Gas Chromatography Determination of the Levels of Polychlorinated Biphenyls (PCBs) in Seawater and Sediment in the Gulf of Aqaba

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The levels of persistent organic pollutants, polychlorinated biphenyls (PCBs), were determined in seawater and marine sediments from different sites along the Jordanian coast of the Gulf of Aqaba. Concentrations of 7 PCBs, namely, PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180, were determined. An automated Soxhlet (Soxtec) extraction method was used for extraction with hexane-acetone as a solvent, and a pre-washed multilayer silica gel column was used for the clean-up step. Samples were analyzed using capillary gas chromatography (GC) with an electron capture detector (ECD) and GC–mass spectrometry (GC–MS) for confirmation. The method's limits of detection (LOD) were determined to be from 0.40 to 1.53 ng/L and from 0.39 to 0.91 ng/g dry weight for seawater and sediment, respectively. Concentrations of PCBs in seawater and sediment samples from all sites were below the LOD. This study provides evidence that very low concentrations of PCBs (<2 ng/g) were found in the water and sediments of the Jordanian coast of the Gulf of Aqaba. PCB concentrations measured in this study can be considered as a baseline for future monitoring and control of PCBs as requested by the Stockholm Convention.

Keywords: polychlorinated biphenyls, gas chromatography, Gulf of Aqaba

1. Introduction

Polychlorinated biphenyls (PCBs) are persistent organic pollutants with significant bioaccumulation potentials in environmental systems [1]. These compounds are frequently detected in a wide variety of environmental matrices such as sediments, soils, biota, water, and air, in both industrial and nonindustrial areas [2]. The synthesis of PCBs was first described in 1881 [3], while their occurrence in the environment was first recognized in biological samples in 1966 [4]. PCBs have long been identified as harmful substances due to their toxicity [5].

The Stockholm Convention [6] registered 12 contaminant group species of persistent organic pollutants (POPs) and prioritized global restrictions and bans in order to limit their impacts over the global environment. These 12 contaminant groups may be allocated into 3 categories: by-products of other processes (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans [PCDD/Fs]), industrial chemicals (PCBs), and organochlorine pesticides (OCPs).

The empirical formula for PCBs is \( C_{12}H_{10-n}Cl_n \), where \( n \) may be any value from 1 to 10. They are made up of 2 phenyl rings with 10 attachment possible points for chlorine atoms. A chlorine atom may be present at a single attachment point or several points, where up to 209 compounds combinations are possible. These combinations are known as congeners [7]. PCBs with 5 or more chlorine atoms per molecule are referred to as “higher chlorobiphenyls” and are relatively more persistent in the environment than the “lower chlorobiphenyls” [7]. For use in industry, multiple congeners were used, and this mixture is most commonly known as an Aroclors, which are numbered (e.g., 1242, 1260, and 1254). The numbers describe the percentage of chlorine by weight, thus Aroclor 1254 is 54% chlorine by weight. The more chlorinated the Aroclors, the more toxic and persistent [7].

PCBs can be among the most abundant and persistent pollutants in the global ecosystem [8]. The principal route of PCB transport to the marine environment begins in streams that are influenced by industrial runoff, where the PCBs are moved downstream by means of solution and re-ad sorption into particles. These particles may also be transported by the sediment itself, until finally reaching estuaries and coastal waters. The marine environment appears to be the ultimate and major sink for PCBs. Recorded levels in the marine environment found levels of 0.05–0.6 ng/L [8].

In the Gulf of Aqaba on the Jordanian coast, there is no previous assessment of PCBs. However, 2 studies investigated the distribution of organochlorine pesticides, where one of the persistent organic pollutants is PCBs [9, 10]. The authors found a range of 0.6–4.7 μg/L for those pesticides in the sediment. This study aimed to estimate the levels of PCBs in seawater and marine sediment in different sites along the Jordanian Coast of the Gulf of Aqaba to establish baselines for these harmful compounds.

