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Original Research

Study of the Polychlorinated Biphenyls (PCBs) Contamination in Barnacles and Sediments in Caspian Sea Commercial and Recreative Ports

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Abstract

Polychlorinated Biphenyls (PCBs) are mutagenic, carcinogenic, and persistent organic pollutants of the marine and coastal environment. No previous research has precisely used barnacles as potential PCBs bioindicators in the Caspian sea. The present survey was done to add the PCBs concentrations amongst the barnacle and sediment samples collected from 5 important Iranian ports besides the Caspian sea. Barnacle and sediment samples were collected from Astara, Anzali, Caspian, Kiashahr, and Chamkhaleh ports. Samples were transferred to the laboratory and analyzed for the presence and concentration of PCBs using GC-MS. Eighty different types of PCB were detected in the examined samples. PCBs concentration ranged from 02.00±00.10 to 53.00±00.60 ng/g. PCB-77 (53.00±00.60 ng/g in Chamkhaleh, 53.00±01.20 ng/g in Caspian, 53.00±02.40 ng/g in Anzali, and 53.00±2.7 ng/g in Astara ports) had the highest concentration in barnacle samples. PCB-52 (53.00±00.70 ng/g in Chamkhaleh port) had the highest concentration in sediment samples. PCB-114 had the lowest concentration amongst the barnacle (02.00±00.10 ng/g in Astara port) and sediment (03.00±00.20 ng/g in Anzali port) samples. This study is the first report evaluating the presence and concentration of PCBs in barnacle in the

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Caspian Sea. The role of barnacles and sediments as biomonitors of PCBs was determined. It seems necessary to set strict rules to prevent pollutants from entering the Caspian Sea.

Keywords: Polychlorinated Biphenyls (PCBs), concentration, Caspian Sea, sediment, barnacle

Introduction

Marine coastal areas are ecologically affected by human activities, so managing and protecting the coastal environment, which includes a sensitive and rich ecosystem in terms of different biological species, is considered a complex challenge [1]. Humans directly and indirectly, through measures such as the development of urban and industrial areas and agricultural activities, release various pollutants in aquatic ecosystems, which have adverse effects on the quality of aquatic habitats and health [2].

Compounds of Polychlorinated Biphenyls (PCBs, C12H10-nCln) are carcinogenic, mutagenic, and persistent organic environmental pollutants [3]. Because of their persistence, long-term transport in an atmosphere, and a high potential for bioaccumulation, toxicity, and nonbiodegradability, they pose thoughtful threats to human health and ecosystems [4, 5]. PCB compounds are transferred in the aquatic food chain more than terrestrial ones, therefore their highest concentration has been reported in natural environments, marine mammals, fish-eating birds, and fish [6]. These compounds tend to accumulate in the food chain due to their stability and lipophilicity. These compounds accumulate in the animal's bodies, particularly fish and shellfish, and as a result, people who consume these foods are exposed to PCBs [7]. Consumption of food containing PCBs may cause toxicity, neurological and reproductive disorders, and mutation and cancer in long-term exposure and consumption [8, 9].

Although the manufacture of PCBs has reportedly been banned, the potential or possibility of their entry into the environment still exists. The physical and chemical properties of PCBs, including high-temperature stability, chemical inertness, non-flammability, high electrical resistance, etc., have led to their increasing use in the industry [10]. It has been recorded that 150 of 209 isomers of these compounds have been reported in the environment [11]. In addition to the PCBs toxicity, there is also the risk of producing dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds as a result of their incomplete oxidation [12].

The Caspian Sea is the largest closed water area in the world, and due to its size and location in several peninsulas, diverse ecosystems have been created in its different areas [13]. The rocky shores of the southern basin of the Caspian Sea have existed in two forms, natural and artificial, and the artificial beaches were created with goals such as preventing the rise of the sea level, building harbors, and other various marine structures [14]. From an ecological view, rocky shores are a very good model for studying the biological changes of the seas, and due to the direct influence of coastal currents and waves, they are considered a rich source of nutrients, oxygen, and sediments [15]. Rocky shores are home to many different populations of mollusks, bivalves, barnacles, seaweeds, algae, and other biological groups with adhesive ability [16]. Barnacles (*Amphibalanus improvisus*) have a global distribution. Species on decapod gills and free-living groups are adapted to clinging to other organisms and to hard substrates and artificial structures [17]. They have been used as heavy metals biomonitors in aquatic environment previously [18], nevertheless, no earlier research has exactly used barnacles as possible PCBs biomonitors. Barnacles are crustaceans which have many attributes to be a good biomonitor such as they are widespread and abundant in all seasons worldwide, having a sessile adult life, easy to collect, relatively tolerant to contaminants, capable of biomagnifying contaminants and reflecting changes in contaminant levels in the aquatic environment [19].

