

Impact of Organic Micropollutants Causing Mass Mortality of the Clams (*Mactra aequisulcata*) Due to Charactersitic Distribution at Karachi Coast, Pakistan

Safia Khanam^{a*}, Alia Bano Munshi^a, Hina Ahsan^b and Razia Begum^b

^aCentre of Excellence in Marine Biology, University of Karachi, Karachi, Pakistan

^bCentre of Environmental Studies, PCSIR Laboratories Complex Karachi, Karachi, Pakistan

(received February 18, 2019; revised June 28, 2019; accepted July 18, 2019)

Abstract. Mass mortality of bivalve clams along with some other marine fauna was observed during the month of May 2016, mainly consisting of the clams (*Mactra aequisulcata*) of family Veneridae surfaced over the Clifton coast. The occurrence of mass mortality of the venerid clams *Mactra aequisulcata* and other marine life has been studied due to the toxicity of chlorinated hydrocarbons (DDTs, HCHs, hexa chloro benzene (HCB), chlordane, dieldrin (4-chlorophenyl), methanol (TCPMOH) and heptachlor epoxide and polychlorinated biphenyls (PCBs). Significant compositional characteristics of PCBs and DDTs were found in all dead clams and other fauna including fish (*Johnius carutta*), crabs (*Portunus pelagicus*), gastropods (*Babylonia spirata*), bivalves (*Anadara antiquata*), (*Mactra aequisulcata*) and the pen shell (*Atrina pectinata*) from Karachi coast, Pakistan. Mean concentrations (ranges) of organochlorine pesticides (OCPs) in crab (*Portunus pelagicus*) and fish samples were 1.1 (<0.01-1.5), 0.22 (<0.01-1.1) and 0.14 (<0.01-1.3) /µg/g. Those in, bivalves (*Anadara antiquata*) and pen shell (*Atrina pectinata*), clams (*Mactra aequisulcata*) were 0.09 (<0.02-1.2), 0.22 (<0.02-1.3) /µg and 0.13 (<0.01-0.27), respectively. The heptachlor epoxide was found in highest concentration in clam samples (25.00 ± 30.92) µg/g, (wet weight) however, in bivalves, (2.30 µg/g, (wet weight), which were higher than those in other gastropods. Polychlorinated biphenyls (PCBs) concentrations were also measured in the same samples of gastropods (*Babylonia spirata*), fish (*Johnius carutta*), crab (*Portunus pelagicus*), clams (*Mactra aequisulcata*), bivalves (*Anadara antiquata*) and the pen shell (*Atrina pectinata*) to determine the possible cause of mass mortality. The DDT to metabolites (DDD & DDE) concentration ratios exceeded upto 1.0 in the sessile fauna that is clams (*Mactra aequisulcata*) and the pen shell (*Atrina pectinata*) from Karachi coast. These organisms also exhibited dichloro diphenyl trichloroethane (DDT) inputs, whereas dichloro diphenyl trichloroethane (DDD) was found to be in degraded component and PCBs were generally in low concentrations. The concentrations of DDTs were higher than the ERL guidelines in the coastal areas of Karachi, suggesting that there is potential of ecological risk present in the prevailing environment.

Keywords: mass mortality, organochlorines and PCBs, clams, fish, shellfish, toxicity

Introduction

This is well known that the Persistent Organic Pollutants (POPs) like PCBs and pesticides have an adverse impact on different species may have resulted in mass mortality of bivalve; clams of the family Veneridae due to strong toxicity. During May 2016, dead clams and other marine fauna were surfaced over the Clifton coast extending between Sea view and Sands pit, Karachi. The endocrine disrupting chemicals (EDCs) (POPs and OCPs and PCBs) are also involved in causing carcinogenesis and mutagenesis (Wang *et al.*, 2012). An incident of mass mortality of clams presumed to be due to different organic pollutants in some coastal areas of Pakistan, however, such disasters have been observed earlier also many times with a huge quantity of fishes haven died

*Author for correspondence; E-mail: safiakh14@hotmail.com

in the past. Between 17 and 18 May 2016, a high tide brought huge numbers of dead clams of same species identified as *Mactra aequisulcata* including few numbers of crabs particularly *Portunus pelagicus*, fishes like sole fishes and some other molluscs including *Babylonia spirata* and *Atrina pectinata* (Fig. 1-2).