2. Materials and Methods

2.1. Study Site and Sample Collection. The study area was selected to comprise different anthropogenic activities along the Jordanian coast of the Gulf of Aqaba (Figure 1). There were a total of 12 sites examined in this study: the ‘tourist sites’, which included Ayla Oasis project (AP), Hotel Area (HA), Royal Yacht Club (RVC), Marine Park Area (MPA), and the study site was selected to comprise different anthropogenic activities along the Jordanian coast of the Gulf of Aqaba (Figure 1). There were a total of 12 sites examined in this study: the ‘tourist sites’, which included Ayla Oasis project (AP), Hotel Area (HA), Royal Yacht Club (RVC), Marine Park Area

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(MPA), and Tala Bay (TB); the ‘port sites’ included Main Port (MP), and Aqaba Container Terminal (ACT); the ‘industrial sites’, which included Old Aqaba Thermal Power Plant (OTP), Oil Terminal (OT), New Aqaba Thermal Power Plant (NTPS), and Industrial Complex Jetty (ICJ); and finally, the Marine Science Station (MSS), which functioned as a reference site because it is located in a marine reserve.

Seawater samples were collected from the surface water using a 1-L glass container. Water samples were only collected from 4 sites because of the expected very low concentration. These are HA, MSS, TB, and ICJ (Figure 1). The samples were kept in an ice box until arrival at the laboratory, where they were stored at 4 °C until analysis [11]. Sediment samples were collected from the 12 sites from 10-m water depth by SCUBA divers. Duplicate samples from each site were collected from the top layer of the sediment (0–5 cm) with a dedicated metal scoop and placed in a 500-mL amber glass container with a Teflon lid. After collection, sediment samples were stored in aluminum foil and transported within 1 h to the laboratory. Upon arrival at the laboratory, the samples were passed through a 2-mm sieve to remove any pebbles and twigs, mixed thoroughly, and then stored at −20 °C until analysis [11].

2.2. Analysis. The analysis of PCBs in seawater and sediment was performed using a gas chromatograph (GC) with a narrow-bore fused silica column and an electron capture detector (ECD); GC–mass spectrometry (GC–MS) was used for confirmation. This analysis is based on US Environmental Protection Agency (EPA) Methods SW846/3510C/3541/8000B/8082 [12]. Extracts were subjected to clean-up procedures (Florisil, tetrabutylammonium [TBA] sulfate, sulfuric acid) based on US-EPA/SW-846 Methods 600C/3620C/3660B/3665A [12].

2.2.1. Solvents and Reagents. All solvents, glass-wool, and filter paper used are of pesticide residue analysis grade. Anhydrous sodium sulfate was heated at 400 °C for 4 h, cooled, and stored in a closed container. A 6 mL Florisil cartridge of 1 g sorbent (Supelco CAT # 57155 or equivalent) was used for clean-up.

2.2.2. Standards. All standards were obtained from AccuStandard (New Haven, USA), including PCB stock calibration standards: 100 μg/mL individual native PCB congeners (IUPAC No. PCB-28, 52, 101, 118, 138, 153, and 180), surrogate stock standard: 300 μg/mL native PCBs (IUPAC No. PCB-30), and PCB internal standard solution: 100 μg/mL native PCBs (IUPAC No. PCB-209).

PCB matrix spike working solution was prepared to get 1 μg/mL in n-hexane stock PCB matrix spiking solution, PCB 30 surrogate working solution was prepared at a concentration of 3 μg/mL in n-hexane, and a solution containing PCB-209 at a concentration of 1 μg/mL in n-Hexane was prepared as the internal standard solution. Six solutions were prepared as PCB working calibration standards: 1, 5, 10, 25, 50, and 100 ng/mL in n-hexane. Each calibration standard contains surrogates at a concentration range of 75 ng/mL in n-hexane and contains PCB internal standard compounds at a concentration of 25 ng/mL in n-hexane.

2.2.3. Apparatus. An automated Soxhlet (Soxtec) extraction system (FOSS – model 2050, Sweden), a rotary evaporator (Strike – Steroglass, Italy), and a gas chromatograph equipped with a glass-lined injection port were used for extraction. Sample analysis was performed using an Agilent 6890 GC mass spectrometer (Agilent technology model No 5975B inert XL MSD, USA) and a fused silica capillary column (DB-5MS: 30-m long 0.25-mm id, 0.5-μm film thickness).

