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Biochemical characterization, microbial diversity and biodegradability of coastal sediments in the Gulf of Gabès, Southern Mediterranean Sea

M. Jeddi¹ · F. Karray¹ · A. Battimelli² · A. Danel² · S. Melliti Ben Garali³ · M. Tedetti⁴ · H. Zaghden¹ · N. Mhiri¹ · P. Sousbie² · D. Patureau² · S. Sayadi⁵

Abstract

Coastal sediments are considered to be final receptacles for organic and inorganic contaminants. Characterizing those sediments and assessing their biodegradation potential have become a great challenge in recent years. In this study, the chemical composition, including the content in polycyclic aromatic hydrocarbons, the microbial community abundance and diversity (using culture-independent approaches targeting 16S rRNA genes), and the aerobic/anaerobic biodegradation potential of coastal sediments collected in the Sfax coastal area (Gulf of Gabès, Southern Mediterranean Sea) were investigated. The highest concentration of total polycyclic aromatic hydrocarbons (981 $\mu\text{g kg}^{-1}$ dw) was recorded in Sidi Mansour harbor sediment, emphasized pyrogenic and petrogenic hydrocarbon sources. Organic matter, including total organic carbon, and the ultimate aerobic biodegradability, with 30% as the highest value in Sidi Salem channel sediment, were in a positive accordance with bacterial communities assigned within *Actinobacteria*, *Clostridia* and *Flavobacteria* classes. The correlation noticed between *Thermocladium* and *Thermogladius* genera and sulfate content explained that Sidi Mansour and PK4 sediments are located in terrestrial acid-sulfate areas. The highest cumulative methane produced with Marseille inoculum and Tunisian inoculum was recorded in Sidi Salem sediment and strongly correlated with methanogens among *Methanobacteria*, *Methanococci* and *Methanomicrobia* classes showing the presence of industrial and municipal sources. The bioavailability of low and moderate polycyclic aromatic hydrocarbons in the current study may explain the occurrence of *Methanobacterium* which positively correlated with the anaerobic biodegradability using Tunisian inoculum with 50% as the highest value in Sidi Mansour sediment.

Keywords Aerobic/anaerobic biodegradation potential · Methanogens · Microbial community · Polycyclic aromatic hydrocarbons · Sfax coastal area

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Introduction

Coastal sediments are known to be receptacles of organic and inorganic contaminants entering the coastal marine environment through sedimentation of particles. At the same time, coastal sediments may be sources of contaminants for the water column during sedimentary resuspension events and subsequent remobilization of contaminants (Dang et al. 2015; Guigue et al. 2017). This potential release of contaminants from sediments to the water column may involve an impact on marine biota and the adaptation of microbial communities (Dang et al. 2014, 2015; Zouch et al. 2018). It has been shown that the diversity and functioning of prokaryotic communities in coastal marine sediments could be affected by their content in contaminants (Abell et al. 2014; Tangherlini et al. 2020). The diversity of carbon

sources for prokaryotic communities could be increased due to the presence of more or less biodegradable organic contaminants, leading to new ecological niches (Acosta-Gonzalez and Marques 2016; Misson et al. 2016). The development of high-throughput sequencing technologies allows us to expand the knowledge about chemical contamination impacts on microbial diversity and potential consequences on ecosystem functioning (Aylagas et al. 2017). However, only a few works have been conducted until now to assess this link between the chemical composition/contamination level and the prokaryotic diversity and abundance in coastal sediments. Misson et al. (2016) showed a strong correlation between bacterial richness and both anthropogenic and natural chemical composition, although weaker relationships were observed between archaeal richness and chemical variables.

In turn, prokaryotes can modify the fate of contaminants in coastal marine sediments by influencing their mobility (toxic metals) and degradation processes (organic contaminants). Indeed, anaerobic biodegradation experiments have been performed to assess the conversion of recalcitrant compounds, such as polycyclic aromatic hydrocarbons (PAHs), pesticides, chlorinated solvents and polychlorinated biphenyls (Vidali 2001). Increasing attention has been given recently to the application of biotechnological strategies based on the biostimulation of microbial communities to accelerate the biodegradation/bioconversion processes of organic and inorganic contaminants in coastal sediments. These strategies include the characterization of microbial communities' structure and the identification of populations associated, with a greater understanding of how physico-chemical factors may affect the biodegradation potential of contaminants. Such investigations were used to clarify the possibilities and limits of anaerobic bioremediation strategies and also to reinforce the knowledge of microbial processes in coastal sediments.

The Gulf of Gabès (Tunisia, Southern Mediterranean Sea) is considered to be a very productive and of great diversity region within the Mediterranean and is the main fishing area of Tunisia (Bel Hassen et al. 2009; D'Ortenzio and D'Alcalà, 2009; El Zrelli et al. 2017; Rabaoui et al. 2014). Nevertheless, the Gulf of Gabès is also strongly affected by anthropogenic inputs, caused by the important population development and the growth of industrial and agricultural activities. The coastal area of Sfax city, located in the northern part of the Gulf of Gabès, is impacted by intensive human activities, fishing and commercial harbors, municipal landfills, chemical and industrial effluents, oil and gas field exploitations, as well as agriculture and aquaculture activities (Aloulou et al. 2012; Fourati et al. 2018). Anthropogenic

activities present harmful threats to the marine ecosystems (Yonglong et al. 2016). For this reason, knowing the levels of contaminants and biodegradation potential of these coastal sediments has become a major issue, especially in the Gulf of Gabès, which remains a poorly explored marine area from this point of view.

In this context, the objectives of this study were first to investigate the chemical composition and the diversity and abundance of prokaryotic communities (Bacteria and Archaea) in three contrasted surface sediments collected along the Sfax coastal zone. The diversity and abundance of prokaryotic communities were determined using culture-independent approaches targeting 16S rRNA genes (Illumina Miseq sequencing and quantitative polymerase chain reaction (Q-PCR), with *mcrA* genes encoding methyl coenzyme M reductase used to determine methanogens abundance; secondly, to assess the aerobic/anaerobic biodegradability of sedimentary organic matter and contaminants; and finally, to determine the potential link between the chemical composition and the microbial community structure of those sediments.

Materials and Methods

Sampling sites and procedures

The three surface sediment samples were collected on January 27, 2016, in the Sfax coastal area, located in the northern part of the Gulf of Gabès (Tunisia, Southern Mediterranean Sea). These sediments have been chosen because they are impacted by different anthropogenic inputs. One sample was collected at the outlet of the rainwater drain channel ("PK4"), which crosses the Sfax city from the southwest to the northeast. PK4 is thus impacted by runoffs and urban discharges. One sample was taken in the Sidi Mansour fishing harbor ("PP") in the northern part of Sfax city. This site is subjected to petroleum inputs. The latter sample was collected in the Sidi Salem channel ("SS"), situated in the southern part of Sfax city. Sidi Salem releases various industrial wastes into the sea, including those of the Sfax slaughter house, agricultural alimentary industries and soap factories, as well as wastewater treatment plant (WWTP) effluent.