A ban has been imposed since the 1970s on production and uses of organo chlorine pesticides (OCPs) and poly chlorinated biphenyls (PCBs) in most countries being ubiquitous environmental contaminants (Kim *et al.*, 2016). A continuing cycle in the ecosphere has been reported (Dhananjayan and Muralidharan, 2013) with declined concentrations of OCPs in relatively slow rate in various ecosystems (Ockenden *et al.*, 2003). Since mass mortalities and declining stocks among several marine mammal populations from highly polluted

areas have been attributed, in part, to contamination by organochlorine contaminants (Kannan *et al.*, 2004), therefore, measurement of chlorinated pesticides in biological samples is important as OCPs and PCBs tend to accumulate in fish and shellfish due to their lipophilicity being more lipophilic and environmentally persistent pollutants (Shi *et al.*, 2013). The main objective of the research is to investigate the fate of all organochlorine compounds as one of the major root cause for the mass mortality of clams and other dead fauna. The work plan of this study is to find out the recent situation of the spatial distribution of organic micropollutants and the potential ecological with compositional characteristics. Additionally, OCPs and PCBs may have hazardous impact on human beings including aquatic or marine life (Ravindran *et al.*, 2016), therefore, OCPs and PCBs were analyzed in the different fishes and shellfishes from the Karachi coast by using a quick method that is the matrix solid-phase dispersion (MSPD). Thus the present study was carried out to study the possible cause of mass mortality of clams and other marine fauna by the data of POPs for the pollution control and remediation for further such disaster in future.

Materials and Methods

Sampling locations. Karachi covers a long coastline with the most populous area, called Clifton, located at 26 km away in the southeast part of Karachi coastline. All samples were collected from Clifton coast extending between Sea view and Sands pit along the Karachi coast. Karachi is located at 24.91°N latitude and 67.00°E longitude and at an elevation of 38 meters above the sea level. Fishes and shellfishes were collected from the location shown in the map (Fig. 3) during the winter season that is between November to March Fig. 1. Map of Karachi with sampling locations. Physical parameters of water and the sediments were also taken into consideration (Table 1).

Sample collection. Samples were collected during 2016, when an incidence of mass mortality of the venerid clams surfaced over the Clifton coast extending between Sea view and Sands pit occurred. Samples of venerid clams and other fish and shellfish were collected in a properly designed buckets and were brought back to the laboratory for further analysis. Identification of species and biometric data was recorded in the lab. After identification, dissection of the samples were done and tissues and organs were removed, wrapped



Fig. 1. Mass mortality of the clams *Macra antiquata* washed ashore in Clifton beach.



Fig. 2. Mortality of other fauna.

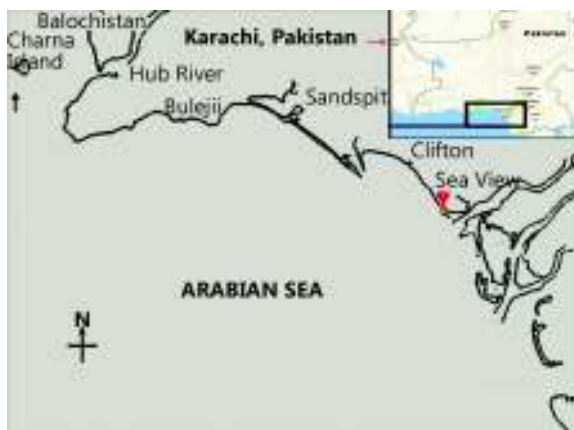


Fig. 3. Map of Karachi where clams mortality occurred.

in aluminum foil and were then frozen below 4 °C for further analysis. The muscle, liver, and gonads were taken out and analyzed individually. Frozen dried samples were ground and sieved by passing through an 80 mesh size sieve and were then fully homogenized.

Sample analyses. Samples storage, preparation for extraction, concentration and chromatographic separation were conducted in the Seafood Quality Laboratory of CEMB and PCSIR -KLC.

