplished in September of 2006 (72 days post the oil spill).

[a,b] Mean total PCB levels in the same row with different alphabet superscripts are significantly different ($0.05 \leq P < 0.1$)

[1,2] Mean total PCB levels in the same column with different numerical superscripts are significantly different ($0.05 \leq P < 0.1$)

72.4 km north of the oil spill source) had a significantly lower level of PCB in *Mullets* spp. compared to the level in the same organism collected from one site in the south (Sarafand, 24.1 km south of the oil spill source) ($P < 0.1$). Moreover, one site in the north (Barbara, 78.3 km north of oil source) had a significantly lower level of PCB in *Siganus rivulatus* compared to the level in the same organism collected from one site in the south (Sarafand) ($P < 0.1$).

Sixty-three regression equations were studied (Table 2), correlating the total PCB values (y) in each of the three selected marine types to individual dimensions (x). The calculated interaction is obtained by multiplication of the number of organisms times the number of sites and number of measured size dimensions (phenotypes: length, maximum width, and whole weight).

Out of the 63 studied regressions, six equations showed statistically significant correlations (1 equation at $P < 0.05$, and 5 equations at $0.05 \leq P < 0.1$) (Table 3). The highest positive correlation with the highest R^2 value of 0.909 was shown in PCB values of *Mullets* spp. harvested from Beirut (30.3 km north of the source of the oil spill) in relation to weight. It is worth noting that one regression equation had a negative slope, negatively correlating the PCB values in *Siganus rivulatus* to their weight in the Sarafand area (24.1 km south of the oil spill source).

Another three regressions were studied correlating the level of total PCB in each marine organism to distance (kms) north of the source of the oil spill, targeted by the south to north sea currents at the time of the spill. Only one regression equation had a significant negative correlation of the total PCB level in *Siganus rivulatus* to distance in kms north of the oil spill, at a value of $R^2 = 0.870$ at $P = 0.1$ (Fig. 3).

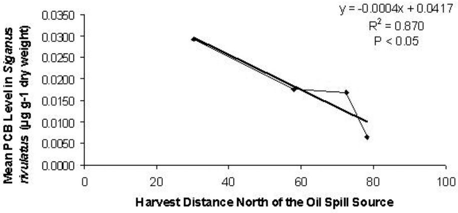

Fig. 3: Line of best fit correlating the Total PCB level (µg g^{-1} dry weight) in Siganus rivulatus to harvest distance (kms) north of the oil spill source.

Discussion

The presence of the highest level of mean total PCB in *Mullets* spp. compared to the other two selected marine organisms, and in 4 out of the 6 studied sites (Table 1), is most likely due to the feeding behavior of this benthic species, usually used for testing sediment pollution. In addition, the high fat content of the *Mullets* spp. could have accounted for such a high deposition of PCB in such a species compared to the other two (PASTOR *et al.,* 1996; PERUGINI *et al.,* 2004). Future investigations will attempt to evaluate the correlation between fat percentage in the harvested organism and PCB levels.

The hypothesis that current targets carrying the spilled oil to northern sites would be resulting in higher total PCB in the three selected organisms harvested from the north, did not apply for the two fish species. In fact, the highest level of total PCB in fish was noticed in Sarafand, located at 24.1 km south of the oil source, a location that was against the sea current at the time of the spill. Further investigation of the Sarafand coast revealed the presence of many gas stations, continuously spilling used engine oils directly to the sea in that area. This source of contamination could be respon-

Table 2
Interactions resulting in 63 studied regression equations.

Organisms	Size dimensions	Harvest sites*	Total interactions**
3	3	6	54
3	3	pool of 6 sites***	9
	Total studied regressions		63

* The harvest of oysters and the two fish species was accomplished in September of the year 2006 (72 days post the oil spill).
** Total interactions is the result of multiplication of number of organisms x measured size parameters x number of harvest sites.
*** Data of the same species pooled for all six locations.

Table 3
Significant regressions of total PCB level (y) in different selected marine organisms to dimensions and weight parameters (x).