The surface sediment samples (0–5 cm) were collected using a clean spatula, in the morning in low tide and clear sky conditions. Samples were transferred into previously washed polypropylene bottles that were stored in the dark in the cold and then immediately frozen at -20°C until analysis and experiments.

Biological and physicochemical analysis

Biochemical methane potential (BMP) analysis

BMP was determined according to Raposo et al. (2011). For this purpose, wet sediments and two types of fresh and pre-incubated inocula were used. One inoculum originated from upflow anaerobic sludge blanket (UASB) digester in sugar industry in Marseille, France (MI), and an inoculum from anaerobic sludge treatment station in Tunis, Tunisia (TI).

Physicochemical analysis

Content in cations and anions, pH and electrical conductivity (EC) were measured with a liquid–solid (LS) ratio of 10 mL extractant (deionized water)/g dry lyophilized sediment. The extraction was conducted during 48 h followed by a centrifugation to separate the solid and liquid phases. Then, a filtration through 0.45- μm polypropylene filtration membrane was operated. Leachate subsamples were collected and stored as appropriate for ionic chromatography analysis (ICS 3000, Dionex, USA) as described elsewhere (Kosson et al. 2002).

Biological analysis

Total solids (TS) and volatile solids (VS) were determined in wet samples using standard methods (APHA 1985). Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) protocols were applied to wet sediments. Total Kjeldahl nitrogen (TKN) was determined in lyophilized sediments as described by Akhiar et al. (2016). Total carbon (TC) and total inorganic carbon (TIC) were measured in lyophilized sediments as described in Battimelli et al. (2010). For total organic carbon (TOC) determination, lyophilized sediments were acidified with 1 N HCl in order to remove inorganic carbon. Hydrogen (H) and sulfur (S) elements were determined on lyophilized and homogenized sediments (fraction < 200 μm) by an SC-144 LECO elemental analyzer at combustion temperatures of 1050 °C (H) or 1350 °C (S) in oxygen atmosphere (Zaghden et al. 2017). C, H and S were expressed in % of dried weight (% dw).

Granulometric analysis

The laser particle size analysis (granulometry) was done after 2-mm sieving and treatment with H_2O_2 and HCl using a granulometric laser. The results are expressed in % of sand (2000–63 μm), silt (63–2 μm) and clay (< 2 μm) of sediment samples.

PAH analysis

For the determination of PAH concentrations, duplicate frozen sediment samples were dried using a ventilated oven (60 h at 40 °C), crushed and sieved on a 2-mm mesh size and stored at 20 °C in the dark until extraction. An Accelerated Solvent Extraction ASE-200 system (Dionex) was used to perform the extraction from dried samples. Hexane and acetone mixture (50:50 v:v) was the extraction solvent used for the experiment. The ASE cells were filled from bottom to the top, a filter of glass fiber (19-mm diameter, Whatmann), 1 g of alumina (Sigma), 1 or 0.5 g of dried sediment and 1.5 g of Hydromatrix (Varian). For extraction procedure, cells were heated at 120 °C and with a pressure of 100 bars for 2 cycles and a static time of extraction of 5 min. The extract was evaporated under nitrogen flow to dryness. The dry extract was then dissolved in 2 mL of acetonitrile and was immediately analyzed using high-performance liquid chromatography (HPLC). The gradient of elution of the 13 PAHs was performed from acetonitrile/water (60/40%, v/v) to acetonitrile (100%). The upper and lower limits of the calibration curves were between 5–750 $\mu\text{g L}^{-1}$ for a quantification limit (LOQ) of 3 $\mu\text{g L}^{-1}$ (Trably et al. 2004).

Quality assurance and quality control of PAHs

During the PAH experiments, wearing nitrile gloves and mask was necessary in order to avoid contaminations and contact with skin. Glassware including glass brown preservative vials were previously cleaned with acetone or heated. Cells were cleaned with acetone in the ultrasonic water bath. Eight standards were used for PAH calibration with concentrations varying between 5, 10, 25, 50, 100, 250, 500 and 750 $\mu\text{g L}^{-1}$ obtained by diluting a concentrated stock solution of 10 mg L^{-1} (PAH MIX 9, CIL Cluzeau). Each ten samples (extracts in acetonitrile), a standard of 50, 100 or 250 $\mu\text{g L}^{-1}$ was injected in order to verify the instrument stability. Each extract was run two times. A recalibration was done with respect to the obtained standard deviation of the control samples.

DNA extraction, Illumina sequencing and data processing

The three coastal sediments were firstly centrifuged at 10.000 g for 5 min; the pellets were stored at –20 °C once the supernatant removed. The EZ-10 Spin Column Soil DNA Mini-preps Kit was used to extract DNA from pellets of the mixed sludge samples; it was performed according to the manufacturer's protocol. The NanoDrop ND-2000 Spectrophotometer (Thermo Scientific) was used to determine the concentration and the quality of rDNA gene.

Sequencing of PCR amplicons of 16S rDNA was conducted with the Illumina MiSeq platform (CBS, Sfax, Tunisia) targeting the V3-V4 hyper variable regions of coastal sediments using universal prokaryotic primers (Pro341/Pro805R) and archaeal specific primers sets (ARC344F/Arch806R) (Takahashi et al. 2014) for 1 × 300-bp single end sequencing (Illumina). PCR amplification was performed in triplicate in 25 µL reactions with 12.5 µL of 2 × KAPA HIFI Hot Start Ready Mix (KAPA Biosystems), 0.2 µM of forward and reverse primers and about 5 ng µL⁻¹ DNA. Thermal cycling consisted of initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s, and finally 72 °C for 5 min. The PCR products were analyzed on 2% agarose gel. Illumina library preparations of the amplified and cleaned PCR products were performed. The library quality was assessed on the Qubit®3.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. QIIME software package 1.9 was used to analyze raw data obtained from the Illumina MiSeq sequencing platform. Sequences were filtered for low-quality sequences and checked for chimera by UCHIME. The high-quality sequences were then clustered into operational taxonomic units (OTUs) using a 97% identity threshold with UCLUST (Edgar 2010). QIIME software package was used to analyze alpha diversity (Chao1, observed OTU Species, Simpson and Shannon index and PD whole tree) (Caporaso et al. 2010). Representative sequences selected from dominant OTUs (> 1% of total sequences) were picked out and the taxonomic assignment was obtained by querying NCBI (National Center for Biotechnology Information) nucleotide. The online sequence analysis resources “BLAST” (Basic Local Alignment Search Tool) was used to define sequence similarity searches (Altschul et al. 1997).

Nucleotide sequence accession number

Resulting nucleotides sequences deposited in Gen Bank database have the following accession numbers: MK092340–MK092367 for bacteria and MK097279–MK097318 for archaea.