Extraction. All stored fish samples were thawed to bring at room temperature and cut into small pieces with a stainless steel knife. Shellfish, removed from

shells and very fine chopped fish tissue was weighed (0.5 g) in a small plastic weighing boat. The weighed tissue and C-18 sorbent (2 g Bondesil® C-18 sorbent (Varian Inc. CA).) were thoroughly ground in a glass mortar and pestle up to a homogenous status or to form a consistency of small grains in appearance (Barker, 2007). At a time 12 Florisil solid phase extraction cartridge (20 mL bond Elute® from Varian Inc. CA) was affixed to a vacuum manifold (Supelco, PA). The sample and C-18 mixture was transferred to the cartridge using a metal spatula. A polyethylene frit (Varian Inc. CA) was firmly tamped into the place above the sample and C-18 mixture with a disposable syringe plunger. All samples (that is 12 in numbers) were eluted from the cartridge under vacuum with successive washes (3×5 mL) of dichloromethane. The eluate was collected in a glass, 25 mL test tube, evaporated up to 1 mL using a nitrogen evaporator (ICH Q2B, 1996). Then transferred quantitatively to a centrifuge tube using about 10 mL *n*-hexane and evaporated in a second time diluted to the final volume and were transferred to an autosampler vial to gas chromatographic analysis.

Gas chromatographic. Extract of the sample was analyzed by gas chromatography for organochlorines (DDTs) and PCBs, the chromatograph equipped with electron capture detector (⁶³Ni, GC-ECD, HP Agilent model 6890). The individual sample was injected in splitless mode with the following temperature programming of instrument shown in Table 1a.

Table 1a. Temperature programming by gas chromatography

GC- Temperature programming conditions	
Injector temperature	200 °C
ECD temperature	350 °C
Capillary column	RTX-5 30 meters × 0.25 mm ID × 0.25 M
Capillary column	DB 35, 30 meters × 0.25 mm ID × 0.25 M
The oven temperature program	60 °C for 0 min, 20 °C/min to 160 °C, hold for 1.00 min. the detector
Carrier gas	make-up gas was nitrogen and the combined carrier and makeup flow rate was 60 mL/min
Hewlett packard chem station software	G 2070AA Rev.A.10.02 for data acquisition and analysis

Certified reference material and standard quantification. This was performed by using an external an organochlorine mixture of pesticide standard containing 20 components and polychlorinated biphenyl standard mixture of Aroclors (1016, 1221, 1242, 1248, 1254, and 1260) from Restack (Bellefonte, PA) and the certified mixed standard employed. The calibration curves were made with dilutions of standards in pesticides grade solvent. Identified compounds were quantified using the external standard technique.

Quality control and assurance (QC & QA). All glassware was washed and completely dried by a standard method and avoiding use of any rubber or plastic items. “Blanks” (for every set of samples, a procedural blank) were employed periodically along with a spiked blank and a matrix spiked sample consisted of all chemicals. A matrix spiked replicate and a reference sample analyzed. This practice is done for assurance of interference and cross-contamination during analysis. Internal spiking and reagent blanks were used to determine recovery values. Recovery was calculated by the spiked amount of surrogate standard calculated on the spread sheet by the difference of samples results minus blank value, which found in the range 95-120% and 90-114% for OC and PCBs pesticides. Every time, at least 20% replicates examined to verify the precision was estimated 1-10 from the multiple analyses of spiked samples for the different compounds of analytical results. Limit of detection (LOD) for PCB single congener and organochlorine compounds was 0.01 ng/g and 1.00 ng/g, respectively. A twelve-(12) port glass manifold assembly was used with the column (syringe) barrels that are Florisil (1 g/packing) designed for environmental samples from Supelco, USA. The vacuum was created using a vacuum pump and columns were first conditioned by passing about 2 mL CH₃OH.

Statistics. Calculations were carried out by using the SPSS, standing for the statistical analysis of the data, Excel on the basis of the linear equation EURACHEM, (1998).