Marine organisms*	Locations (distance from oil source)**	Relating parameter in organisms (x)	Regression equation (y = total PCB level)	R^2	$0.05 \leq P < 0.1$	$P<0.05$
Siganus rivulatus	Sarafand (24.1,S)	Weight	y = -0.0037x+ 0.2403	0.883	0.055	
Mullets spp.	Beirut (30.3, N)	Weight	y = 0.3692x -11.248	0.909	0.060	
		Width	y = 0.0503x - 0.251	0.838	0.084	
	Tabarja (57.9, N)	Width	y = 0.0237x - 0.1083	0.811	0.099	
Oysters	Tyre (47.8,S)	Weight	y = 0.0003x - 0.0274	0.754		0.038
		Width	y = 0.0207x - 0.0995	0.719	0.068	

* The harvest of oysters and the two fish species was accomplished in September of 2006 (72 days post the oil spill).
** Distance from source could be to the north (N) or south (S), and is reported in kms. The direction of the sea current at the time of the oil spill was south to north.

sible for raising the total PCB levels in the two types of fish harvested from that area (ATSDR, 2001).

The above-mentioned hypothesis did apply for oysters, in which the PCB was the highest in oysters harvested from the 4 northern sites compared to Tyre-reference southern site; in fact, the highest level of

PCB was seen in oysters from Amchit (0.0448 μg g^{-1} dry weight), located at 72.4 km north of the oil spill source. The filter feeding behavior and sedentary life of oysters (CHU *et al.,* 2000) could have helped in establishing such a total PCB pattern following the oil spill, a pattern that was absent in the two fish species.

It is worth noting, however, that in spite of the significant oil spill all means of total PCB in the 2 selected fish species, at different locations, and in oysters, were below the guidance level of 2 ppm set by the US-FDA (Table 1) (USFDA, 2001). The collective range of the total PCB level in the examined marine organisms of both harvests was 0.006 – 0.044 μg g^{-1} dry weight, a range that is below the US-EPA consumption limit set at 20 μg g^{-1} (ppm) for total PCB (USEPA, 2000). This indicates that a consumption of 454.5 g of fish of the type of *Mullets* spp. containing the maximum level of PCB (0.044 μg g^{-1} dry weight) obtained in this study will reach the EPA consumption limit of 20 μg day^{-1}.

The establishment of a total of 6 significant regression equations (Table 3) helped in prediction of the total PCB level in different organisms at different locations from the size parameters of the three species. It is worth noting that 5 out of the 6 regression equations had positive slopes, indicating that more of a total PCB level is to be expected with larger size of the organisms, or increased age. Previous literature points at positive correlations between organic contaminants from oil spills and marine organism dimensions, or age (GRAFTON *et al.,* 2005).

In attempting to find a correlation, in each marine organism, between the total PCB level and the harvest distance from the source of the oil spill, it was found that only *Siganus rivulatus* showed a significant correlation with R^2 value of 0.870 at a P = 0.1 (Fig. 3). The slope of the correlation was negative (total PCB level = -0.0004 distance (km) + 0.0417), indicating that the farther the harvest of *Siganus rivulatus* is from the source of the oil spill, the lower the level of the total PCB will be in the dorsal muscles. Previous literature has also reported a negative correlation in marine organisms between a contaminant level and distance of the harvest from the source of contamination (FOSSATO and SIVIERO 1974; BAZZANTI *et al.,* 1997). This correlation existed in *Siganus rivulatus* at 72 days post the oil spill, a relationship that could be susceptible to change by time, which could be used in evaluation of self-purification processes of total PCB in marine organisms.

In conclusion, the harvest of the three selected marine organisms had the highest total PCB level in one of the current target northern sites. However, all total PCB means in different organisms were lower than the US-FDA guidance level of 2 ppm. The established regression equations were helpful in predicting the PCB level in certain organisms, at certain locations, from the measured size parameters. Moreover, only the *Siganus rivulatus* had a significant negative correlation of PCB level to harvest distance from the source of the oil spill.

Future investigations will study the decay curves of the total PCB level in selected marine organisms, and will see the impact of self-purification of total PCB on the nature of the change in regression equations. Due to the possible immuno-suppression that could occur from the low detected levels of PCB in the three marine organisms, future works will investigate the impact of such PCB levels on the health of selected marine organisms in the Eastern Mediterranean Sea.

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