ORIGINAL PAPER

Natural Attenuation Potential
of Polychlorinated Biphenyl-Polluted Marine Sediments

SARAH ALDHAFIRI[1], HUDA MAHMOUD[2], MOHAMMED AL-SARAWI[3] and WAEL A. ISMAIL[1]*

[1] Environmental Biotechnology Program, Life Sciences Department, College of Graduate Studies,
Arabian Gulf University, Manama, Kingdom of Bahrain
[2] Department of Biological Sciences, College of Science, Kuwait University, Kuwait
[3] Department of Earth and Environmental Sciences, College of Science, Kuwait University, Kuwait

A b s t r a c t

The marine environment in Kuwait is polluted with various hazardous chemicals of industrial origin. These include petroleum hydrocarbons, halogenated compounds and heavy metals. Bioremediation with dedicated microorganisms can be effectively applied for reclamation of the polluted marine sediments. However, information on the autochthonous microbes and their ecophysiology is largely lacking. We analyzed sediments from Shuwaikh harbor to detect polychlorinated biphenyls (PCBs) and total petroleum hydrocarbons (TPHs). Then we adopted both culture-dependent and culture-independent (PCR-DGGE) approaches to identify bacterial inhabitants of the polluted marine sediments from Shuwaikh harbor. The chemical analysis revealed spatial variation among the sampling stations in terms of total amount of PCBs, TPHs and the PCB congener fingerprints. Moreover, in all analyzed sediments, the medium-chlorine PCB congeners were more abundant than the low-chlorine and high-chlorine counterparts. PCR-DGGE showed the presence of members of the Proteobacteria, Spirochaetes, Firmicutes and Bacteroidetes in the analyzed sediments. However, Chloroflexi-related bacteria dominated the detected bacterial community. We also enriched a biphenyl-utilizing mixed culture using the W2 station sediment as an inoculum in chemically defined medium using biphenyl as a sole carbon and energy source. The enriched mixed culture consisted mainly of the Firmicute *Paenibacillus* spp. Sequences of genes encoding putative aromatic ring-hydroxylating dioxygenases were detected in sediments from most sampling stations and the enriched mixed culture. The results suggest the potential of bioremediation as a means for natural attenuation of Shuwaikh harbor sediments polluted with PCBs and TPHs.

K e y   w o r d s: aromatic ring oxygenases, bacterial communities, bioremediation, Chloroflexi, DGGE, polluted marine sediments

Introduction

The industrial, urban and agricultural development in Kuwait and other Arabian Gulf countries, following the discovery of oil, has burdened the marine environment in this region with many hazardous pollutants. Many different chemicals including heavy metals, petrochemicals and halogenated organic compounds have been introduced into coastal marine sediments (Al-Muzaini *et al.*, 1995; Beg *et al.*, 2001). Since the early 1980s, several studies have been conducted to assess the quality of coastal sediments in Kuwait. These investigations concluded that certain stretches of the coastline are polluted with high concentrations of toxic chemicals from municipal and industrial wastewater discharges (Shunbo and Literathy, 1984; Clark, 2001; Beolchini *et al.*, 2009). Most of the studies carried out in the Arabian Gulf region have focused on oil and related chemicals as the source of pollution in the territorial water of the Gulf. Other persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) have received much less attention (Fowler *et al.*, 1993). The presence of PCBs in Kuwait's costal marine sediments has been reported (Gevao *et al.*, 2012; 2006). In a study by Helaleh *et al.* (2012), samples from some marine biota (collected from local fish market in Kuwait) were analyzed for the occurrence of organochlorine pesticides and PCBs. The authors detected PCBs (ng g^{-1}) in fish (2.53), squid (0.25), bivalves (0.24), shells (0.24), octopus (0.17) and shrimp (1.38).

PCBs were first manufactured commercially in 1929. The chemical structure of PCBs is based on a biphenyl core with 1 to 10 chlorine atoms introduced at different degrees of chlorination and substitution to produce 209 products called congeners (Furukawa and Fujihara, 2008). PCB congeners having identical number of

* Corresponding author: W.A. Ismail, Environmental Biotechnology Program, Life Sciences Department, College of Graduate Studies, Arabian Gulf University, Manama, Kingdom of Bahrain, **e-mail**: waelame@agu.edu.bh

chlorine atoms are referred to as homologues, whereas homologues with different chlorine positions are called isomers (Borja *et al.*, 2005). In the environment, PCBs generally exist as complex mixtures of different congeners such as Aroclor produced by Monsanto (USA) (Borja *et al.*, 2005). Due to their unique physicochemical properties, PCBs have been adopted for a variety of industrial applications such as lubricants, dielectric fluids and plasticizers (Gevao *et al.*, 2012). Because of their toxicity and persistence in the environment, PCBs were banned in most countries in the late 1970s (Ross, 2004). PCBs can be very hazardous to the health of human and other biota due to their bioaccumulation in different tissues (Passatore *et al.*, 2014).

Like other anthropogenic compounds, PCBs can enter the aquatic environment from different sources including: (i) direct deposition from the atmosphere, (ii) runoff from land, (iii) directly from industrial and wastewater treatment plant discharges, sewer branches, thermal and chlorinated effluents from power plants as well as shipping and dockyard activities in harbors (Bush and Kadlec, 1995). Among all the environmental media, sediments are recognized as significant reservoirs and sinks for a large variety of POPs such as PCBs. This is due to their hydrophobicity. They have a strong affinity for particulate matter and ultimately accumulate in bottom sediments (Bush and Kadlec, 1995; Zennegg *et al.*, 2007).

In Kuwait, only a few studies have addressed pollution of the marine environment with PCBs. However, the objective of those studies was to assess the occurrence, distribution and toxicity of PCBs without paying attention to the fate of these compounds in the environment. Biological transformation and degradation by microorganisms can play an important role in the elimination and/or detoxification of PCBs found in polluted ecosystems (Furukawa and Fujihara, 2008). Nonetheless, there is dearth of information concerning PCBs-degrading microbial communities in the Kuwait's marine environment. Moreover, the majority of the POPs biodegradation studies on the polluted ecosystems in Kuwait relied on the conventional enrichment and isolation techniques (culture-dependent procedures), which are laborious and usually underestimate the quantity and quality of the microbial communities inhabiting a polluted ecosystem (Dennis *et al.*, 2003; Van Hamme *et al.*, 2003).

The comprehensive understanding of the structure and function of microbial communities is a prerequisite for the development of an efficient bioremediation process. In this context, Dell'Anno *et al.* (2012) investigated the dynamics of bacterial abundance and biodiversity during bioremediation of hydrocarbon-contaminated marine harbor sediment. The authors reported that the incubation temperature was the principal determi-

nant of bacterial abundance, diversity and community structure. Furthermore, they found that hydrocarbon biodegradation efficiency was promoted as the bacterial richness and evenness increased. In a study on PCBs bioremediation, Wang and He (2013) studied the reductive dechlorination patterns and microorganisms involved in the dechlorination of Aroclor 1260 in sediment/soil microcosms and sediment-free enrichment cultures. They reported significant PCBs dechlorination activity with distinct patterns such as the N, H and T processes. They also identified *Dehalogenimonas* and *Dehalococcoides* spp. as major dechlorinators in the microcosms. Recently, Sydow *et al.* (2016) reported that a diesel-degrading bacterial consortium maintained its structural and functional integrity after short-term exposure to different hydrocarbon feeds.

In this study, bacterial diversity and abundance in PCBs-polluted sediments from Shuwaikh harbor in Kuwait were investigated. Spatial shift in community structure was also addressed. Moreover, biodegradation potential of the bacteria inhabiting the sediments was assessed *via* fingerprinting of the functional genes of aromatic ring oxygenases.