Results and Discussion

The biometric data of various fauna analyzed in this study is presented in Table 1b. The spiked sample results to determine the recovery, lower detection limit (LOD) and (LOR) lower reportable limit of the method are given in Table 2. Recovery range is 93-210% in the spiked surrogate standard with the concentration of 4 ng/g and LOD for organochlorines was determined

in a range of 0. 1-1.00 ng/g (Fig. 4). The matrix solid phase dispersion (MSPD) is a significant reliable method for the confirmation and quantification of chlorinated pesticides (Oyuna *et al.*, 2004). Total levels

Table 1b. Biometry of fish and shellfish analyzed

Fauna	Weight (g)	Total size (cm)
Gastropods : <i>Babylonia spirata</i>	54.6±2.31	6.5±0.98
Pen shell : <i>Atrina pectinata</i>	53±0.65	8.4±1.02
Crab : <i>Portunus pelagicus</i>	46±1.03	6.0±1.35
Fish : <i>Johnius carutta</i>	46±1.98	16±1.25
Clams : <i>Macra aequisulcata</i>	43±2.01	6.5±0.59
Bivalves : <i>Anadara antiquata</i>	50±3.01	8.0±1.03

Table 2. List of 20 organo chlorine pesticides (OCPs) and 7 poly chlorinated biphenyls (PCBs) with statistics

Organochlorine	Reproducibility	Recoveries	R.S.D (%)	LOD ng/g	LOR ng/g
Aldrin	03	93%	4.6	0.10	1.00
Alpha BHC	05	115%	5.1	0.099	0.99
Alpha chlordane	03	119%	5.0	0.015	1.05
Beta BHC	03	115%	4.1	0.098	0.98
Delta BHC	04	169%	5.2	0.099	0.99
Dieldrin	03	145%	4.5	0.100	1.00
Endosulfan I	05	138%	5.2	0.099	0.99
Endosulfan II	03	140%	6.0	0.089	0.89
Endosulfan sulfate	03	210%	4.3	0.105	1.05
Endrin	04	113%	5.4	0.105	1.05
Endrin aldehyde	03	181%	4.7	0.089	0.89
Endrin ketone	05	192%	5.2	0.100	1.00
Gamma BHC	03	149%	6.0	0.089	0.89
Gamma-chlordane	05	117%	4.3	0.099	0.99
Heptachlor	03	118%	4.7	0.100	1.00
Heptachlor epoxide	03	114%	5.2	0.100	1.00
Methoxychlor	04	NR*	6.0	0.098	0.98
p,p DDD	03	149%	4.3	0.099	0.99
p,p DDE	05	121%	5.4	0.100	1.00
p,p DDT	03	NR	4.7	0.099	0.99
		Polychlorobiphenyl			
Aroclor1016	03	122%	4.7	0.01	0.10
Aroclor1221	05	145%	5.2	0.009	0.09
Aroclor1232	03	98%	6.0	0.01	0.10
Aroclor1242	03	172%	4.3	0.008	0.08
Aroclor1248	04	108%	5.4	0.011	0.11
Aroclor1254	03	134%	4.7	0.01	0.10
Aroclor1260	03	209%	5.2	0.01	0.10

of OCPs and PCBs in samples were determined and results summarized in Table 3-4. Heptachlor epoxies and Σ BHC were found to be the most dominant OCPs in this study. Other chlorinated pesticides like Dieldrin, DDTs, chlordane and Endrin were present at low concentrations in all samples as compared to the results for mean concentrations of OC pesticides determined in the fish from different parts of the world are shown in (Table 5). The fish (*Johnius carutta*) contained the

highest concentration of BHCs with an average value of 56 ng/g w.w, however, in gastropods, it was in slightly lower level, which is 54 ng/g w.w. The concentration of Σ BHC in pen shell (*Atrina pectinata*) was much lower that is 19 ng/g w.w. α -BHC was found in gastropods and crab (*Portunus pelagicus*), while β -BHC found in gastropods, crab, and the fish (*Johnius carutta*). The δ -BHC was found in *Johnius carutta* and γ -BHC in gastropods and fish (*Johnius carutta*) (Table 3).