2.2.4. Sample Extraction and Clean-up. For extractions, a 500-mL seawater sample was spiked with 25 μL of the 3 μg/mL surrogate working solution (PCB-30) and transferred into a 1-L separatory funnel. After the addition of 60 mL methylene chloride, the funnel was shaken for 2 min with periodic venting to release excess pressure. The organic bottom layer was separated from the liquid phase. The organic extract was
Table 1. Method detection limits (2 g/L) for the investigated PCBs for seawater and sediment

| Congress | Matrix       | Detection Limit (ng/L) |
|----------|--------------|------------------------|
| 2,4,5'-trichlorobiphenyl | Water         | 0.99                   |
| 2,2',5,5'-tetrachlorobiphenyl | Water        | 0.80                   |
| 2,3',4',4'-pentachlorobiphenyl | Water        | 0.97                   |
| 2,2',4,4,5,5'-hexachlorobiphenyl | Water       | 0.40                   |
| 2,2',3,4,4,5,5'-heptachlorobiphenyl | Water | 1.50                   |
| 2,2',3,4,4,5,5'-heptachlorobiphenyl | Sediment   | 0.53                   |
| 2,2',3,4,4,5,5'-heptachlorobiphenyl | Sediment   | 0.91                   |
| 2,2',5,5'-tetrachlorobiphenyl | Sediment    | 0.67                   |
| 2,2',4,4,5,5'-pentachlorobiphenyl | Sediment   | 0.39                   |
| 2,2',4,4,5,5'-pentachlorobiphenyl | Sediment   | 0.59                   |
| 2,2',4,4,5,5'-pentachlorobiphenyl | Sediment   | 0.50                   |
| 2,2',3,4,4',5,6-hexachlorobiphenyl | Sediment  | 0.53                   |
| 2,2',3,4,4',5,6-hexachlorobiphenyl | Sediment   | 0.40                   |

filtered through anhydrous sodium sulfate and then collected in a 250-mL round bottom flask.

The extract was then concentrated to 2–3 mL using a rotary evaporator under a stream of nitrogen gas. The solvent was replaced by adding 10 mL acetone and evaporated to below 2 mL. Another solvent replacement was performed using 10 mL hexane and evaporated to 1 mL hexane. The extract was then purified on a Florisil cartridge (1 g sorbent, 6 mL cartridge). Finally, 25 μL of the 3 μg/mL internal standard working solution (PCB-209) was added, and the volume was adjusted to 1 mL hexane. The samples were analyzed using GC–ECD and confirmed by GC–MS.

For sediment samples, 10 g of dry sediment was mixed with 10 g of anhydrous sodium sulfate and then spiked with 25 μL of the 3 μg/mL surrogate working solution (PCB-30). The sediment sample was then extracted with 80 mL (1:1) of acetone–hexane for 2 h in a Soxtec apparatus. The solvents were evaporated to 2–3 mL n-hexane under vacuum. The extracts were then combined and desulfurized by using tetrabutylammonium (TBA) sulfite clean-up. Humic acids were removed from the extracts by concentrated H2SO4 (98%). This step was repeated several times until the n-hexane layer became colorless. The extracts were concentrated under a gentle nitrogen stream to 1 mL and were further purified on a Florisil cartridge (1 × 6 mL). After that, 25 μL of the 3 μg/mL internal standard working solution was then added (PCB-209), and the volume was adjusted to 1 mL hexane for GC analysis.

2.2.5. Qualitative and Quantitative Analysis. Sample preparation, clean up, and analysis were conducted based on USEPA Methods SW846 /3510C/3541/8000B/ and 8082 [12].

Qualitative determination and quantification methods were used for the target compounds. They were identified with an electron capture detector (ECD) according to their identified corresponding congener retention time (RT) using calibration standards, and GC–MS was used for confirmation of all samples.

Quantitative determination was carried out based on the relative peak area and the relative concentration. The average concentration for 2 analytical trials was considered as the final result.

2.2.6. Evaluation of Total Analytical Procedures. The recovery percentage of the compounds under investigation was determined using matrix spiking techniques of PCB-free seawater and thermally clean sand samples with a reference standard mixture of the target compounds (native) and internal standards. The analysis of the samples was performed per USEPA reference standards [12].

The mean recovery of PCBs from the spiked seawater samples varied from 87% to 95%, while the mean recovery of PCBs from spiked sediment samples varied from 80% to 94%.

3. Results and Discussion

3.1. Detection Limits. The limit of detection (LOD) was calculated as a concentration of specified PCB (ng/L) and (ng/g) at a signal-to-noise ratio equal to 3:1 [13]. The results of the LODs for seawater and sediment are represented in Table 1.

3.2. PCB Levels in Seawater and Sediment. The levels of the 7 investigated PCB congeners for water and sediments were below the method detection limits (Table 1 above). An example chromatogram for seawater and sediment at Marine Science Station (total ion current) is shown in Figure 2a and b, respectively.