Experimental

Materials and Methods

Chemicals and molecular biology materials. Chemicals and microbiological media were obtained from Fluka (Switzerland), Sigma-Aldrich (USA) and Promega (USA). Total community DNA was isolated from sediment samples with PowerSoil DNA Isolation Kit (MoBio, USA). Molecular biology enzymes and reagents were purchased from Qiagen (USA) and GE Healthcare (UK). The PCBs analytical standard (EC-4133) was purchased from Cambridge Isotope Laboratory (CIL, Andover, MA). The internal standard Mirex and the recovery standard EC 4058 were purchased from CIL.

Study area and collection of sediment samples. Kuwait Bay is considered an ideal seaport in terms of its nature and geography. It encloses Shuwaikh harbor within an urban industrial area in the Al-Asimah Governorate (Capital Governorate) of Kuwait. Shuwaikh harbor lies on the south shore of Kuwait Bay at Latitude 29° 21′ North and Longitude 47° 56′ East. It is considered the main commercial port in the country. It is one of the busiest ports in the Middle East with 21 deepwater berths. For the PCR-DGGE experiments, sediment samples were collected at five different locations (W1, W2, W3, W4 and W5) from inside the Shuwaikh harbor using a sediment core sampler (Fig. 1). For the chemical analysis, a Van Veen Grab was used to collect
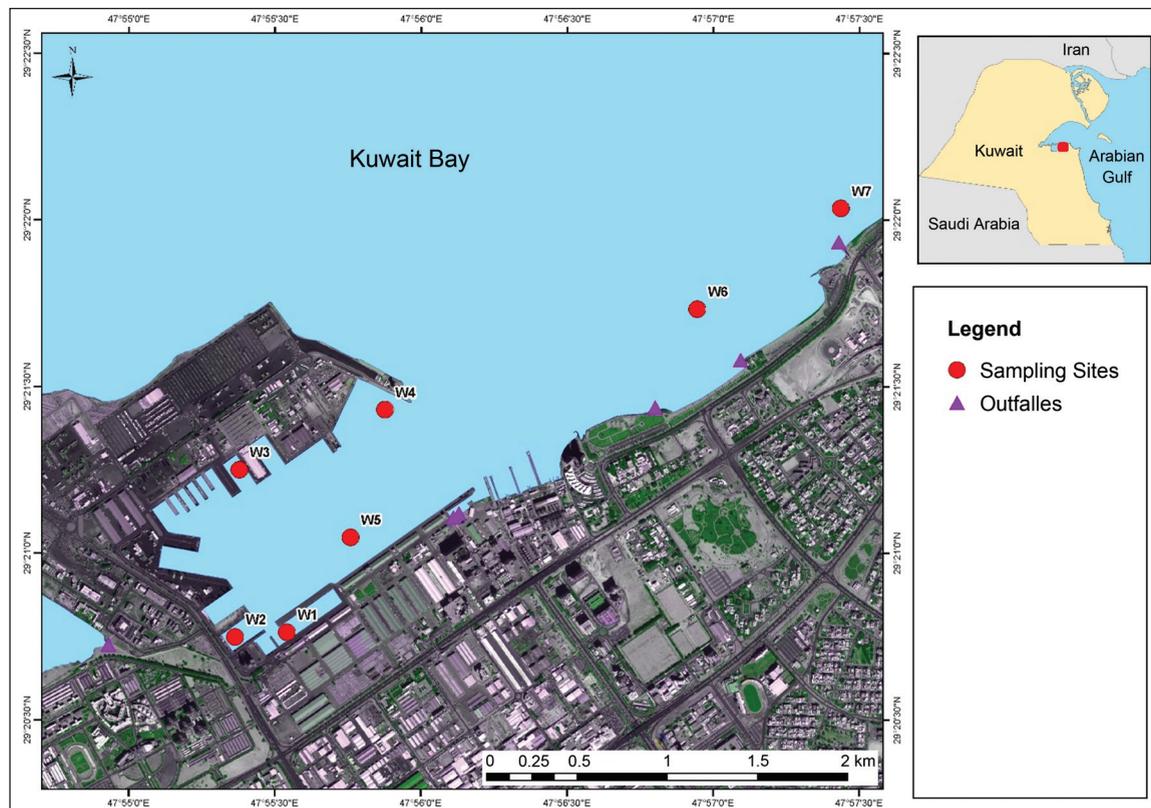
Fig. 1. A map showing Shuwaikh harbor and the 7 sampling sites (red points).

sediment samples from the W1-W5 sites in addition to two locations (W6 and W7) outside the harbor (Fig. 1). The cores were secured and returned back to the laboratory for immediate processing. The sediment cores were divided into layers or sections (5-cm long), which were kept in sterile glass Petri dishes. Each section was then homogenized into one composite sample using a sterile wooden spatula. Samples were transferred into 50-ml sterile tubes and marked according to the sampling location.

Culture media and growth conditions. Luria-Bertani (LB), trypticase soy broth (TSB) and trypticase soy agar (TSA) were prepared according to the instructions of the supplier and were used for growth and isolation of bacteria. Chemically defined medium (CDM) had the following composition per litre of deionized water: KH_2PO_4, 1.35 g; K_2HPO_4, 7.0 g; NH_4Cl, 0.54 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $CaCl_2 \cdot 2H_2O$, 0.044 g; vitamins (Cyanocobalamine 0.2 mg, pyridoxine-HCl 0.6 mg, thiamine-HCl 0.4 mg, nicotinic acid 0.4 mg, *p*-aminobenzoate 0.32 mg, biotin 0.04 mg, Ca-pantothenate 0.4 mg) and trace elements ($FeSO_4 \cdot 7H_2O$ 2.0 mg, $ZnSO_4 \cdot 7H_2O$ 150 µg, $MnSO_4 \cdot H_2O$ 85 µg, $CuSO_4 \cdot 5H_2O$ 37 µg, $CoCl_2 \cdot 6H_2O$ 200 µg, $Na_2MoO_4 \cdot 2H_2O$ 40 µg, $NiCl_2 \cdot 6H_2O$ 20 µg and H_3BO_3 20 µg). The carbon sources were added either as solid in case of biphenyl (1 g/l) or from acetone stock solutions for Aroclor 1242 (0.1 ml/100 ml of CDM, commercial PCBs mixture of

5 mg/100 ml acetone, Sigma-Aldrich) and 2,4-dichlorobiphenyl (0.1 ml/100 ml of CDM, commercially available as 2.5 mg/100 ml acetone stock, Sigma-Aldrich). All liquid culture media (100 ml) were routinely prepared in 250-ml Erlenmeyer flasks and incubated at 30°C in an orbital shaker (200 rpm). Agar plates were incubated at 30°C for 48 hours.

Enrichment and isolation of biphenyl-degrading bacteria. Two grams from the sediment samples W1, W2, W3, W4 and W5 (upper 5 cm of the core) were inoculated individually into CDM containing biphenyl as the sole carbon source. After 10 days of incubation, 10 ml aliquots from those initial enrichments were transferred to fresh medium followed by further incubation for 10 days. This sub-culturing step was repeated four times. Aliquots (1 ml) from the 4th enrichment (subculture) were serially diluted in sterile normal saline (0.9% NaCl) and samples (100 µl) from each dilution were spread on TSA plates. After incubation, single colonies (morphologically distinct) from the different plates were purified by subsequent streaking on TSA plates. The isolated single colonies were then grown in TSB (trypticase soy broth, 20 ml) medium for 48 h. The cells were harvested from culture samples (1 ml) by centrifugation (10,000 rpm, 5 min) and washed once with 10 ml of 0.1 M K-phosphate buffer (pH 6.8). The washed cells were suspended in phosphate buffer and inoculated into CDM containing biphenyl as a sole carbon source.

Enrichment of PCB-degrading bacteria. Portions (2 g) of the sediment samples collected from sites W1, W5 (uppermost layer of the core, 5 cm) and W6 were inoculated into CDM containing either Aroclor 1242 or 2,4-dichlorobiphenyl as a sole carbon source. After two weeks of incubation, 10-ml aliquots of those initial enrichments were transferred to fresh medium and further incubated for two weeks. This sub-culturing step was performed three times.