Table 3. Level of organo chlorines in fish and shellfish (ng/g wet weight)

Organochlorines	Gastropod : <i>Babylonia spirata</i>	Fish : <i>Johnius carutta</i>	Bivalves : <i>Anadara antiquata</i>	Pen shell : <i>Atrina pectinata</i>	Crab : <i>Portunus pelagicus</i>	Clams : <i>Macra aequisulcata</i>
Aldrin	10.11±0.05	5.10±0.02	6.00±0.12	5.43±0.03	11.12±0.05	6.32±0.03
α -BHC	<LOD*	<LOD	<LOD	<LOD	2.63±0.01	<LOD
β -BHC	<LOD	12.30±0.02	12.10±0.03	<LOD	6.00±0.02	3.62±0.03
δ -BHC	9.15±0.05	20.31±0.02	<LOD	14.20±0.02	<LOD	1.80±0.01
γ -BHC	ND	<LOD	23.70±0.31	<LOD	<LOD	3.51±0.12
α -chlordane	10.95±0.05	19.36±0.20	0.00	6.20±0.07	<LOD	2.98±0.03
γ -chlordane	13.95±0.05	2.54±0.05	5.12±0.05	<LOD	10.25±0.05	<LOD
p,p-DDD	<LOD	ND	<LOD	<LOD	6.52±0.04	3.25±0.05
p,p-DDE	7.75±0.01	3.30±0.05	3.72±0.03	6.57±0.10	9.23±0.10	<LOD
p,p DDT	<LOD	<LOD	<LOD	<LOD	1.25±0.03	2.30±0.04
Dieldrin	5.47±0.01	5.00±0.04	4.52±0.01	4.21±0.01	<LOD	8.47±0.05
Endosulfan I	6.62±0.03	6.12±0.12	3.15±0.11	3.62±0.05	16.46±0.05	6.62±0.12
Endosulfan II	1.50±0.02	2.00±0.02	7.47±0.01	<LOD	<LOD	8.20±0.03
Endosulfan sulfate	ND	<LOD	<LOD	<LOD	2.53±0.05	<LOD
Endrin	7.53±0.01	<LOD	<LOD	5.27±0.12	<LOD	1.80±0.01
Endrin aldehyde	<LOD	<LOD	<LOD	<LOD	3.56±0.05	1.25±0.22
Endrin ketone	<LOD	<LOD	<LOD	<LOD	1.98	<LOD
Heptachlor	5.53±0.02	10.74±0.05	4.11±0.05	4.50±0.05	2.65±0.05±0.05	1.25±0.04
Heptachlor epoxide	<LOD	12.50±0.05	25.00±0.05	<LOD	<LOD	1.48±0.03
Methoxychlor	23.37±0.01	<LOD	<LOD	1.60±0.03	3.65±0.02	<LOD

* Lower detection limit.

Table 4. Results for concentrations of polychlorinated biphenyl (PCBs) in Fish and Shellfish (ng/g wet weight)

Organochlorines	Gastropod : <i>Babylonia spirata</i>	Fish : <i>Johnius carutta</i>	Bivalves : <i>Anadara antiquata</i>	Pen shell : <i>Atrina pectinata</i>	Crab : <i>Portunus pelagicus</i>	Clams : <i>Macra aequisulcata</i>
Aroclor1016	<LOD*	1.34±0.05	1.32±0.12	2.14±0.07	0.002±0.01	N.D
Aroclor1221	<LOD	ND	4.13±0.10	ND	0.20±0.01	3.05±0.08
Aroclor1232	236.90±0.72	<LOD	<LOD	5.96±0.04	0.01±0.01	5.40±0.10
Aroclor1242	0.147±0.01	ND	ND	7.12±0.05	7.40±0.10	0.02±0.01
Aroclor1248	40.00±0.52	11.3±0.05	127.67±0.15	0.03±0.01	4.30±0.02	45.17±0.03
Aroclor1254	ND	5.32±0.05	6.27±0.06	<LOD	4.13±0.03	0.04±0.01
Aroclor1260	8.45±0.18	5.37±0.04	8.68±0.08	0.03±0.01	<LOD	<LOD

* Lower limit.

Fishes have a significant correlation between the concentrations of the various OCPs as shown the correlation factors (r^2) (Fig. 5) and the significant correlation was calculated by r^2 value between matrix of organochlorines proportional to types of fish and shellfish were found on the site (Table 6).