Real-Time PCR quantification

Real-time PCR (RT-PCR) assays targeting archaeal and bacterial 16S RNA gene were performed using C1000™ Thermo Cycleur CFX96-Real-Time System (Bio-Rad). The primer sets 344F/519R and 331F/797R were used to

quantify copy numbers of bacterial and archaeal 16S rRNA genes, respectively. The Q-PCR mixture of a total volume of 10 µL consisted on Sso Advanced SYBR Green Supermix kit (Bio-Rad) including 0.25 µL of each primer (final concentration, 500 nM each), 5 µL of Sso Advanced universal SYBR Green supermix (final concentration, 1x) and 1 µL of template DNA. Negative controls without template DNA were included with each Q-PCR conducted. The bacterial RT-PCRs consisted on an initial denaturing step of 10 min at 95 °C, followed by 35 cycles of 30 s of denaturing at 95 °C, 30 s of annealing phase at 60 °C and 30 s of primer extension at 72 °C. The archaeal RT-PCRs were started by an initial denaturation at 98 °C for 2 min and 39 cycles of a two-step PCR protocol with a 5 s denaturation phase at 98 °C and followed by an annealing/elongation phase for 30 s at 56 °C (Jeddi et al. 2020). The amplification of standard DNA fragments was performed from *Halomonas alimentaria* YKJ-16^T for bacterial 16S rRNA gene Q-PCR and from *Halorubrum chaoviator* DSM 19316 for archaeal 16S rRNA gene Q-PCR (Jeddi et al. 2020). *McrA* functional gene of the group-specific methanogens was amplified by proceeding to an initial denaturation for 10 min at 95 °C followed by 40 cycles of 5 s at 95 °C, 30 s at annealing temperature of 55 °C and an extension for 30 s at 72 °C. Primer pairs McrAF–McrAR targeting methanogens were used to amplify standard DNA fragments from *Methanosarcina acetivorans*. Template DNA prepared from samples was analyzed in duplicate. Primers used in this research for RT-PCR of each domain and their characteristics are found in supplementary material (S1). Standard curves were generated and their quality parameters were judged with the slope and the correlation coefficient (R²) which was higher than 99%.

Statistical analyses

Spearman’s rank correlation coefficient (also called Spearman’s rho) is a nonparametric measure for evaluating the degree of linear correlation between two independent variables (Gauthier 2001). Spearman’s correlation test was performed using SPSS 18.0 software. For these different analyses and tests conducted, the significance threshold was set at $p < 0.05$. For a size n of 3 data samples (SS, PK4 and PP), the Spearman correlation was used to assess the relationships between various biochemical variables (TOC, SO₄²⁻, PAH, BOD₄₂/COD, cumulative CH₄-TI, cumulative CH₄-MI, BMP-TI and anaerobic biodegradability-TI) and microbial community variables (Bacteria and Archaea).

Table 1 Total 13 PAH, physicochemical parameters, cations and anions, biochemical analysis, particle size distribution and microbial abundance of coastal sediments

Coastal sediment parameters	Sidi Salem channel	PK4 outfall	Sidi Mansour harbor
<i>Total 13 PAH ($\mu\text{g kg}^{-1} \text{ dw}$)</i>	435 ± 5.6	224 ± 1.9	981 ± 4.5
Fluorene	19 ± 0.2	8 ± 0.3	16 ± 0.1
Phenanthrene	102 ± 0.9	35 ± 0.2	181 ± 2.3
Anthracene	8 ± 0.2	3 ± 0.1	194 ± 1.4
Fluoranthene	27 ± 0.5	13 ± 0.7	55 ± 0.4
Pyrene	103 ± 0.7	108 ± 2.4	139 ± 0.6
Benzo(a)anthracene	–	–	65 ± 0.34
Chrysene	75 ± 1.2	15 ± 0.5	83 ± 0.6
Benzo(b)fluoranthene	27 ± 0.5	13 ± 0.7	55 ± 0.4
Benzo(k)fluoranthene	–	–	25 ± 0.5
Benzo(a)pyrene	15 ± 0.4	–	60 ± 0.3
DiBenzo(a,h)anthracene	6 ± 0.4	3 ± 0.2	14 ± 0.3
Benzo(g,h,i)perylene	39 ± 4.1	20 ± 1.5	58 ± 0.9
Indeno(123 cd)pyrene	15 ± 0.2	7 ± 0.3	37 ± 0.3
<i>Physicochemical parameters</i>			
pH	7.85	8.05	8.48
Conductivity (mS cm^{-1})	6.79	3.97	3.26
<i>Cations and Anions (g L^{-1})</i>			
Na ⁺	1.10	0.62	0.54
K ⁺	0.08	0.06	0.03
NH ₄ ⁺	0.04	0.02	0.02
Cl ⁻	1.84	1.02	0.90
SO ₄ ²⁻	0.04	0.15	0.12
<i>Biochemical analysis</i>			
COD _T ($\text{g O}_2/\text{L}$)	77.00	39.00	13.2
BOD ₅ ($\text{g O}_2/\text{L}$)	7.60	4.00	1.40
TS (% ww)	50.20	57.5	67.0
VS (% ww)	7.80	4.90	3.20
TC (% dw)	6.71	5.72	1.54
TIC (% dw)	1.37	3.61	1.19
TOC (% dw)	5.34	2.11	0.35
TKN (% dw)	0.55	0.25	0.13
S (% dw)	1.07	0.59	0.29
H (% dw)	0.83	0.41	0.20
<i>Particle size distribution (%)</i>			
Sand (2000–63 μm)	45.19	69.86	72.52
Silt (63–2 μm)	51.98	28.43	26.01
Clay (<2 μm)	2.83	1.72	1.47
<i>Microbial abundance (copies/g)</i>			
Bacteria	62 ± 10 × 10 ⁷	1130 ± 630 × 10 ⁷	74 ± 15 × 10 ⁷
Archaea	0.8 ± 0.4 × 10 ⁷	4.2 ± 2 × 10 ⁷	3.1 ± 10 ⁷
Methanogens	12.5 ± 1.4 × 10 ³	600 ± 10 ³	45.7 ± 1.2 × 10 ³

Results and discussion

Chemical characterization and contribution to coastal sediment contamination

PP sediment presented the highest TS concentration with a rate of 67.1% wet weight (Table 1). As shown in Table 1,

TOC content was 0.35% in PP, 2.1% in PK4 and 5.3% in SS samples. These values represented, respectively, 7.3, 25 and 34% of TS. The high TOC content recorded in SS was associated with a low TIC content (1.4%). The high organic content in SS sediment may be explained by important terrestrial inputs (Sanchez-Mata et al. 1999). In contrast, the highest TIC level was observed for PK4 sediment. The highest VS

values were found in SS with an average of 7.8% wet matter, while the lowest ones were recorded in PP (3.2%). The TOC contents measured here in the three sediments are close to those already reported in sediments of Sfax harbor and Thyna channel, which ranged from 2.75 to 8%, but lower than those observed previously in Sidi Mansour sediment (Zaghden et al. 2016) and higher than those determined in the sediments of Bizerte Lagoon, Tunisia (0.4–3.9%) (Barhoumi et al. 2014). The rather high TOC concentrations can be due to the eutrophication of this coastal area caused by multiple nutrient inputs (Bel Hassen et al. 2009; D'Ortenzio and D'Alcalà, 2009).