3.3. Comparison with Guidelines and Previous Studies. The sediment data of this study showed that the PCBs were below the method detection limits (~0.4–0.9 ng/g dry wt.). By comparing the PCB level in the present study with quality guidelines of Canadian Interim Sediment Quality Guidelines [14], the PCB concentrations in all sites were below the screening criterion of 21.5 ng/g dry wt. Given the concentration levels of PCBs at most sites along the Jordanian coast, there is little to no possibility for the occurrence of a toxic or adverse biological effect of PCBs. Also, the PCB results of this study were very low compared to many other studies throughout the world (Table 2). PCB range of 13–1600 ng/g was found in Cortiou, Marseille, France [20], which can be considered very high, compared with other studies. This may be due to the industrial and harbor activities in this city, which developed earlier than other location studies to date. Also, relatively high PCB values of 1–1210 ng/g were found in Alexandria harbor, Egypt [26]. The authors related this to the extensive harbor activities at this site. In the Gulf of Aden, Yemen PCB values were relatively low, ranging from 0.4 to 5.0 ng/g [19]. The only research conducted in the Red Sea by El Nemr et al. [15] showed that PCB concentrations were relatively low (0.4–60 ng/g).

Jordan has always valued the importance of its coastline. The Gulf of Aqaba is a unique marine environment [30]. The need to balance the Kingdom’s only sea ports (a growing industrial zone, which is important to the national economy) with the increasing demand for tourism (due to Jordan’s pristine yet fragile marine ecosystem) has always been taken seriously by the Government of Jordan [30]. Since the inception of the Aqaba Special Economic Zone Authority (ASEZA), a number of important steps have been considered to increase the protection of the coastline and the sea, and the zero discharge policy has played an important role in reducing contamination in the Gulf of Aqaba [30]. The undetectable PCBs is a result of the restricted standard of the environmental protection policy in ASEZA, as well as the prevention of the PCBs in Jordan since the 1800s [10]. This unique environmental condition without a direct source point of pollution gives Jordan the chance to protect the coral reef and support a marine protection area that encourages recreation, increases tourism, and keeps the marine ecosystem healthy.

4. Conclusion

In conclusion, this study provides evidence that very low concentrations of PCBs (< 2 ng/g) were found in the water and sediments of the Jordanian coast of the Gulf of Aqaba. The restricted standard for environmental protection policy and regulation in ASEZA cause the undetectable PCB content. The results of this study can be utilized as a baseline for future monitoring and control of PCBs, as requested by the Stockholm Convention [6] (2009).
GC Determination of the Levels of PCBs

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Figure 2. GC-ECD chromatogram for MSS a) seawater spiked sample of 0.05 μg/L concentrations and b) Marine sediment of concentrations of 2.5 μg/Kg DW with 0.075 μg surrogate standard (PCB 30) and 0.075 μg Internal Standard (PCB 209)

Table 2. Comparison of PCB levels (ng/g dry wt) in marine sediment of this study with different studies worldwide

| Location                          | PCBs (ng/g dry wt) | References |
|-----------------------------------|-------------------|------------|
| Red sea, Jordan                   | < DL (~0.4–0.9)*  | Present study |
| Red Sea, Egypt                    | 0.4–6.0           | [15]       |
| Tamentfoust port, Algiers Bay, Algeria | 0–70              | [16]       |
| Vietnam, Ha Long Bay              | 0–10              | [17]       |
| Coastal Barcelona, Spain          | 2–44              | [18]       |
| Gulf of Aden, Yemen               | 0–4.97            | [19]       |
| Cortiou, Marseille, France        | 12–1559           | [20]       |
| Hugli estuary, West Bengal, northeast India | 0–2         | [21]       |
| Salton Sea, California, USA       | 10–40             | [22]       |
| Port of Bagnoli, Gulf of Naples, south Italy | 4–100       | [23]       |
| Masan Bay, Korea                  | 1–41              | [24]       |
| Black Sea, Turkey                 | 0–4               | [25]       |
| Alexandria harbor, Egypt          | 1–1210            | [26]       |
| Daya Bay, China                   | 1–27.4            | [27]       |
| Caspian Sea, Russia               | 1–6               | [28]       |
| Sousse, Tunisian coast            | 40                | [29]       |
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