The maximum level of heptachlor was determined found in all fish with an average of 4.61 ng/g, however its maximum concentration was found in crab that is 10.74 ng/g w.w (Table 3). Relativity in the concentration of Σ OCPs was found in order of Heptachlor epoxide > Σ BHC > Σ endosulfan > Σ chlordane. Total DDT concentration may be an indicator of all possible sources of the DDT because DDT is metabolized to DDE with a

ratio. A critical limit of the total value of DDT that is 120.30 ng/g has been reported in previous studies (Reis Souza *et al.*, 2013). A significant Σ DDT ratio individual compound of the same group (0.9) indicated that there was no recent inputs of DDT in the study environment. However, in crab (*Portunus pelagicus*), fish and other shellfish, this ratio was calculated in a sequence of 0.99, 0.24 and zero. It may be concluded that the Karachi coast at Clifton beach has no new significant DDT inputs as proved by the DDE / DDT ratio consideration. All fishes were found to be contaminated with chlordane group, for example, the pen shell (*Atrina pectinata*) contained 25 ng/g w.w, the highest concentrations of chlordane as similar to PCBs congeners, was determined in fish tissue. Summed concentrations of seven chlorobiphenyl congeners (Σ PCBs) ranged from 7.17 ng/g w.w. to 276.20 ng/g w.w, whereas, the lowest concentration in the pen shell (*Atrina pectinata*) and same level in fish (*Johnius carutta*) and the highest value in gastropods followed by the crab. The PCBs congeners 1248 and 1232 levels in the gastropods were markedly higher than those found in the other fish due to the significantly higher lipid content. The PCB congener's significant relative distributions with average concentrations are reported in the analyzed fish. Aroclor 1232, 1260 and 1254 are predominated up to 42.93%, 4.33% and 3.29% of Σ PCBs followed by the lowest ratio of Σ PCB as (3.27% and 1.12%) and individual Aroclor 1242 and 1016. The Σ PCBs concentrations were found to be in the same range in pen shell *Atrina pectinata*, fish (*Johnius carutta*), clams (*Mactra aequisulcata*) and crab (*Portunus pelagicus*). The Σ Aroclor was determined at a minimum level in the fish and maximum Σ Aroclor in gastropods however Aroclor 1016 was found in lowest amount (7.2 ng/g) or below the detection limit in gastropods as similar in case of pen shell, crabs, clams and fishes. The Aroclor 1221 concentration varied between 5.96-24.01 ng/g in crab and gastropods. It may be concluded that minimum Aroclor was found in fish and maximum in gastropods as 250.89 ng/g, however Aroclor congener patterns were the same in the fish (*Johnius carutta*), pen shell and bivalves. The, unlike congener profiles, has been demonstrated in other studies and the total Aroclor content does differ dramatically amongst fish and that Σ PCB differences among fish can vary with the site. Furthermore, the greater metabolic activity for the lower molecular chlorinated congeners has exhibited a clear trend of occurrence (Oyuna *et al.*, 2004) and the very low concentrations of PCBs are critical to recognize in fish samples. Bioaccumulation

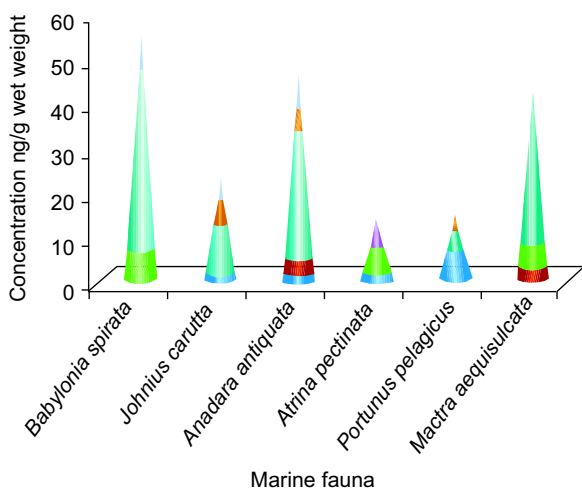


Fig. 4. Level of PCBs in fish and shellfish.