Isolation of total community DNA from sediments and bacterial cultures. Total genomic DNA of microbial communities inhabiting Shuwaikh harbor sediment samples was isolated with the Power-Soil DNA Isolation Kit. The initial step of extraction involved homogenizing 0.25 g of sediment samples using bead beater at 2500 bpm for 3 min. Then the rest of the extraction process was achieved following the manufacturer's protocol. The DNA from the unknown purified bacterial cultures was extracted using Prep-Man Ultra Sample Preparation Reagent (Applied Biosystems, USA) following the manufacturer's protocol.

Partial amplification of the 16S rRNA gene. Partial 16S rRNA gene fragments were amplified using the universal bacterial primers 907R (5'-CCCCGTCAATTC-MTTTGAGTTT-3') and GM5F (5'-CCTACGGGAG-GCAGCAG-3') (Schafer and Muyzer, 2001) and the Pure Taq Ready-To-Go PCR Beads (Amersham Biosciences, UK). Aliquots (30 pmol) of both forward and reverse primers were added to the beads along with 1 μl (25 ng DNA) of DNA and the final volume in the vials was brought up to 25 μl with sterile water (Sigma, USA). The vials were incubated in a Thermocycler (Gene Amp PCR system 9700, Applied Biosystem, USA), where a standard PCR program was applied. The initial denaturing step took place at 94°C for 5 min followed by 25 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec. Eventually, a final extension step was run at 72°C for 7 min.

Amplification of genes encoding putative aromatic ring oxygenases. Segments from genes encoding aromatic ring-hydroxylating oxygenases were amplified by PCR using DNA obtained from the sediment samples as a template. Total DNA was isolated from sediment samples collected from the locations W1, W2, W3, W4, W5, W6 and W7 with the PowerSoil DNA Isolation Kit. The forward primer 888 and the reverse primer 300R were used (Kitagawa *et al.*, 2001). The primer 888 consisted of the 40-bp GC-clamp sequence (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G-3') and the sequence (5'-TGC ASS TWT CAC GGS TGG-3') and the primer 300R had the sequence (5'-CTC GAC TCC GAG CTT CCA GTT-3') conserved among aromatic ring-hydroxylating dioxygenases. The touchdown PCR procedure was used: 20 cycles at 94°C for 40 sec, 60°C for 40 sec (decreased

by 1°C every 2 cycles), 72°C for 40 sec and 10 cycles at 94°C for 40 sec, 50°C for 40 sec and 72°C for 40 sec. The expected 340-bp DNA fragments containing the 300-bp target plus 40-bp GC-clamp sequences were amplified from the extracted DNA (Kitagawa *et al.*, 2001).

Denaturing gradient gel electrophoresis (DGGE). DGGE was performed on Dcode Universal Mutation System (Bio-Rad, USA) according to the manufacturer's instructions where the denaturant's concentrations increase from the top of the gel toward the bottom. Three 6% polyacrylamide gel solutions with different denaturants concentrations (0%, 30% and 50%) were prepared. Samples (300–600 ng of the amplified DNA) were run at 50V and 60°C for 16 hours. The DGGE bands were excised for sequencing and were visualized using Dark Reader (Clare Chemical Reader, USA). Gels were post-stained for 20 min in 1×TAE buffer supplied with SYBR Green (Invitrogen, USA). The DNA was allowed to passively diffuse from the gel pieces into the water at 4°C overnight. The resulting solution was used as a template to re-amplify the content of each band using the same PCR primers and programs mentioned previously. The DGGE data obtained were analyzed using Phoretix 1D analysis software. The DGGE profile was transformed to binary matrix depending on the presence (1) and absence (0) of the bands on the examined profile before being analyzed using hierarchical cluster analysis.

DNA sequencing. PCR products were purified using QIA Quick Purification Kit (Qiagen, USA) following the manufacturer's protocol to remove the excess Taq polymerase, primers and DNTPs that might interfere with the sequencing steps. BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA) was used for labelling and amplifying the purified product. The PCR program applied included 1 cycle of denaturation at 96°C for 1 min, followed by 25 cycles of denaturation at 96ºC for 1 min, annealing at 50°C for 5 sec and extension at 60°C for 4 min. The final products were further purified using sodium acetate (pH 5.2) and absolute ethanol. Then 20 μl of absolute ethanol were added to each sample. After the denaturation step, samples were kept on ice and loaded directly in the 3130*xl* genetic analyzer (Applied Biosystems, USA) and the results were obtained using Sequencing Analysis v5.2 Software (Applied Biosystems, USA). The obtained sequences were compared with others in the GenBank database using BLAST (Altschul *et al.*, 1997). The partial 16S rRNA gene sequences were deposited in the GenBank (accession numbers – see Supplementary Material).

Analysis of PCBs and total petroleum hydrocarbons (TPHs). For the chemical analysis, sediment samples from the seven sampling stations W1-W7 were collected with a Van Veen grab. Analysis of the PCBs was performed in the Central Analytical Laboratory-Kuwait
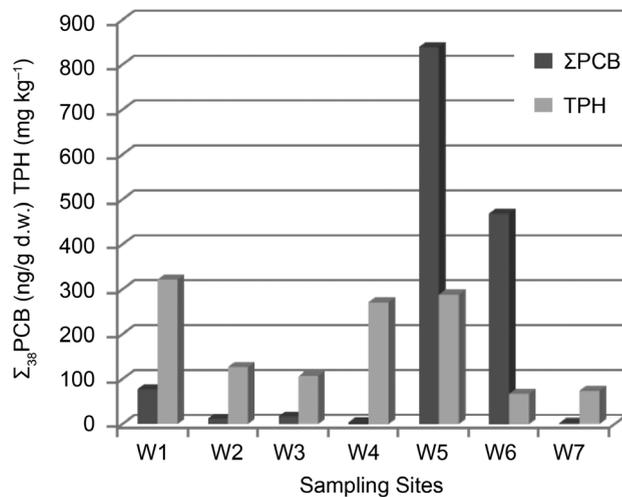
Fig. 2. Total PCBs concentrations (38 congeners) and TPHs measured for the 7 sites sampled at the chosen Shuwaikh harbor location, d.w. (dry weight).

Institute for Scientific Research (Kuwait) according to the methods described in Gevao *et al.* (2012). TPHs in sediment samples were measured by the Infrared Spectroscopic Method recommended by United States Environmental Protection Agency (USEPA, 1978). The calibration plot was used to calculate the concentration of hydrocarbons in sediments with the following equation:

$$mg/kg = I.V/a.w$$

where I = integral value from IR measurement, V = volume of the extract (100 ml), a = slope of the calibration curve, w = weight of the sediment sample (about 5 to 10 g).