pH values ranged from 7.85 to 8.48 and for EC, SS site showed a value of 6.79 mS cm⁻¹, whereas for PK4 and PP, values were 3.97 and 3.26 mS cm⁻¹, respectively. The highest EC detected in SS site showed the high water mineralization degree when comparing to the established standard limits (Zaghden et al. 2016), due to Cl⁻ (1.84 g L⁻¹) and Na⁺ (1.10 g L⁻¹) concentrations (Table 1) which contribute to sediment salinity and electrical conductivity. Furthermore, the important EC in sediments can be explained by the wealth of Na⁺, Ca²⁺, Mg²⁺ and K⁺ minerals. TKN detected in the studied sediments showed values between 0.09% dw in PP, 0.25% dw in PK4 and 0.55% dw in SS site (Table 1). This latter value was higher than that reported for harbor sediments in a Mediterranean French site (i.e., 0.3% dw of TKN) (Padox et al. 2010). Hence, we may consider that SS site is more influenced by anthropogenic inputs, including domestic and industrial wastes, than the two other sites.

The organic matter origin and sources in the studied sediments can be highlighted by C/N ratio. The highest TOC/TKN ratio in SS site (9.7), in accordance with the highest conductivity in SS sediment, suggested organic matter of both marine and continental origin. Fine sediment fractions (silt and clay) dominated in SS sediment accounting, respectively, for 52.0 and 2.8% of particle distribution. This fine fraction predominance is known to contribute to the accumulation of contaminants in the sediment (Ujevic et al. 2000). On the other side, sand was the predominant sediment fraction in PK4 and PP sites, with, respectively, 69.9 and 72.5% of particle distribution. This difference of particle size distribution between the southern (SS) and northern sites (PK4, PP) may be related to different hydrodynamic conditions, with in the north, the presence of an intertidal preventing the deposition of the finest fraction (Aloulou et al. 2012). The highest contents of H and S were recorded in SS sediment with, respectively, 0.83 and 1.07%. The S content in the studied sediments ranged from 0.29 and 1.07% and

was close to that recorded for superficial sediments of the Sfax–Kerkennah channel (Zaghden et al. 2017).

COD and BOD₅ are useful parameters to estimate the rate of total oxidizable matter in sediment affected by urban and industrial wastes. COD values were 77 × 10³ mg L⁻¹ in SS, 39 × 10³ mg L⁻¹ in PK4 and 13.2 × 10³ mg L⁻¹ in PP (Table 1), and were higher than those reported previously in the same Sfax coastal area (from 0.5 to 1.9 × 10³ mg L⁻¹) (Drira et al. 2016; Zaghden et al. 2016). The BOD₅ values reached 7.6 × 10³ mg L⁻¹ in SS, 4 × 10³ mg L⁻¹ in PK4 and 1.4 × 10³ mg L⁻¹ in PP (Table 1), far exceeding the average value measured in Sidi Mansour (200 mg L⁻¹), Sfax harbor (190 mg L⁻¹) and Ouad Maou channel (300 mg L⁻¹) (Zaghden et al. 2016). In conclusion, the fingerprint of anthropogenic inputs was clearly identified and higher for the SS surface sediment (Sfax southern area).

Concentrations in PAHs

The total (\sum 13) PAH concentrations determined in the studied sediments varied between 224 and 981 µg kg⁻¹ dw. The highest PAH concentrations were found in PP and SS (Table 1). PAHs of low molecular weight (two and three-ring, LMW-PAHs) accounted for 21–40% of the total PAHs in the studied sites. PAHs of moderate molecular weight (four-ring, MMW-PAHs) represented 20–54% of the total PAHs, whereas high molecular weight PAHs (five and six-ring, HMW-PAHs) accounted for 26–41%. PK4 sediment had the highest contribution of MMW-PAHs (54%). The highest concentration (194 µg kg⁻¹ dw) was observed for anthracene series in PP site. Benzo(a)anthracene and benzo(k)fluoranthene were only recorded in PP sediment and were associated with petroleum contamination called petrogenic sources (De Luca et al. 2004). For pyrene series, values of 103, 108 and 139 µg kg⁻¹ dw were observed for SS, PK4 and PP stations, respectively. Pyrene was the most abundant compound in SS and PK4 sediments.

Phe/Ant (phenanthrene vs anthracene concentrations) and Flt/Pyr (fluoranthene vs pyrene concentrations) isomeric ratios were determined to assess contamination sources. For PP sediment, a Phe/Ant ratio of 0.9 highlighted strong pyrogenic inputs associated with the combustion of petroleum, wood, coal like creosote and coal tar (Baumard et al. 1998). In addition, the studied stations (SS, PK4 and PP) displayed \sum LMW/ \sum HMW ratio < 1, Flt/Flt + Pyr ratio < 0.4 and Flt/Pyr ratio between 0.1 and 0.4, which illustrated both petrogenic and pyrogenic sources of PAHs (Li et al. 2006; Yunker et al. 2002).

Table 2 Comparison of total PAH concentrations (in $\mu\text{g kg}^{-1}$ sed. dw) in surface sediments from different regions of the Tunisian coastline

Tunisian site	Number of analyzed PAHs	Range ($\mu\text{g kg}^{-1}$ sed. dw)	Pollution level ^a	Reference
Coastal zone of Sfax	13	224–981	Moderate	This study
Gulf of Gabès, Sfax harbor	17	175–10,769	Moderate to very high	(Zaghden et al. 2017)
Gulf of Gabès, Sfax–Kerkennah channel	17	175–245	Moderate	(Zaghden et al. 2017)
Gulf of Gabès, Sfax coastline and Kerkennah channel	17	113–10,720	Moderate to very high	(Zaghden et al. 2007)
Gulf of Gabès, Sfax coastline	16	811–5608	Moderate to very high	(Zaghden et al. 2014)
Between the coastal zone of Sfax and the Sfax–Kerkennah channel	17	150,3–2100	Moderate to high	(Zaghden et al. 2007)
Near the coastal zone of the Kerkennah Island	17	560,8–955,3	Moderate	(Zaghden et al. 2007)
The coastal zone of the Kerkennah Island	17	406,2	Moderate	(Zaghden et al. 2007)
Gulf of Tunis (Rades, Goulette, Sidi Bou Saïd harbors)	22	363–7026	Moderate to very high	(Mzoughi and Chouba 2011)
Jarzouna-Bizerte coastal area	17	916–3146	Moderate to high	(Zrafi-nouira et al. 2010)
Coasts of Sfax, Luza, Khniss and Sousse	14	160–1990	Moderate to high	(Kessabi et al. 2013)
Ghar El Melh Lagoon	17	40–655	Low to moderate	(Ben Ameur et al. 2010)
Bizerte Lagoon	16	2–537	Low to moderate	(Louiz et al. 2008)
Bizerte Lagoon	16	83–447	Low to moderate	(Trabelsi and Driss 2005)
Bizerte Lagoon	14	17–394	Low to moderate	(Barhoumi et al. 2014)
Khniss coast	17	7–15	Low	(Zrafi et al. 2013)