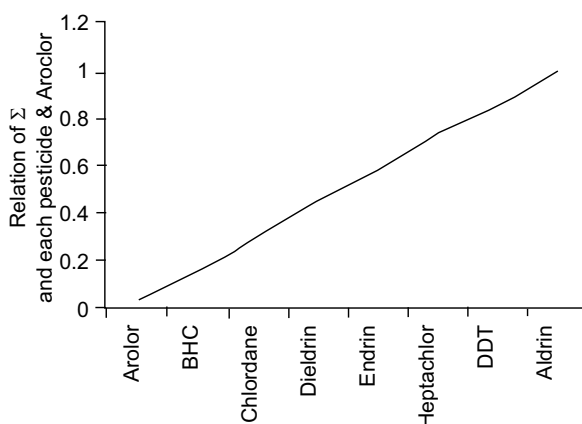


Fig. 5. Correlation (r) of sum of (OCP & Aroclor) and individual pesticide.

pollutants and/or feeding habits. Scientists and researchers are continuously doing their efforts to determine the accurate level of pollution and a meaningful progress in the development of analytical methodologies has been made during the last decade to determine OCPs in the environment and in biological tissues with 100% accuracy. Analyst and scientists preferred to use solid phase extraction technique in place of the Soxhlet extraction method with the application of advanced software. Results of the present study, has interpreted that OCPs and Archlor can be found in various environmental compartments at any time being the most prevalent environmental pollutants and extremely persistent and lipophilic in nature are widespread. The Persistent Organic Pollutants (POPs) bioaccumulate in the adipose tissues of fish resulting in the enrichment throughout the food chain (Shi *et al.*, 2013) and pollutants may interfere the normal physiology and biochemistry due to prolonged exposure (Wang *et al.*, 2012). The lipid composition is another factor that influenced the bioaccumulation of organo-chlorine compounds (Zorn, 1997). Thus, it is postulated that the feeding habit, metabolic ability, reproductive cycles and many other factors have impacts on contaminant burden of an organism.

Conclusion

The Σ Aroclors and Σ OCPs levels were detected in different fish and shellfish tissues from the Clifton beach and Sands pit along the Karachi coast. Concentrations of organochlorine contaminants in fish and shellfish gradually decreased as in crab (*Portunus pelagicus*) > pen shell (*Pinna pectinata*) > gastropods (*Babylonia spirata*) > fish (*Johnius carutta*) > crab. Σ Aroclors level also decreased in pattern of such as gastropods > crab (*Portunus pelagicus*) > fish (*Johnius carutta*) > pen shell (*Atrina pectinata*). The OCPs and Aroclors concentration were generally very low and fish tissue contained a very low concentration of individual OCP and Aroclor. However, values were found below the limit of detection in many fish and shellfish analyzed during this study. The distance from the anthropogenic source of contamination, atmospheric sources of pollution, and large dilution factor reflected relatively smaller concentrations. Scientific studies on of OCPs and PCBs being endocrine disrupter chemicals (EDCs) for possible occurrence in seafood have triggered the public concerns. It is necessary to control organochlorine and Aroclors pollution with serious efforts and with all possible remedial measures to be taken to control and stop dumping of

plastic and polymer wastes into the ocean which contain high levels of OCPs and PCBs. As, an issue of OCPs and PCBs pollution in the marine environment is still under discussion and currently there is no international legislation specifically to OCPs and PCBs in the fishery. Further research in this field is necessary.

Acknowledgment

Thanks are due to the Director, Centre of Excellence in Marine Biology, and the University of Karachi in proving the financial facilities in preparation of the manuscript.

Conflict of Interest. The authors declare no conflict of interest.