Results

Pollution of Shuwaikh marine sediments with PCBs and TPHs. Figure 2 shows that stations W5 and W6 had the highest measured PCB levels (W5: 59.1% of Σ_{38}PCB – all sites, W6: 33.0%). Taking a closer look at the distribution of congener concentrations for the locations across the different homologue series represented (those PCBs containing the same number of chlorine atoms), it can be seen that PCB-138 (2, 2', 3, 4, 4', 5'–hexachlorobiphenyl) was the dominant pollutant congener with a ΣPCB of 165.4 ng/g (Fig. 3, Table S1). Other congeners, which exceeded 100 ng/g were PCB-101, 110, 118, 153 and 209. However, it is important to note that PCB-138, PCB-101 and PCB-153 were only present in the two most contaminated sites (W5 and W6), whereas PCB-110 and PCB-118 were observed in all of the sampling sites (Fig. 3). The sum of Cl_5 and Cl_6 congeners (16 out of the 38 measured congeners, 42%) accounted for more than two-thirds of all the detected PCB pollutants. No Cl_9 congeners were observed above the measurement detection limit. In terms of the PCB recovery data (Table S2), the average site recovery (for all congeners) was 84.5% (range of 55.7% [W2] to 101.4% [W7]). Coincidentally, the average congener recovery for all sites was also 84.5% (range of 67.1% [PCB-52] to 104.3% [PCB-180]). Total TPHs measured at the location (summed over seven sampling sites) was 1255.9 mg/kg with an average of 179.4 mg/kg. The lowest levels were observed at the two sites most distant from the harbor (Fig. 2).
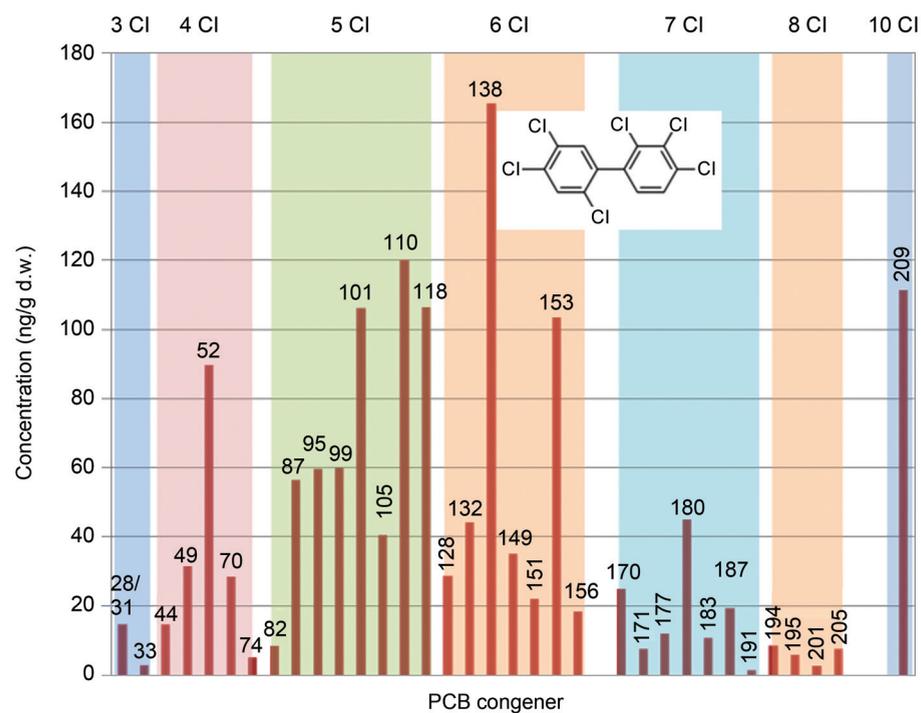

Fig. 3. Distribution of PCBs congeners in Shuwaikh harbor sediments (summed across all sampling sites), d.w. (dry weight).

Enrichment of biphenyl-degrading bacteria. Sediment samples from different sites were inoculated into CDM containing biphenyl as a sole carbon source to enrich biphenyl-utilizing bacteria. Culture turbidity due to bacterial growth was sustained only in the enrichment containing sediment sample collected from the W2 station. The W2 biphenyl enrichment culture (whole culture) appeared yellow in color. This was evident even after the third sub-culturing. When samples from the W2 biphenyl enrichment (fourth subculture) were serially diluted and spread on TSA plates, it was possible to differentiate three morphologically distinct colonies. After purification, none of those three strains could grow individually on biphenyl as a sole carbon source in CDM. Enrichments with Aroclor 1242 or 2,4-dichlorobiphenyl as carbon sources did not reveal any bacterial growth even after repeated sub-culturing. Moreover, the W2 biphenyl enrichment culture did not grow on either Aroclor 1242 or 2,4-dichlorobiphenyl as a sole carbon source even after prolonged incubation.

Natural bacterial communities dwelling in Shuwaikh harbor sediments. The bacterial community inhabiting different depths (sections) of the sediment cores was investigated by comparing the DGGE band pattern obtained from various lamina recovered from each core (Table I). In terms of the number of bands, the DGGE profile showed almost the same pattern among the different sampling sites. Generally, the uppermost layer in the cores revealed a larger number of bands. The five sampling sites showed no remarkable variation in the average number of bands. In terms of total band numbers, site W4 revealed the highest, while W3 had the lowest number.

The DNA extracted from different sections of each core was pooled and the DGGE band patterns for such pooled samples were compared with their counterparts from other sites. The results (Fig. 4) revealed that sites W1 and W5 shared a similar band pattern. Only one additional band (band c) was found in site W1 and band a was found in site W5. Furthermore, sites W1 and W5 clusters were very close to each other (Fig. 4). Sites W2 and W3 clusters were also quite close to sites W1 and W5, whereas site W4 was the most divergent in terms of DGGE band pattern. In total, 32 bands were excised and sequenced. Table II summarizes the identity of only 18 bands that showed good sequences. The Genbank match search for the sequenced bands showed the dominance of bacteria belonging to Chloroflexi members (58.3%). In addition, sequences related to members of Spirochaetaceae, Proteobacteria (both γ and δ), Firmicutes and Bacteroidetes were detected. Site W1 was dominated by sequences affiliated to *Dehalogenimonas*

Table I
Number of DGGE bands obtained from the various layers of the sediment cores.

Layer (section) number*	Site W1	Site W2	Site W3	Site W4	Site W5
1	12	14	8	19	12
2	12	11	8	15	12
3	10	9	4	6	8
4	9	8	9	12	12
5	3	7		10	3
6	5	12		13	9
7	9			8	
8				13	
9				7	
10				4	
11				8	
12				8	
13				9	
14				9	
15				8	
16				3	
17				3	
Total	60	61	29	155	56
Average	8.57	10.16	7.25	9.11	9.33
(Min-Max)	(3–12)	(7–14)	(4–9)	(3–19)	(3–12)
SD	3.4	2.6	2.2	4.2	3.5

* Layer 1 is at the top of the sediment core

Table II
Phylogenetic affiliation of partial 16S rRNA gene sequences retrieved from Shuwaikh harbor sediments.

DGGE Band Code	Sample Name	Total Base Pair	Phylum/ Subdivision	Base Compared	Nearest GenBank Match	Similarity (%)	Accession no.
1m	*Dehalococcoides* sp. SPS1F2	485	Chloroflexi	430/495	*Dehalococcoides* sp. BHI80-15	87	AJ431246
1c	*Vibrio* sp. SPS1F3	513	γ-Proteobacteria	391/438	*Vibrio campbellii* ATCC baa-1116 chromosome 1	89	CP006605
1g	*Dehalogenimonas* sp. W1F4	502	Chloroflexi	337/391	*Dehalogenimonas alkenigignens* SBP1	68	JQ994267
2m	*Dehalococcoides* sp. W2F9	506	Chloroflexi	347/399	*Dehalococcoides* sp. BHI80-52	87	AJ431247
3b	*Dictyoglomus* sp. W3F13	492	Dictyoglomi	334/405	*Dictyoglomus thermophilum* strain H-12	82	NR_074876
3k	*Chloroflexi* sp. W3R15	488	Chloroflexi	399/479	Chloroflexi bacterium SCJCAAA240-M02	83	HQ675620
3d	Spirochaetaceae W3F-17	507	Spirochaetes	475/498	Spirochaetaceae bacterium Tc33_10	95	AB518752
4a	*Desulfatibacillum* sp. W4R18	500	δ-Proteobacteria	415/486	*Desulfatibacillum alkenivorans* AK-01	85	NR_074962
4f	*Halothermothrix* sp. W4R-19	489	Firmicutes	321/407	*Halothermothrix orenii* strain H 168	79	NR_074915
4h	*Thalassiosira* sp. W4F24	479	Chloroplast	470/480	*Thalassiosira gravida* isolate C140 chloroplast	97	FJ002211
4g	*Lutimonas* sp. W4R24	499	Bacteroidetes	473/500	*Lutimonas* sp. MOLA 107	95	AM990881
5g	*Dehalogenimonas* sp. W5F27	479	Chloroflexi	404/480	*Dehalogenimonas* sp. SBP1	84	JQ994267
5m	*Dehalococcoides* sp. W5F28	477	Chloroflexi	411/465	*Dehalococcoides* sp .BHI80-52	88	AJ431247
5h	*Thalassiosira* sp. W5F30	501	Chloroplast	479/489	*Thalassiosira gravida* isolate C140 chloroplast	98	FJ002211
5a	*Desulfofaba* sp. W5R31	509	δ-Proteobacteria	400/473	*Desulfofaba gelida* strain PSv29	85	NR_O28730
2k	*Chloroflexi* sp. W2S	467	Chloroflexi	456/467	Uncultured Chloroflexi bacterium clone D28	98	GQ249570.1
4k	*Chloroflexi* sp. W4F23	486	Chloroflexi	383/462	Chloroflexi bacterium SCJCAAA240-C11	83	HQ675557
4m	*Dehalococcoides* sp. W4F21	491	Chloroflexi	434/498	*Dehalococcoides* sp. BHI80-15	87	AJ431246