The coastal sediments of the Caspian Sea are affected by various agricultural activities, shipping, petrochemical industries, oil extraction, and industrial settlements, each of which has a significant contribution to the pollution of the region. Thus the present survey was done to investigate PCBs accumulation in barnacle and sediment biomarkers in the main ports of the Caspian Sea in the coastal lines of Gilan province, Iran.

Materials and Methods

Study Area

The Caspian Sea is the largest lake on the planet, which is called a sea due to its geographical and ecological features. This lake is located at 41.9350ºN and 50.6689ºE longitude and latitude. Fig. 1 shows the study location and stations. Totally, Astara. Anzali, Caspian, Kiashahr, and Chamkhaleh stations were selected to assess the PCBs concentration among barnacles and sedimentation samples (Fig. 1).

Table 1 shows the geographical properties of 5 selected stations.

In this research, a station in each coastal port and 3 repetitions in each station were considered to reduce the error rate. In the case of sediment and barnacles, each one was taken separately and sampled.

Sampling Procedure

First, preliminary action was taken in the spring season to visit and identify the sampling site and

Fig. 1. Study location and stations.

determine and record the stations accurately using GPS (Garmin 600, USA). Then, with the warming of the weather and the blooming of barnacles in the summer season and the months of August and September, the sampling operation was carried out with the appropriate sampling tool using the Van Veen Grab for sedimentation and choosing the standard sampling method for the biomarker. In this research, 5 stations were considered and in each station, 3 repetitions were randomly done for sediment and 3 repetitions for barnacles, and a total of 30 samples were collected. After collection, the samples were first washed with seawater and then with distilled water and stored in aluminum containers at -4ºC. All samples were transferred to the laboratory.

Table 1. Geographical properties of 5 selected stations.

Station name	Geographical properties
Astara port	X = 314207 , Y = 4251734
Anzali port	X = 365786 , Y = 4149059
Caspian port	X =382353 , Y = 4146471
Kiashahr port	X = 409098 , Y = 4143476
Chamkhaleh port	X = 435711 , Y = 4119098

Sample Preparation

Before extracting, the sediment and barnacle samples were frozen for 48 hours using a freeze dryer (Christ Alpha 1-2, Germany) until the samples were completely dry, then the sediment samples were filttered using 250 m filter. The barnacle samples were also ground.

PCBs Determination in Sediments and Barnacles

PCBs extraction method from sea sediments and barnacle tissue was done using an ultrasonic probe. Briefly, 5 g of granulated sample is transferred to a 40 mL vial. To obtain extraction recovery, 60 µl of 10 ppb standard surrogate (PCB-209) was added to the sediment inside the vial. Then, the sediment was added 3 times with 20 ml of a 50:50 mixture of dichloromethane: acetone solvents, and the sediment was exposed to ultrasonic waves from the ultrasonic probe for 5 min. Then, using a centrifuge, the sediment was separated and the extracting solvent was collected in a 100 mL rotary flask. To fully extract PCBs, the extraction and decantation steps were repeated three times. Then, the solvent inside the balloon was dried using a rotary, then the rotary balloon was washed with 5 mL of n-hexane, and the resulting solution was transferred to the clean-up stage.

To clean up materials, some glass wool was transferred into the Pasteur paste, then 500 mg of silica gel was transferred into the pipette. Before passing the extracted solution, the silica gel stationary phase was conditioned with 3 mL of n-hexane. Then, the extracted solution was passed through the column and the column was washed again with 10 mL of n-hexane and the resulting solution was collected inside a vial. Finally, the resulting solution was brought to a volume of 200 µL using N^2 blowing. To analyze the compounds of PCBs, one microliter of the resulting solution was injected into the GC-MS device and 18 isomers of PCB compounds were obtained.

GC-MS Analysis

A GC device (Agilent Technologies 6890N, USA) coupled to MS (Agilent Technologies 5973N, USA) equipped with the Rxi 5ms column (30 m long × 0.25 mm i.d × 0.25 µm df) was used to determine the PCBs concertation amongst the examined samples. The oven temperature program is shown in Table 2. The carrier gas was helium, and the flow rate was 1 mL/min [20].