^aThe pollution levels are those defined by (Baumard et al. 1998): low: 0–100 $\mu\text{g kg}^{-1}$; moderate: 100–1000 $\mu\text{g kg}^{-1}$; high: 1000–5000 $\mu\text{g kg}^{-1}$; very high: > 5000 $\mu\text{g kg}^{-1}$; bld: below detection limit

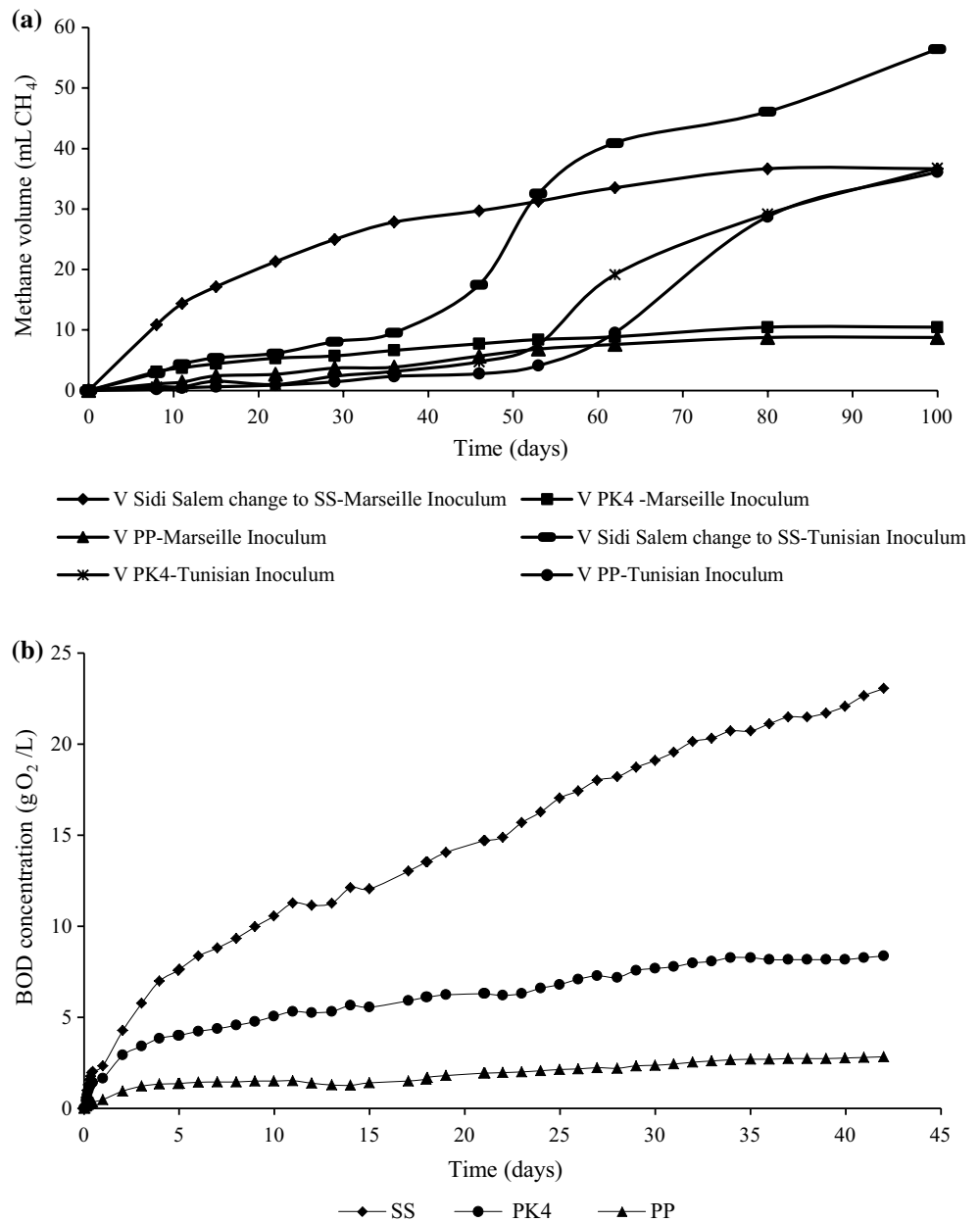
When comparing with other marine areas in the Tunisian coastline as shown in Table 2, the PAH concentrations in this study were generally higher than those found in the Sfax–Kerkennah channel (sum of 17 PAHs between 175 and 245 $\mu\text{g kg}^{-1}$ sed. dw), but lower than those observed in the Sfax harbor (sum of 17 PAHs of 10,769 $\mu\text{g kg}^{-1}$ sed. dw) (Zaghden et al. 2017), in the Thyna bay (sum of 16 PAHs of 1929 $\mu\text{g kg}^{-1}$ sed. dw) and in the vicinity of the industrial area of phosphogypsum discharge (sum of 16 PAHs of 5608 $\mu\text{g kg}^{-1}$ sed. dw) in the Sfax coastline (Zaghden et al. 2014). Moreover, the PAHs concentrations in this work, were higher than those indicated for Khniss coast, Tunisia (sum of 17 PAHs between 7 and 15 $\mu\text{g kg}^{-1}$ sed. dw), but rather lower than those determined in the coasts of Sfax, Luza, Khniss and Sousse (sum of 17 PAHs of 160–1990 $\mu\text{g kg}^{-1}$ sed. dw) (Kessabi et al. 2013). Therefore, the PAH concentrations reported here are relatively

moderate compared with those observed in other sediments of the Tunisian coast (Baumard et al. 1998).

Contribution of COD, BOD and BMP to organic matter biodegradability

The conducted BMP experiment explored the influence of using two different anaerobic inoculums on the digestion of coastal sediments. The results presented in Fig. 1a showed the diversity of biodegradation behaviors of the selected coastal sediments. BOD and BMP variables were expressed with reference to COD concentrations in order to compare the aerobic and anaerobic biodegradability of the organic matter contained in the samples. The specific methane volume produced with both inoculums is expressed in mL $\text{CH}_4/\text{g COD}$ added. The aerobic and anaerobic biodegradability rates were expressed in percentages compared to the theoretical methane volume. The results presented

Fig. 1 a Average methane production curves for sediments inoculated with Marseille and Tunisian inocula. **b** Average biochemical oxygen demand in coastal sediments



in Table 3 showed that under aerobic conditions, BOD₂₁/COD ratios varied from 14 to 19% for the three sites studied reflecting coastal sediments slowly biodegradable in 21 days of incubation (Fig. 1b). Thus, as BOD₅/COD and BOD₂₁/COD ratio provides information about the anthropogenic compounds biodegradability and the pollution origin (Yigiterhan et al. 2011; Rhaouat et al. 2014), the BOD₅/COD ratio lower than 50% in all sites highlighted a pollution of

chemical origin (Rhaouat et al. 2014). Moreover, the BOD₄₂/COD ratio between 21 and 30% indicated a slow moderately biodegradability of sediments after 42 days (Fig. 1b). PP sediment with the highest PAH concentration presented 75% of recalcitrant chemical components compared to other samples (73% for PK4 and 61% for SS). So, most of the organic matter in the sediment samples from sites were non-biodegradable with very low content of rapidly

biodegradable matter with an average of 10%. This may be caused by intense fishing, maritime traffic and industrial effluents generating hardly biodegradable chemical pollution (Zaghden et al. 2014).