sp., *Dehalococcoides* sp. and *Vibrio* sp., where each comprised 33% of the obtained sequences. *Vibrio* sp. was found only in site W1. Site W2 contained 50% each of Chloroflexi-related bacteria and *Dehalococcoides* sp. Site W3 contained 33% of Spirochaetes-related sequences and 34% of *Dictyoglomus* sp., both of which were not found in the other four sites. Site W3 did not contain *Dehalococcoides*-related sequences, which were present in all the other sites. Site W4 harbored larger diversity of bacteria than the rest of the sites based on the sequencing results. Apart from *Dehalococcoides* sp., site W4 contained three unique species that were not found in the rest of the sites. These were *Lutimonas* sp., *Halothermothrix* sp. and *Desulfatibacillum* sp. Site W5 with

four different kinds of bacteria, had the second most diverse bacterial community. It contained 40% of the *Dehalococcoides* sp. and 20% of *Desulfofaba* sp. Chloroflexi bacteria were found in three of the five sites and were the second most abundant organisms found in the investigated area. *Thalassiosira* sp. was the third most abundant organism and was found in two sites. *Dehalococcoides* sp. dominated sequences obtained from sites 1, 2, 4 and 5, while Chloroflexi members were found in sites 2, 3 and 4.

Structure of the biphenyl-utilizing mixed culture. Sediment samples (uppermost part) from site W2 were enriched in batch cultures supplied with biphenyl as a sole carbon source. The enriched bacteria were
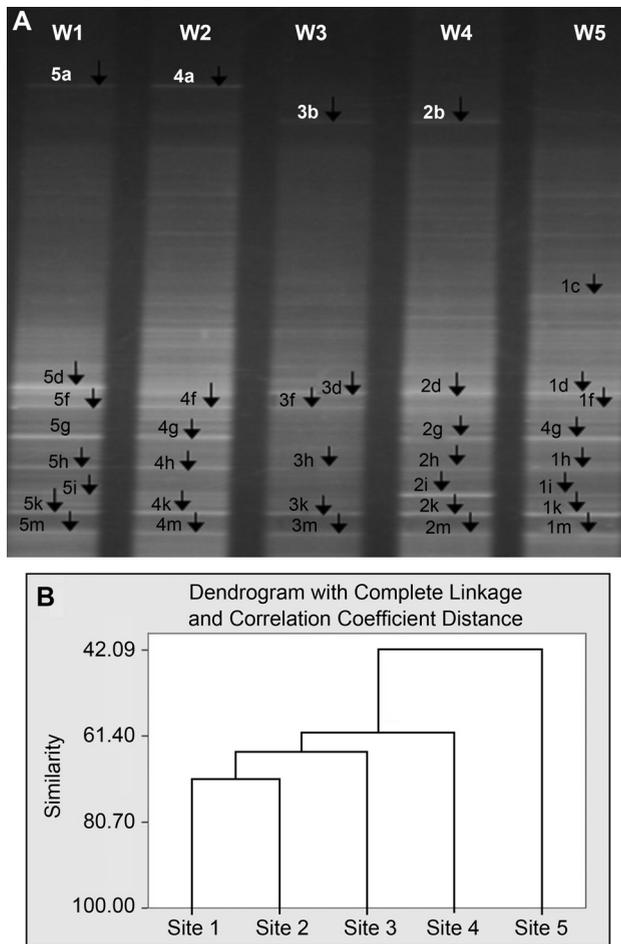
Fig. 4. (A) DGGE profile based on DNA pooled from all the sediment core layers for the 5 sampling sites W1-W5 at Shuwaikh harbor. (B) Cluster analysis performed using binary matrix (0, 1) of DGGE showing variation between bacterial populations inhabiting the five sampling locations W1-W5 (site1 – site 5).
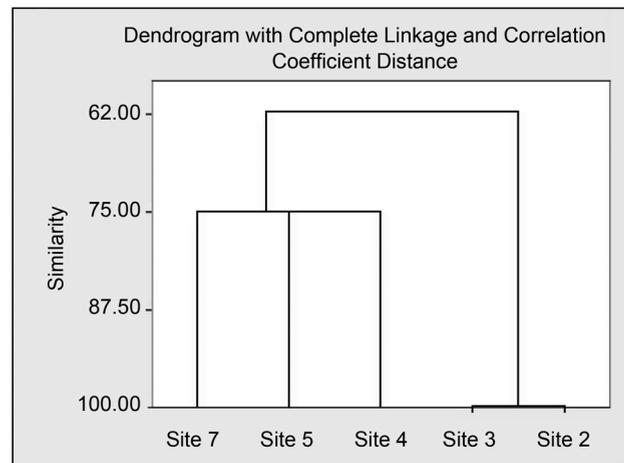

Fig. 5. Cluster analysis of sequences related to aromatic ring-hydroxylating dioxygenases retrieved from different sampling sites at Shuwaikh harbor (sites 2, 3, 4, 5 and 7 designate sites W2, W3, W4, W5 and W7, respectively).

characterized by DGGE. The total number of bands recovered from the mixed culture was 18 as compared to the average number of bands of 14 obtained from the natural sediment bacterial community of site W2. Sequences of bacteria affiliated to Firmicutes (~82%) and Proteobacteria (9%) were detected. *Paenibacillus* sp.-related sequences dominated the sequenced bands.

Aromatic ring-hydroxylating dioxygenases in Shuwaikh harbor sediments and the biphenyl-degrading mixed culture. Table III lists the sequences retrieved from the sediment samples along with their closest matches. The genes detected encode proteins similar to phenylpropionate dioxygenase and related aromatic ring-hydroxylating dioxygenases. In addition, protein sequences similar to the large subunit of aromatic ring oxygenases were detected. The biphenyl-utilizing mixed culture contained one sequence that is related to hydrocarbon degradation (the large subunit of aromatic ring oxygenases) (Table III). Cluster analysis showed that sites W2 and W3 shared similar band pattern, while sites W4, W5 and W7 clustered together (Fig. 5).

Discussion

Pollution of Shuwaikh harbor sediments with PCBs and TPHs. It was evident from the results that Shuwaikh harbor sediments are polluted with PCBs and TPHs. The presence PCBs in Shuwaikh harbor sediments has not been reported before. However, there are a few reports on PCBs pollution in Kuwait coastal sediments (Gevao *et al.*, 2012) and marine biota (Helaleh *et al.*, 2012). There was a spatial variation among the sampling sites in terms of total PCBs amounts and congener distribution. This could be due to different pollution sources contaminating the different sampling stations. Other factors may be involved such as microbial biodegradation, the pollution history, *in situ* abiotic degradation, diffusion through the sediment column, lateral movement and resuspension of the sediments, and resuspension of PCBs into the water column (Li *et al.*, 2009). Pollution of the Kuwait's coast with petroleum hydrocarbons has been frequently reported (Ahmed *et al.*, 1998; Mahmoud *et al.*, 2009; Michel, 2011). Oil spills, accidental or deliberate, and oil transport and processing operations are major sources of pollution of the Arabian Gulf with petroleum hydrocarbons. The decreasing trend of pollution in the stations from inside to the outside of the harbor is consistent with previous reports (Beg *et al.*, 2001).