Data Analysis

Analyzing the test results of sediment and barnacle samples was done using Excel, SPSS, and Anderson-darling statistical analysis.

Results and Discussion

The present study was done to assess the PCBs concentrations amongst the sediment and barnacle samples collected from 5 important ports of the Gilan province, Iran (Caspian Sea).

Table 3 shows the percent recoveries, limit of detection (LOD) and limit of quantitation (LOQ) values of PCBs. Table 4 shows the PCBs concentrations amongst the barnacle and sediment samples collected from different ports. The findings showed that PCBs concentration in examined samples had a range between 02.00±0.10 to 53.00±00.60 ng/g. Amongst the barnacle samples, PCB-77 (53.00±00.60 ng/g in Chamkhaleh, 53.00±01.20 ng/g in Caspian, 53.00±02.40 ng/g in Anzali, and 53.00±02.70 ng/g in Astara ports), had the highest concentration, while PCB-114 (02.00±00.10 ng/g in Astara port) had the lowest. Amongst the sediment samples, PCB-52 (53.00±00.70 ng/g in Chamkhaleh port) had the highest concentration, while PCB-114 (03.00±00.20 ng/g in Anzali port) had the lowest. Statistically significant differences were observed

Table 2. GC-MS oven temperature program.

Stage		Temperature (ºC)
GC	Initial	130ºC (2 min hold)
	Increased at 15ºC/min	160ºC
	Increased at 5ºC/min	300ºC (30 min hold)
MS transfer line	300ºC	
Ion source	230ºC	
Quadrupole	150ºC	
Injector port	270ºC	

Table 3. The percent recoveries, LOD and LOQ values of PCBs.

PCB types	Revocery rate (%)±RSD[a]	LOD[b] (ng/g)	LOQ[c] (ng/g)
PCB-28	88.01±04.80	0.60	2.00
PCB-52	85.24±02.40	0.60	2.00
PCB-81	75.51±17.70	0.40	1.30
PCB-77	83.20±05.90	0.40	1.30
PCB-101	79.50±07.10	0.60	2.00
PCB-105	83.12±12.50	0.40	1.30
PCB-114	86.37±03.90	0.40	1.30
PCB-118	86.93±07.20	0.60	2.00
PCB-123	95.26±08.60	0.80	2.70
PCB-126	85.35±09.70	0.40	1.30
PCB-138	82.42±14.60	0.60	2.00
PCB-153	76.31±15.10	0.60	2.00
PCB-156	80.20±05.50	0.80	2.70
PCB-157	81.40±03.90	0.60	2.00
PCB-167	78.30±08.20	0.4	1.30
PCB-169	80.60±05.70	0.80	2.70
PCB-180	79.90±10.10	1.60	5.00
PCB-189	90.60±14.20	0.60	2.00

[a]Relative standard deviations
[b]Limit of detection
[c]Limit of quantitation

Table 4. PCBs concentrations amongst the barnacle and sediment samples collected from different ports.