Under anaerobic conditions with Marseille inoculum, a biodegradability of 7–21% was noted with a BMP of 74 mL CH₄ / g COD for the most biodegradable SS sediment (Table 3). With Tunisian inoculum, 26–50% of biodegradability was observed with the highest BMP of 175 mL CH₄/g COD (Table 3). The difference in biodegradability was related to a high mineral content in matrices, which was in accordance with the origin of the recalcitrance of organic matter for anaerobic biodegradation. Indeed, the incubation time for Tunisian inoculum was greater than 40 days in order to observe a significant variation in methane production (Fig. 1a). Whereas in the case of Marseille inoculum, a low methanogenic potential was observed for PK4 and PP sediments and a rapid initiation of the methanogenic potential was recorded from the beginning of the incubation for SS sediment, which showed the highest methane production (Fig. 1a). Thus, a sufficient acclimatization period for microflora is required in order to sustain to the changes in the system in the case of a low methanogens metabolism. The overall performance of the ecosystem depended not only on the time of contamination, but also on the degree of contamination allowing the selection of efficient microorganisms (Hayes et al. 1999). Furthermore, the biotransformation of PAHs under anaerobic conditions depended on a sufficiently long adaptation period and appropriate electron acceptors availability (Karthikeyan and Bhandari 2001).

Taxonomic diversity and microbial abundance of bacterial and archaeal communities in coastal sediments

In order to study the taxonomic diversity of 16S rRNA gene in coastal sediments, prokaryotic universal primers were used for high-throughput sequencing. As shown in Table 4, for prokaryotic communities, OTU numbers ranged between 1052 and 1579 and sequence numbers between 27,258 and 69,122. The highest richness (observed OTU) and diversity (Simpson and Shannon indexes) were recorded in PK4 sediment. Phylum level derived from different coastal sediments for prokaryotic universal primers are shown in Fig. 2a. In this study, for prokaryotic communities, the abundance of *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* phyla was noted with different percentages among coastal sediments. Therefore, *Firmicutes* phylum presented a relative abundance within the studied coastal sediments ranging between 3.6 and 38.6%. SS sequences were mostly assigned to *Firmicutes* with the highest abundance (38.6%). The phylum *Proteobacteria* dominated especially in PK4 sediment with 60.1% and less in SS (27.6%) and PP (46.4%). PK4 sequences were principally assigned to *Proteobacteria* with 18.5% for *Alphaproteobacteria*, 12% for *Betaproteobacteria* and 15% for *Deltaproteobacteria*. PP sequences were mostly affiliated to *Gammaproteobacteria* with 25.6%. Furthermore, *Bacteroidetes* phylum varied between 11.1% in SS, 13% in PK4 and 14.8% in PP and *Chloroflexi* phylum was noticed at 8.9% in PP, 4.01% in PK4 and 2.36% in SS.

Taxonomic classification at genus level for OTU > 1% from microbial DNA sequences in the studied coastal sediments compared by blast with NCBI (supplementary

Table 3 Biological analysis, aerobic biodegradability and anaerobic biodegradation potential with Marseille and Tunisian inoculums

Coastal sediment analysis	Sidi Salem channel	PK4 outfall	Fishing Port Sidi Mansour
<i>Biological analysis and aerobic biodegradability</i>			
COD _T (g O ₂ /L)	77.0	39.0	13.2
BOD ₅ (g O ₂ /L)	7.6	4.0	1.4
BOD ₂₁ (g O ₂ /L)	14.7	6.3	1.9
BOD ₄₂ (g O ₂ /L)	23.1	8.4	2.8
Rapidly aerobic biodegradability BOD ₂₁ /COD _T (%)	19.0	16.0	14.0
Ultimate aerobic biodegradability BOD ₄₂ /COD _T (%)	30	21	21
<i>Anaerobic biodegradation potential with MI</i>			
Cumulative CH ₄ -MI (mL CH ₄)	37	10	9
BMP-MI (mL CH ₄ /g COD)	74	26	42
Anaerobic biodegradability-MI (%)	21	7	12
<i>Anaerobic biodegradation potential with TI</i>			
Cumulative CH ₄ -TI (mL CH ₄)	56	37	36
BMP-TI (mL CH ₄ /g COD)	114	92	175
Anaerobic biodegradability-TI (%)	33	26	50

Table 4 Richness and diversity of microbial communities in SS, PK4 and PP samples using prokaryotic universal primers and archaeal specific primers

Microbes	Sample	Sequence	OTU	OTU > 1%	Chao	Coverage(%)	Shannon	Simpson
Prokaryotic universal primers	SS	27,258	1052	14	1126,039	93.43	8.083	0.990
	PK4	64,887	1579,9	9	1711,702	92.3	8.968	0.994
	PP	69,122	1476	9	1553,716	95	8.950	0.994
Archaeal specific primers	SS	82,744	420	17	424,773	98.88	5.812	0.960
	PK4	111,416	594	18	605,053	98.18	6.006	0.952
	PP	137,834	614	15	618,125	99.34	6.524	0.961