The biphenyl-utilizing mixed culture. A biphenyl-utilizing culture was enriched from sampling site W2 sediments. This indicates the presence of biphenyl-, and probably PCBs-, degrading bacteria in the W2 sediment. The yellow color observed in the biphenyl whole culture could be due to the meta-cleavage of the aromatic ring of 2,3-dihydroxybiphenyl, which produces 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate (Kolar *et al.*, 2007).

Table III
Gene sequences encoding putative aromatic ring hydroxylases/oxygenases recovered from sediments of the Shuwaikh harbor and a biphenyl-utilizing mixed culture enriched from the sediment of the W2 sampling site.

DGGE Band Code	Sample Name	Number of Bases	Microbial Source	Nearest GenBank Match	Base Compared	Similarity (%)	Accession no
3b	R32	194	Uncultured marine microorganism	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases (large subunit)	44/63	70	CAM58106
2a	R211	193	Uncultured marine microorganism	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases (large subunit)	40/64	63	CAM58106
5a	R512	193	Uncultured marine microorganism	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases (large subunit)	43/64	67	CAM58106
4d	R413	200	Uncultured marine microorganism	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases (large subunit)	35/63	56	CAM58106
3a	R311	201	Uncultured marine microorganism	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases (large subunit)	44/65	68	CAM58106
3d	R313	203	Uncultured marine microorganism	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases (large subunit)	43/66	65	CAM58106
4a	R431	198	Uncultured marine microorganism	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenases (large subunit)	42/65	65	CAM58106
2d	R2111	203	Uncultured marine microorganism	Phenylpropionate dioxygenase and related ring-hydroxylating dioxygenase (large subunit)	38/62	61	CAM58106
–	RB1*	189	*Luminiphilus syltensis*	Large subunit aromatic oxygenase	34/56	61	WP_009018933
–	RB2*	192	*Luminiphilus syltensis*	Large subunit aromatic oxygenase	41/63	65	WP_009018933
–	FB3*	225	*Pseudomonas putida* DOT-T1E	Large subunit aromatic oxygenase	33/50	66	YP_006532342

* Sequences obtained from biphenyl-degrading mixed culture

The W2 culture was shown to be a bacterial consortium as confirmed by the presence of three different bacteria on TSA plates. Kolar *et al.* (2007) reported the isolation of biphenyl-degrading mixed culture from marine sediments. It was not possible to enrich PCBs-degrading cultures from the tested sediments. This could be due to toxicity of the substrate and/or growth conditions that did not allow the growth of PCBs degraders. Alternatively, it is possible that the adopted culture medium and growth conditions were not conductive for the establishment of relevant consortia that can cooperatively degrade the PCB substrate *via* synergistic interactions, provision of necessary growth factors, or elimination of toxic products (McGenity *et al.*, 2012; Mikesková *et al.*, 2012; Van Hamme *et al.*, 2003).

The biphenyl-utilizing mixed culture was dominated by *Paenibacillus naphthalenovorans*-affiliated sequences. This limited taxonomic diversity in the biphenyl-degrading culture W2 is consistent with the results reported by Kolar *et al.* (2007). These authors found that in a biphe-

nyl-degrading mixed culture, six of the seven isolates were affiliated to the genus *Rhodococcus*. Other bacteria that were found in the biphenyl W2 culture represent the minor fraction and are related to *Pseudomonas* and *Azoarcus* spp. Some members of the genera *Paenibacillus*, *Pseudomonas* and *Azoarcus* are known to degrade aromatic compounds under aerobic conditions such as polyaromatic hydrocarbons (PAHs), biphenyl, and PCBs (Daane *et al.*, 2002; Sakai *et al.*, 2005; Ismail and Gescher, 2012; Koubek *et al.*, 2013; Nam *et al.*, 2014). Accordingly, the detection of sequences related to these bacteria in the aerobically grown biphenyl-utilizing mixed culture is not surprising.

Natural bacterial communities inhabiting Shuwaikh harbor sediments. The observed variations in number of DGGE bands among the sections of the same sediment core or cores from the different sampling sites might reflect changes in bacterial community structure. The latter could be due to fluctuations in oxygen tension, types and concentrations of the

pollutants (*e.g.*, PCBs congeners), organic carbon content, availability of nutrients, electron acceptors and electron donors, as well as resuspension and sedimentation processes (Black *et al.*, 2017; Correa *et al.*, 2010; Zhang *et al.*, 2015). Decrease in the number of bands with increasing the core depth might reflect the anoxic conditions in the deep layers, which restrict bacterial growth and reproduction.

In general, there was a little spatial variation among the sampling sites in terms of the number of the DGGE bands. Considering the phylum level, the sequenced DGGE bands were affiliated to Chloroflexi, Spirochaetes, Proteobacteria, Firmicutes and Bacteroidetes. Bacteria belonging to these groups have been identified before in sediments polluted with PCBs (and other hydrocarbons) and in enrichment cultures containing PCBs or TPHs. For instance, Zanaroli *et al.* (2012) applied DGGE and T-RFLP to characterize the microbial community enriched in PCBs-containing slurry microcosms from marine sediments. The authors identified members of α-, β-,γ- and ε-divisions of Proteobacteria, Firmicutes and Chloroflexi. Moreover, members of Chloroflexi, Firmicutes, Proteobacteria, Spirochaetes and Bacteroidetes were identified in sediment-free PCB-dechlorinating cultures (Wang and He, 2013).

At the genus level, the observed dominance of *Dehalococcoides*-related sequences and other Chloroflexi members is consistent with the chemical analysis, which revealed PCBs pollution of the tested sediments. These bacteria are known key players in the reductive dechlorination of various PCB congeners. To date, all identified PCB-dechlorinating bacteria belong to *Dehalococcoides* spp. and the phylogenetically related Chloroflexi bacteria like DF-1 and o-17 (Bedard, 2008; Zanaroli *et al.*, 2012). Wang and He (2013) identified *Dehalococcoides* spp., *Dehalobacter* spp. and *Dehalogenimonas* spp. in sediment-free cultures dechlorinating Aroclor 1260. Also recently, LaRoe *et al.* (2014) isolated *Dehalococcoides mccartyi* strain JNA in a pure culture dechlorinating Aroclor 1260. The literature, however, does not contain any reports on PCB-dechlorinating bacteria in marine sediments from Kuwait.

Members of the different Proteobacteria subdivisions have been identified by other investigators in hydrocarbon-polluted soil (Correa *et al.*, 2010) and marine sediments even in the Arabian Gulf water (Al-Awadhi *et al.*, 2013; Kolar *et al.*, 2007). Other dominant genera that were detected in the current study include *Dictyoglomus* sp. and a Spirochaetaceae bacterium, which were unique to sampling site W3. *Dictyoglomus thermophilum* is an anaerobic, chemoorganotrophic thermophilic bacterium isolated from a hot spring in Japan (Coil *et al.* 2014). Spirochaetes were identified by Wang and He (2013) in sediment-free cultures dechlorinating Aroclor 1260.

The community of site W4 was characterized by two sequences that were missing in the other sites. *Desulfatibacillum alkenivorans* AK-01 is a sulfate-reducing alkane-degrading bacterium isolated from estuarine sediment (Callaghan *et al.*, 2012). Accordingly, it might be involved in the sulfate reduction-coupled anaerobic biodegradation of aliphatic hydrocarbons in the sediment at site W4. *Halothermothrix orenii* is related to Clostridia. It is a strictly anaerobic thermophilic bacterium isolated from sediment of a Tunisian salt lake (Mavromatis *et al.*, 2009). *Desulfofaba*-related sequence was found only in site W5 sediments. These bacteria are sulfate reducers and were isolated from marine sediments (Knoblauch *et al.*, 1999).