PCB types	Concentrations in samples collected from different ports (ng/g)									
	Astara		Anzali		Capian		Kishahr		Chamkhaleh	
	Barnacle	Sediment	Barnacle	Sediment	Barnacle	Sediment	Barnacle	Sediment	Barnacle	Sediment
PCB-28	50.00±01.20a*	19.00±00.20c	47.00±01.20a	53.00±01.00a	50.00±01.10a	53.00±02.70a	53.00±02.50a	53.00±02.70a	50.00±02.20a	37.33±01.90b
PCB-52	44.00±01.70b	52.33±01.20a	44.00±01.30b	48.83±01.40a	47.00±00.80a	50.00±02.50a	41.00±01.40b	50.00±01.80a	47.00±01.10a	53.00±00.70a
PCB-101	27.30±01.20c	47.00±±00.90a	23.00±01.60c	34.67±01.10b	11.33±00.70d	28.33±00.80c	11.00±00.50d	33.83±00.50b	07.17±00.20e	35.67±02.70b
PCB-81	47.00±01.50a	44.00±01.80a	50.00±02.30a	48.17±00.70a	32.00±01.60b	47.00±01.70a	38.00±01.20b	44.00±01.50a	41.00±00.40a	12.33±00.20c
PCB-77	53.00±02.70a	34.83±01.90c	53.00±02.40a	31.83±00.80c	53.00±01.20a	12.67±0.50f	47.00±01.50b	19.17±00.60e	53.00±00.60a	28.50±01.80d
PCB-123	23.00±00.50c	38.33±01.30a	26.00±00.40c	40.00±00.20a	23.00±00.10c	38.67±000.60a	33.33±00.20b	25.00±00.40c	26.00±00.90c	27.33±00.50c
PCB-118	38.00±00.60c	26.33±00.80d	36.67±02.20c	24.50±00.50d	38.67±01.20c	20.67±00.80d	50.00±01.30a	37.50±01.20c	44.00±00.20b	44.33±00.40b
PCB-114	02.00±00.10d	04.50±00.20d	02.67±00.10d	03.00±00.20d	03.17±00.10d	04.83±00.20d	33.67±01.90a	11.17±01.00c	26.33±01.50b	04.33±00.30d
PCB-153	32.00±01.10a	31.50±01.50a	29.00±00.50a	30.33±01.70a	4.33±00.20d	24.00±01.30b	14.00±00.20c	5.67±00.10d	13.83±00.50c	11.33±00.30c
PCB-105	10.33±00.70c	23.00±00.60b	20.00±00.60b	12.17±00.40c	27.33±01.30b	12.83±00.20c	20.00±00.10b	11.17±00.70c	34.67±00.20a	07.33±00.50d
PCB-138	41.00±01.20a	40.67±02.70a	38.33±01.20a	35.33±01.20a	35.00±01.90a	44.00±01.40a	05.00±00.40c	30.67±01.30b	24.00±00.80b	28.00±00.70b
PCB-126	27.67±00.70c	50.67±02.80a	39.00±02.30b	44.00±00.50b	44.00±01.10b	35.50±00.50c	44.00±00.60b	47.00±00.90b	32.33±01.00c	50.00±00.50a
PCB-167	16.83±00.20b	11.33±00.50c	13.67±00.10c	9.00±00.20d	17.00±00.80b	8.00±00.20d	25.67±01.70a	13.50±00.80c	13.17±00.50c	22.67±01.20a
PCB-156	06.17±00.20b	07.33±00.50b	04.33±00.30c	04.00±00.20c	08.83±0.40b	02.17±00.20d	02.00±00.10d	03.50±00.10c	02.00±00.10d	12.33±01.10a
PCB-157	13.00±01.10d	13.50±01.20d	8.00±00.60e	14.83±01.10d	12.33±00.60d	30.17±02.10a	17.00±00.70c	21.00±01.50b	5.83±00.30e	11.33±01.10d
PCB-180	08.67±00.50d	03.33±00.20e	12.67±00.40c	27.33±01.70b	20.00±00.10b	26.83±02.30b	08.00±00.50d	40.33±00.30a	16.00±00.60c	46.67±00.60a
PCB-169	20.00±01.20b	29.33±00.50b	32.00±00.50a	18.17±01.30b	27.67±00.10b	39.83±01.90a	23.33±00.90b	15.83±01.40c	38.00±00.80a	22.33±00.80b
PCB-189	35.00±00.70b	18.00±00.90c	15.67±00.30c	15.83±01.50c	40.33±00.40a	16.50±01.20c	29.00±01.70b	32.67±01.10b	20.67±00.10c	40.17±02.20a

*Dissimilar small letters in each row show statistically significant differences about $P<0.05$.

between PCBs concentration and types of samples ($P<0.05$).

The strategic position of the Caspian Sea, including its rich source of oil and gas and commercial source of diverse kinds of fish and shrimp, makes it important to assess the ecological properties. Rendering the huge volume of oil and gas exchanges, as well as the activities of ships and factories around the Caspian Sea, the possibility of contamination of the ecosystem of this sea with various types of environmental pollutants, especially PCBs, is very high. As PCBs can be filtered by filter feeders from water and according to their accumulation in lipid-rich tissues of aquatic organisms, these organisms can be used as bioindicators of persistent PCBs pollutants in marine and coastal environments [21]. The majority of the preceding investigations used to fish and shellfish species as PCBs bioindicators [7, 22, 23]. However, in the present survey, barnacles and sediment of the Caspian sea were used to assess the PCBs concertation. Barnacles have been used as heavy metals bioindicators in the marine environment beforehand [24], nevertheless, no previous research has precisely used barnacles as potential PCBs bioindicators in the Caspian sea. Extensive and plentiful distribution in all seasons globally, a sessile adult life, easy collection procedure, tolerant nature against contaminants, and the property of reflecting the existing contaminant conditions make barnacles to be the best bioindicator of marine and coastal environment [25].