References

- Barker, S.A. 2007. A review article: matrix solid phase dispersion (MSPD). *Journal of Biochemical and Biophysical Methods*, **70**: 151-162.
- Dhananjayan, V., Muralidharan, S. 2013. *Bio Medical Research International*, **2013**: 190-353.
- EURACHEM, 1998. *EURACHEM Guidance Document No.1/WELAC Guidance Document No.WGD 2:1998*. Accreditation for chemical laboratories: Guidance on the interpretation of the EN 45000. Series of standards and ISO/IEC Guide 25, 1998. Available from the EURACHEM Secretariat, PO Box 46, Teddington, Middlesex, TW11 ONH, UK.
- ICH Q2B. 1996. *International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology*, ICH-Q2 (R1), 17 pp., Geneva 1996.
- Kannan, K., Kajiwara, N., Le Boeuf, B.J., Tanabe, S. 2004. Organochlorine pesticides and polychlorinated biphenyls in California sea lion. *Journal of Environmental Pollution*, **131**: 425-434.
- Kim, J., Sun, Q., Yue, Y., Yoon, K.S., Whang, K.Y., Marshall, C.J., Park, Y. 2016. Dichlorodiphenyl-trichloroethane (DDT) and 4,4'-dichlorodiphenyl-dichloroethylene (DDE) promote adipogenesis in 3T3-L1 adipocyte cell culture. *Pesticide Biochemistry and Physiology*, **131**: 40-45. <https://doi.org/10.1016/j.pestbp.2016.01>.
- Long, E.D., MacDonald, D.D. 1998. Recommended uses of empirically derived, sediment quality guidelines for marine and estuarine ecosystems.

- Human and Ecological Risk Assessment*, **4**: 1019-1093.
- Meijer, S.N., Ockenden, W.A., Sweetman, A., Breivik, K., Grimalt, J.O., Jones, K.C. 2003. Global distribution and budget of PCBs and HCB in background surface soils: implications for sources and environmental processes. *Environmental Science and Technology*, **37**: 667-672.
- Munshi, A.B., Perveen, F., Usmani, T.H. 2005. Accumulation of organochlorines in edible fishes and mussels from coastal waters of Karachi, Pakistan. *Journal of the Chemical Society of Pakistan*, **2**: 404-408.
- Ockenden, W.A., Breivik, K., Meijer, S.N., Steinnes, E., Sweetman, A.J., Jones, K.C. 2003. The global re-cycling of persistent organic pollutants is strongly retarded by soils. *Environmental Pollution*, **121**: 75-80.
- Oyuna, T., Tu Binh, M., Natsuko, K., Shinsuke, T. 2004. Recent contamination by persistent organochlorines in Baikal seal (*Phoca sibirica*) from Lake Baikal, Russia. *Marine Pollution Bulletin*, **48**: 749-58 DOI: 10.1016/j.marpolbul.2003.10.027.
- Rajendiran, S., Mahin, B., Iqbal, M., Sugumar, V. 2016. Induced thermal stress on *Portunus pelagicus*, *Biochemistry and Biophysics Reports*, **5**: 425-429.
- Ravindran, J., Pankajshan, M., Puthur, S. 2016. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinary Toxicology*, **9**: 90-100 doi: 10.1515/intox-2016-0012.
- Reis Souza, M.R., dos Moreira, C.O., de Lima, T.G., Aquino, A., Dórea, H.S. 2013. Validation of a matrix solid phase dispersion (MSPD) technique for determination of pesticides in lyophilized eggs of the chicken *Gallus gallus domesticus*. *Microchemical Journal*, **110**: 395-401 DOI: 10.1016/j.microc.2013.05.001.
- Ritter, R., Scheringer, M., MacLeod, M., Hungerbühler, K. 2011. Assessment of non occupational exposure to DDT in the tropics and the north: relevance of uptake *via* inhalation from indoor residual spraying. *Environmental Health Perspectives*, **119**: 707-712.
- Shi, J., Li, Y., Liang, H., Wu, Y., Liu, W. 2013. OCPs and PCBs in marine edible fish and human health risk assessment in the eastern Guangdong, China. *Archives of Environmental Contamination and Toxicology*, **64**: 632-642.
- Wang, G., Peng, M., Zhang, Q., Lewis, J., Lacey, M., Furukawa, Y., O'Reilly, S.E., Meaux, S., McLachlan, J., Zhang, S. 2012. Endocrine disrupting chemicals in new Orleans surface waters and Mississippi sound sediments. *Journal of Environmental Monitoring*, **14**: 1353-1364.
- Zorn, M.E., Gibbons, R.D., Sonzogni, W.C. 1997. Weighted least-squares approach to calculating limits of detection and quantification by modeling variability as a function of concentration. *Analytical Chemistry*, **69**: 3069-3075.