Aromatic ring-hydroxylating oxygenases in Shuwaikh sediments. DGGE revealed the presence of sequences related to aromatic ring hydroxylation proteins in the sediments and biphenyl mixed culture. Aromatic ring-hydroxylating dioxygenases are key enzymes in the aerobic degradation of aromatic compounds by many microorganisms (Ismail and Gescher, 2012). Hence, the detection of oxygenases-encoding genes in the sediments and the biphenyl-utilizing culture suggests that they might be involved in the degradation of PCBs, biphenyl and other hydrocarbon pollutants in the Shuwaikh sediment. In accordance with these results, Correa *et al.* (2010) reported higher levels of biphenyl dioxygenases in soil microcosms exposed to Aroclor 1242 and individual PCB congeners. Studies on aromatic ring oxygenases in Kuwait's marine sediments are largely lacking.

Natural attenuation potential in polluted Shuwaikh sediments. We have provided some lines of indirect evidence suggesting that Shuwaikh sediments polluted with PCBs and TPHs might exhibit natural attenuation *via* biodegradation/biotransformation. First, sequences affiliated to bacteria that are known as hydrocarbon and PCBs degraders were detected in the sediment and the enriched biphenyl-degrading mixed culture. Second, genes, which encode putative aromatic ring-hydroxylating oxygenases were detected in the sediments and the biphenyl-degrading mixed culture. Third, the observed decrease in the amounts of the high-chlorine PCB congeners (7–10 Cl) as compared to the medium-chlorine ones (4–6 Cl) could be due to microbial reductive dechlorination (Adler *et al.*, 1993; Liang *et al.*, 2014). Moreover, the decreased amount of the low-chlorine PCBs (1–3 Cl) resulting from anaerobic dechlorination is probably because of co-metabolic degradation, which is mediated by oxygenases of the aerobic biphenyl-degrading bacteria (Liang *et al.*, 2014). These are only indirect indicators and further investigations are still needed to unambiguously reveal the microbial biodegradation of organic pollutants in Shuwaikh harbor sediments *in situ*. Future research may

include laboratory microcosms, monitoring temporal changes in pollutants levels and microbial community structure and detection of signature metabolites and key catabolic genes. For better understanding of the ecophysiology and systems biology of the natural microbial communities of the sediments, metaproteomics, metatranscriptomics and metabolomics can be of great value in this context. Furthermore, the application of metagenomics combined with the recent next generation sequencing techniques can help overcome the limitations of DGGE and provide deeper insight into the functional microbial communities.

Conclusion

Marine sediments of the Shuwaikh harbor in Kuwait are polluted with PCBs and TPHs. These polluted sediments are inhabited by diverse hydrocarbon-degrading bacteria having potential to cope with the toxicity of the pollutants and probably utilize them as carbon sources (TPHs and PCBs) and/or electron acceptors (PCBs).

Supplementary Materials

Supplementary materials contain tables S1 and S2. Supplementary materials accompanies the paper on Polish Journal of Microbiology website.

Literature

Adler A.C., M.M. Haggblom, S.R. Oppenheimer and L.Y. Young. 1993. Reductive dechlorination of polychlorinated biphenyls in anaerobic sediments. *Environ. Sci. Technol*. 27: 530–538.

Ahmed M.H., M. El-Raey, S.M. Nasr and O.E. Frihy. 1998. Socioeconomic impact of pollution on ecosystems of the Arabian Gulf. *Environ. Int*. 24(1–2): 229–237.

Al-Awadhi H., N. Dashti, M. Khanafer, D. AL-Mailem, N. Ali and S. Radwan. 2013. Bias problems in culture-independent analysis of environmental bacterial communities: a representative study on hydrocarbonoclastic bacteria. *Springerplus* 2: 369. doi: 10.1186/2193-1801-2-369.

Al-Muzaini S., M.U. Beg, A. Al-Mutairi and A. Al-Mullalah. 1995. Sea water quality at industrial discharge zone. *Water Sci. Technol*. 32: 21–26.

Altschul S.F., L. Thomas, A.A. Madden, Z.Z. Schäffer, W. Miller and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*. 25: 3389–3402.

Bedard D.L. 2008. A case study for microbial biodegradation: anaerobic bacterial reductive dechlorination of polychlorinated biphenyls-from sediment to defined medium. *Annu. Rev. Microbiol*. 62: 253–270.

Beg M.U., T. Saeed, S. Al-Muzaini, K.R. Beg, T. Al-Obaid and A. Kurian. 2001. Extraction, fractionation, and toxicity determination of organic contaminants in sediment from coastal area receiving industrial effluents in Kuwait. *Bull. Environ. Contam. Toxicol*. 67: 881–888.

Beolchini F., A. Dell'Anno, L. De Propris, S. Ubaldini, F. Cerrone and R. Dnovaro. 2009. Auto-and heterotrophic acidophilic bacteria enhance the bioremediation efficiency of sediments contaminated by heavy metals. *Chemosphere* 74: 1321–1326.

Black E.M., M.S. Chimenti and C.L. Just. 2017. Effect of freshwater mussels on the vertical distribution of anaerobic ammonia oxidizers and other nitrogen- transforming microorganisms in upper Mississippi river sediment. *Peer J*. 5: e3536. doi:10.7717/peerj.3536.

Borja J., D.M. Taleon, J. Auresenia and S. Gallardo. 2005. Polychlorinated biphenyls and their biodegradation. *Process Biochem*. 40: 1999–2013.

Bush B. and M.J. Kadlec. 1995. Dynamics of PCBs in the aquatic environment. *Great Lakes Res*. 1(2): 24–30.

Callaghan A.V., B.E. Morris, I.A. Pereira, M.J. Mclnerney, R.N. Austin, J.T. Groves, J.J. Kukor, J.M. Suflita, L.Y. Young, G.J. Zylstra and others. 2012. The genome sequence of *Desulfatibacillum alkanivorans* AK-01: a blueprint for anaerobic alkane oxidation. *Environ. Microbiol*. 14(1): 101–113.

Clark R. 2001. *Marine Pollution*. Oxford University Press, Oxford.

Coil D.R., J.H. Badger, H.C. Forberger, F. Riggs, R. Madupa, N. Fedorova, N. Ward, F.T. Robb and J.A. Eisen. 2014. Complete genome sequence of the extreme thermophile *Dictyoglomus thermophilum* H-6-12. *Genome Announc*. 2(1): e00109-14.

Correa P.A., L. Lin, C.L. Just, D. Hu and K.C. Hornbuckle. 2010. The effects of individual PCB congeners on the soil bacterial community structure and the abundance of biphenyl dioxygenase genes. *Environ. Int*. 36: 901–906.

Daane L.L., I. Harjono, S.M. Barns, L.A. Launen, N.J. Palleron and M.M. Häggblom. 2002. PAH-degradaion by *Paenibacillus* spp. and description of *Paenibacillus naphthalenovorans* sp. nov., a naphthalene-degrading bacterium from the rhizosphere of salt marsh plant. *Int. J. Syst. Evol. Microbiol*. 52(1): 131–139.

Dell'Anno A., F. Beolchini, L. Rocchetti, G.M. Luna and R. Danovaro. 2012. High bacterial biodiversity increases degradation performance of hydrocarbons during bioremediation of contaminated harbor marine sediments. *Environ Pollut*. 167: 85–92.

Dennis P., E.A. Edwards, S.N. Liss, R. Fulthorpe. 2003. Monitoring gene expression in mixed microbial communities by using DNA microarrays. *Appl. Environ. Microbiol*. 69: 769–778.

Fowler S.W., J.W. Readman, B. Oregioni, J.P. Villeneuve and K. McKay. 1993. Petroleum hydrocarbons and trace metals in near shore Gulf sediments and biota before and after the 1991 war: An assessment of temporal and spatial trends. *Mar. Pollut. Bull*. 27: 171–182.

Furukawa K. and H. Fujihara. 2008. Microbial degradation of polychlorinated biphenyls: biochemical and molecular features. *J. Biosci. Bioeng*. 105(5): 433–449.