material S2, S3) is illustrated in Fig. 2b. For OTU > 1%, in SS sediment, the presence of a fermentative halophilic anaerobic bacteria called *Halanaerobium* with 3.17% was detected, and this genus is affiliated to *Firmicutes* phylum, order *Clostridiales*. *Clostridiisalibacter* genus with 4.9% and *Fusibacter* genus with 1.54% seems to be able to use thiosulfate and elemental sulfur as terminal electron acceptors to produce sulfide (Fadhlaoui et al. 2015). These genera were present in other coastal sediments (PK4 and PP) at very low percentages that did not exceed 1%. Highly abundance of *Thioalkalivibrio* genus was found in PP sediment with 3.99%, and this genus belongs to *Gammaproteobacteria* class and is characterized by the ability to remove sulfide from bio and industrial waste gases in full-scale bioreactor (Muyzer et al. 2011). The organic matter degradation in sediments included anaerobic bacterium which are *Youngiibacter*, related to *Firmicutes* phylum, and were only present in SS sediment at 3.36%, and also *Clostridium* and *Acetobacterium* genera at 3.17 and 2.38%, respectively. *Bifidobacterium* genus related to *Actinobacteria* phylum with a relative abundance of 1.36% was mostly present in SS sediment. Moreover, marine bacterium of the family *Flavobacteriaceae* involved *Robiginitalea* and *Actibacter* with relative abundance of, respectively, 1.8 and 1.67% were presented mostly in PP sediment and are involved in anaerobic digestion steps which are hydrolysis and acidogenesis (Dias et al. 2016; Jabari et al. 2016). The aerobic bacterium *Cellulophaga* genus generally found in marine habitats was present with 2.5% in SS sediment (Kahng et al. 2009). In addition, bacterial species related to degrading aromatic compounds metabolism were more abundant in PK4 than in SS and PP sites; *Thauera aromatica* and *Thauera phenylacetica* contributed to 6.6% of *Thauera* genus relative abundance within *Betaproteobacteria* phylum (Mechichi et al. 2002). Sulfate-reducing bacteria present within *Deltaproteobacteria* phylum included *Desulfomicrobium* genus, which is isolated from the anoxic contaminated sediments (Duran et al. 2008). Moreover, *Desulfotignum* genus, with the ability to oxidize the monoaromatic hydrocarbon toluene coupled to hydrogen sulfide production (Ommedal and Torsvik 2007), was mostly present in PK4 at 1.6%. *Desulfosarcina* genus, known

to oxidize organic compounds or hydrogen and to reduce sulfate into sulfide (Devereux et al. 1989), was noticed at 1.33% in PP sediment and at 0.64% in PK4 sediment. Then, other bacterial species contributed to amino acid degradation in bioreactors such as *Aminobacterium* genus within *Synergistaceae* family with 1.99% in PP sediment and very low percentages of 0.37% in PK4 and 0.2% in SS sediments (Chertkov et al. 2010). *Leptolinea* and *Thermomarinilinea* genera, with relative abundance of 1.27 and 1.07%, respectively, in PP sediment exceeding those in SS and PK4 sediments, were affiliated to the bacterial *Chloroflexi* taxon and may contribute as well to degradation of carbohydrates (Yamada et al. 2005). *Marivita* genus related to *Alphaproteobacteria* phylum with polysaccharide-degrading microorganisms capacities was present in PK4 sediment with 2.25% (Yoon et al. 2012).

In addition, the relative abundance of archaeal communities using prokaryotic universal primers showed percentages of 3.12% in SS, 0.97% in PK4 and 4.16% in PP sediment from total prokaryotic communities assigned principally to *Euryarchaeota* phylum and less to *Crenarchaeota* and *Parvarchaeota* phyla. With the aim of studying the archaeal communities in the different sites, archaeal specific primers were used. Sequence analysis exhibited that archaeal communities were mostly affiliated to *Euryarchaeota* phylum with 95, 86.9 and 57.2%, whereas *Crenarchaeota* phylum showed 4, 11 and 32.6% in SS, PK4 and PP sediments, respectively. As well, methanogenic communities recorded using archaeal specific primers represented highly percentages of 82, 62 and 29% from total archaeal communities in SS, PK4 and PP sediments, respectively. Phylum level derived from different coastal sediments for archaea domain is shown in Fig. 2c. As shown in Table 4, for archaeal communities, OTU numbers varied between 420 and 614 and sequence numbers between 82,744 and 137,834. PP sediment presented the highest sequence and OTU numbers, richness (observed OTU) and diversity (Simpson and Shannon indexes). Taxonomic classification using archaeal specific primers at genus level for OTU > 1% (Fig. 2d) showed that methanogenic communities included within *Euryarchaeota* phylum predominate in the studied sediments.

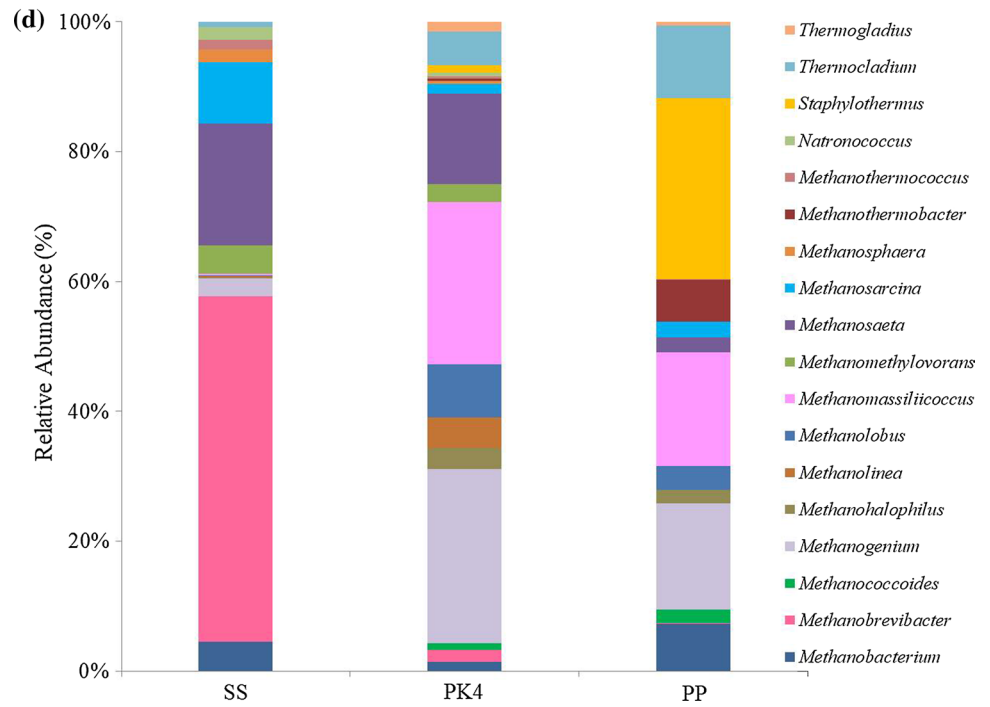
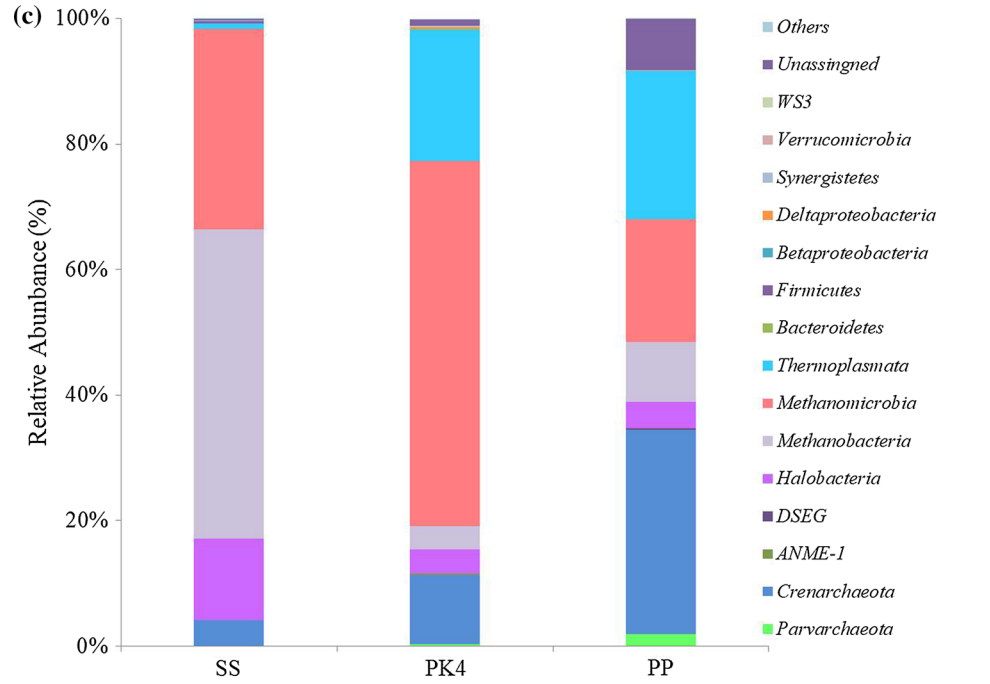
Fig. 2 a Relative abundance of microbial communities in SS, PK4 and PP samples using prokaryotic universal primers. Relative phylogenetic abundance was based on frequencies of 16S rRNA gene sequences affiliated with archaeal and bacterial phyla or proteobacterial classes in SS, PK4 and PP samples. **b** Relative abundance of microbial communities in SS, PK4 and PP samples using prokaryotic universal primers. Relative phylogenetic abundance was based on frequencies of 16S rRNA gene sequences affiliated with major genera from dominant OTU (> 1% of all sequences) in SS, PK4 and PP samples. **c** Relative abundance of microbial communities in SS, PK4 and PP samples using archaeal specific primers. Relative phylogenetic abundance was based on frequencies of 16S rRNA gene sequences affiliated with archaea and bacterial phyla or proteobacterial classes in SS, PK4 and PP samples. **d** Relative abundance of microbial communities in SS, PK4 and PP samples using archaeal specific primers. Relative phylogenetic abundance was based on frequencies of 16S rRNA gene sequences affiliated with major genera from dominant OTU (> 1% of all sequences) in SS, PK4 and PP samples