The findings of the present survey showed that the sediment and barnacle samples collected from ports near the Caspian Sea were polluted with diverse classes of PCBs. In some of the studied ports, the sediment samples and others the barnacle samples were more polluted in terms of a specific type of PCBs. Therefore, no regular pattern was seen in this case. Additionally, no regular results were obtained regarding which of the sediment or barnacle samples had more PCBs pollution. However, PCB-77 and PCB-52 amongst the barnacle and sediment samples were the most frequently identified, respectively. Revelry, PCB-114 amongst both barnacle and sediment samples had the lowest distribution. PCBs concentration in examined samples had a range between 02.00±00.10 to 53.00±00.60 ng/g.

Because of the high diversity of PCBs type analyzed in previous investigations, along with dissimilar measurement units and methods, and finally, the absence of published works about the PCBs concentrations in barnacle samples, comparing the findings of the present research with previous studies is complicated and could not be reasonable. In this regard, a survey conducted in China [26] showed that the dioxin-like (DL)-PCBs concentration amongst the fish samples was 00.88 ng/g wet weight, which was lower than our examined rate in the majority of cases. The PCBs concentration measured in barnacle and sediments of the Caspian sea, Iran (02.00±00.10 to 53.00±00.60 ng/g dw) was high compared to other aquatic environments, including Chapala Lake, Mexico (09.00-27.00 ng/g) [27], Larak Island, Persian Gulf, Iran (02.95-07.95 ng/g) [28], Southern Yellow Sea, Western Pacific Ocean (00.51-05.84 ng/g) [29], Indian sea, Hugli estuary (00.18-02.33 ng/g) [30], Belgium (00.03-03.10 ng/g) [31], and Japan (0.04-00.64 ng/g) [32]. However, PCB's concentrations were low compared to values found in surveys conducted in China Pearl river (16.15-477.85 ng/g) [33], San Diego Bay, USA (23.00-1387.00 ng/g) [34], and Bangladesh (32.17-199.40 ng/g) [35]. A survey conducted in Anzali port, Caspian sea, Iran [36], showed that The mean DL-PCBs concentration amongst the collected fish samples ranged from 232.00±16.00 to 1156.00±14.00 pg/g lipids. Another Iranian survey conducted in Gilan province, Caspian Sea, Iran [37] showed that the mean PCBs concentration amongst the land uses samples ranged from 05.10±00.00 to 28.00±07.00 µg/kg. Vaezzadeh et al. (2021) [38] showed that PCBs concentrations amongst the barnacle samples collected from Malaysia ranged from 07.90±00.00 to 300.00±00.00 ng/g. Similar PCBs profiles have been reported in previous investigations. In this regard, PCB-101, PCB-138, and PCB-153 were also identified in Philippine oyster [39], Spanish bivalve [40], European mussels [41], and Brazilian bivalve [42] samples. PCB-28 was also detected in Chinese mollusk [43], while PCB-52 and PCB-101 were detected in Ghana bivalve [44] samples. PCB-87 and PCB-101 were also detected in Thai mussels [45] and PCB-180 was detected in Senegal mollusks [46]. Similar to our research, PCB-52 and PCB-77 were detected in samples collected from China [47, 48], Egypt [49], Saudi Arabia [50], Korea [51], and the United States [52].

Sewage outfalls, surface runoff, shipping activities, and wastewater discharges have been identified as the main contributors to PCBs distribution in seawater. Such differences found in the PCBs concentration between diverse investigations may be due to differences between geographical origin, environment condition, the season of sampling, type of collected samples, organism's lipid content, living and feeding habits, method of analysis, types of detected PCBs, and biodegradability, water solubility, and chlorinated levels of PCB congeners in the aquatic ecosystem.

Conclusions

In the present study, barnacles for the first time as PCBs bioindicators in the Caspian Sea were assessed. The findings showed that samples of all examined 5 ports besides the Caspian Sea were polluted with PCBs. Relatively high PCBs concentrations amongst the examined samples may also suggest the high contamination rate of the marine and coastal environments, fish, and other aquatic organisms of this region, which may pose an important public health issue. This, coupled with the increasing environmental pollution around Gilan province, the Caspian Sea, could become a concern for its population that traditionally consumes large quantities of fish. Thus, it is essential to determine the limitations of the PCBs concentrations in both barnacles and sediments as biomonitors to assess the quality of the ecosystem. Because the season, sampling location, and organism species were not similar in the present research and the previous studies, supplementary surveys on PCB levels in barnacles and sediments from similar locations are essential.

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Conflict of Interest

The authors declare no conflict of interest.

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