Gevao B., A.A. Aba, A.N. Al-Ghadban and S. Uddin. 2012. Depositional history of polychlorinated biphenyls in a dated sediment core from the Northwestern Arabian Gulf. *Arch. Environ. Contam. Toxicol*. 62: 549–556.

Gevao B., M.U. Beg, A. Al-Omair, M. Helaleh and J. Zafar. 2006. Spatial distribution of polychlorinated biphenyls in coastal marine sediments receiving industrial effluents in Kuwait. *Arch. Environ. Contam. Toxicol*. 50: 166–174.

Helaleh M., A. Al-Rashdan and A. Ibtisam. 2012. Simultaneous analysis of organochlorinated pesticides (OCPS) and polychlorinated biphenyls (PCBs) from marine samples using automated pressurized liquid extraction (PLE) and Power Prep™ clean-up. *Talanta* 94: 44–49.

Ismail W. and J. Gescher. 2012. Epoxy-coenzyme A thioester pathways for degradation of aromatic compounds. *Appl. Environ. Microbiol*. 78(15): 5043–5051.

Kitagawa W., A. Suzuki, T. Hoaki, E. Masai and M. Fukuda. 2001. Multiplicity of aromatic ring hydroxylation dioxygenase genes in a strong PCB Degrader, *Rhodococcus* sp. strain RHA1 demonstrated by denaturing gradient gel electrophoresis. *Biosci. Biotechnol. Biochem.* 65(8): 1907–1911.

Knoblauch C., K. Sahm and B.B. Jøgensen. 1999. Psychrophilic sulfate-reducing bacteria isolated from permanently cold arctic marine sediments: description of *Desulfofrigus oceanense* gen. nov., sp. nov., *Desulfofrigus fragile* sp. nov., *Desulfofaba gelida* gen. nov., sp. nov., *Desulfotalea psychrophila* gen. nov., sp. nov. and *Desulfotalea arctica* sp. nov. *Int. J. Syst. Bacteriol.* 49(4): 1631–1643.

Kolar A.B., D. Hrsak, S. Fingler, H. Cetkovic, I. Petric and N.U. Kolic. 2007. PCB-degrading potential of aerobic bacteria enriched from marine sediments. *Int. Biodeterior. Biodegradation* 60: 16–24.

Koubek J., M. Makova, T. Macek and O. Uhlik. 2013. Diversity of chlorobiphenyl-metabolization bacteria and their biphenyl dioxygenases in contaminated sediment. *Chemosphere* 93: 1548–1555.

LaRoe S.L., A.D. Fricker and D.L. Bedard. 2014. *Dehalococcoides mccarty* strain JNA in pure culture extensively dechlorinates Aroclor 1260 according to polychlorinated biphenyl (PCB) dechlorination process N. *Environ. Sci. Technol.* 48(16): 9187–96.

Li A., K.J. Rockne, N. Sturchio, W. Song, J.C. Ford and H. Wei. 2009. PCBs in sediments of the Great Lakes-Distribution and trends, homolog and chlorine patterns, and in situ degradation. *Environ. Pollut.* 157: 141–147.

Liang Y., A. Martinez, K.C. Hornbuckle and T.E. Mattes. 2014. Potential for polychlorinated biphenyl biodegradation in sediments from Indiana Harbor and Ship Canal. *Int. Biodeterior. Biodegradation* 89: 50–57.

Mahmoud H.M., R.H. Al-Hasan, N.A. Sorkhoh, M. Eliyas and S. Radwan. 2009. Attenuation of oil pollutants in the Arabian Gulf water by bacteria naturally associated with live fish. *Int. Biodeterior. Biodegradation* 63: 615–620.

Mavromatis K., N. Ivanova, I. Anderson, A. Lykidis, S.D. Hooper, H. Sun, V. Kunin, A. Lapidus, P. Hugenholtz, B. Patel and others. 2009. Genome analysis of the anaerobic thermohalophilic bacterium *Halothermothrix orenii. PLoS One* 4(1): e4192. doi: 10.1371/journal.pone.0004192.

McGenity T.J., B.D. Folwell, B.A. McKew and G.O. Sanni. 2012. Marine crude-oil biodegradation: a central role for interspecies interactions. *Aquat. Biosyst.* 8: 10–28.

Michel J. 2011. 1991 Gulf war oil spill, pp. 1127–1132. In: Fingas M (ed), *Oil Spill Science and Technology*. Elsevier, USA.

Mikesková H., C. Novotny and K. Svobodova. 2012. Interspecific interactions in mixed microbial cultures in a biodegradation perspective. *Appl. Microbiol. Biotechnol.* 95(4): 861–870.

Nam I.H., C.M. Chon, K.Y. Jung and J.G. Kim. 2014. Biodegradation of biphenyl and and 2-chlorobiphenyl by a *Pseudomonas* sp. KM-04 isolated from PCBs-contaminated coal mine soil. *Bull. Environ. Contam. Toxicol.* 93(1): 89–94.

Passatore L., S. Rossettic, A. Juwarkard and A. Massaccia. 2014. Phytoremediation and bioremediation of polychlorinated biphenyls (PCBs): State of knowledge and research perspectives. *J. Hazard. Mater.* 278: 189–202.

Ross G. 2004. The public health implications of polychlorinated biphenyls (PCBs) in the environment. *Ecotoxicol. Environ. Saf.* 59: 275–291.

Sakai M., S. Ezaki, N. Suzuki and R. Kurane. 2005. Isolation and characterization of a novel polychlorinated biphenyl-degrading bacterium, *Paenibacillus* sp. KBC101. *Appl. Microbiol. Biotechnol.* 68(1): 111–116.

Schafer H. and G. Muyzer. 2001. Denaturing gradient gel electrophoresis in marine microbiology ecology, pp. 425–468. In: Paul J.H. (ed), *Methods in Microbiology*. Vol 30 *Marine Microbiology*. Academic Press, London.

Shunbo F. and P. Literathy. 1984. Baseline studies of oil and non-oil pollutants in the territorial waters of Kuwait. Kuwait Institute for Scientific Research, Final Report no. KISR/PPI 1662.

Sydow M., M. Owsianiak, Z. Szczepaniak, G. Framski, B.F. Smets, L. Ławniczak, P. Lisiecki, A. Szulc, P. Cyplik and L. Chrzanowski. 2016. Evaluating robustness of diesel-degrading bacterial consortium isolated from contaminated soil. *N. Biotechnol.* 33(6): 852–859.

USEPA, United States Environmental Protection Agency. 1978. Total petroleum hydrocarbons in sediment, chemical analysis methodology, USA.

Van Hamme J.D., A. Singh and O.P. Ward. 2003. Recent advances in petroleum microbiology. *Microbiol. Mol. Biol. Rev.* 67: 503–549.

Wang S. and J. He. 2013. Phylogenetically distinct bacteria involve extensive dechlorination of Aroclor 1260 in sediment-free cultures. *PLoS One* 8(3): e59178. doi: 10.1371/journalpone.0059178.

Zanaroli G., A. Balloi, A. Negroni, L. Borruso, D. Daffonochio and F. Fava. 2012. A Chloroflexi bacterium dechlorinates polychlorinated biphenyls in marine sediments under in situ-like biogeochemical conditions. *J. Hazard. Mater.* 209–210: 449–457.

Zennegg M., M. Kohler, P. Hartmann, M. Sturm, E. Gujer, P. Schmid, A.C. Gerecke, N.V. Heeb, H.P. Kohler and W. Giger. 2007. The historical record of PCB and PCDD/F deposition at Greifensee, a lake of the Swiss plateau, between 1848 and 1999. *Chemosphere* 67(9): 1754–1761.

Zhang J., Y. Yang, L. Zhao, Y. Li, S. Xie and Y. Liu. 2015. Distribution of sediment bacterial and archaeal communities in plateau freshwater lakes. *Appl. Microbiol. Biotechnol.* 99(70): 3291–3302.