Therefore, *Methanobrevibacter* genus was the most abundant with 38% in SS sediment, and this genus grows with H_2 , CO_2 and formate and probably present in human faeces

and originated from a municipal sewage digester (Miller and Lin 2002). In addition, *Methanosarcina* genus was dominant in SS sediment with the highest percentage of 6.8%. It is

Fig. 2 (continued)



worth noting a percentage of 1% in PK4 and PP sediments. The predominance of genus *Methanosarcina* throughout digestion strongly indicated that this archaea has important functions in methanogenesis of organic matter (Wintsche et al. 2016). *Methanosaeta* genus was present too with relatively high percentage in both SS (13.5%) and PK4 (10%). *Methanosaeta* genus was characterized by an exclusive use of acetate as a substrate for producing methane and was widely distributed in nature as methane producer (Mori et al. 2012). Hydrogenotrophic methanogens were represented by *Methanobacterium* genus, isolated from sludge deposited in a crude oil storage tank and mostly detected in PP sediment at 4% and in SS sediment at 3.2% (Mori and Harayama 2011). Within *Methanomicrobia* class in PK4 sediment, *Methanolinea* genus was present at 3.3% of abundance, and this genus is implicated in the final step of organic substances degradation and generally obtained from methanogenic digester of municipal sewage sludge functioning in mesophilic conditions (Imachi et al. 2008). Mesophilic methylotrophic methanogen called *Methanobolus* genus was highly present at 5.8%, generally found in saline environments (Mochimaru et al. 2009). Moreover, thermophilic methanogens were also present mostly in PP sediment; *Methanothermobacter* genus conserved energy by reducing CO₂ from H₂ to produce CH₄ and synthesized all of its cellular components from these same gaseous substrates as well as N₂ or NH₄⁺ and inorganic salts (Smith et al. 1997). *Methanomassiliicoccus* genus was rare (<1%) in SS sediment compared to an abundance of 18% in PK4 and 9.7% in PP. The marine halophilic *Methanogenium* genus was noticed at 19.26% in PK4 sediment and at 9.03% in PP sediment. This genus was isolated from marine sediments and was able to grow by reducing CO₂ with H₂ or formate as electron donor (Chong et al. 2002). Furthermore, *Natronococcus* genus including aerobic halophilic archaea was classified within *Halobacteria* class and was present in SS sediment at 1.39% and in PK4 sediment at 0.37%. This genus is generally found in alkaline, hypersaline lakes and soils (Kamekura 1998). However, within *Crenarchaeota* phylum, the dominance of the hyperthermophilic and heterotrophic *Staphylothermus* genus in PP sediment at 15.4% was noted. The extremely thermophilic *Thermocladium* genus, isolated from acidic areas with 6.2% in PP sediment and 3.7% in PK4, utilized carbon sources for growth and required sulfur, thiosulfate as possible electron acceptors (Itoh et al. 1998). Likewise, the hyperthermophilic heterotrophic *Thermogladius* genus was recorded in PK4 sediment at 1.1% PK4 and in PP sediment at 0.3%. This genus is primarily found in terrestrial acid-sulfate areas and implicated in fermentation

metabolism to produce organic acids from a variety of complex organic substrates (Osburn and Amend 2011).

The total microbial population quantitatively present in sediments expressed as 16S rRNA gene concentration Q-PCR analysis is reported in Table 1. Thus, PK4 sediment presented both the highest mean number of bacterial 16S rRNA gene copies of 1130 copie gene/g dw ($\pm 630 \times 10^7$) and archaeal 16S rRNA gene copies with a mean number of 4.2 copie gene/g dw ($\pm 2 \times 10^7$). Wastewater outfalls may influence the total and active bacterial community abundance and it seems that the highest bacterial and archaeal abundance was noticed in sediments affected by terrestrial inputs, receiving sewage discharges with a high toxic potential. The archaeal community represented 1.4, 0.4 and 4% of the total prokaryotic population (Bacteria and Archaea) for SS, PK4 and PP sediments, respectively. The methanogenic community abundance presented 1.42% of total archaeal communities in PK4 sediment. Thus, Q-PCR showed that the bacteria dominated the prokaryotic community at the studied areas. The highest archaea percentage of 4% is observed in PP site which is the most contaminated site with PAHs and TS content, certainly due to combined effects of terrigenous organic matter inputs and harbor activities pollution (Zhang et al. 2009).

Correlation between biochemical characterization and microbial communities' diversity in sediments

Marine coastal sediments are subject to various natural and anthropogenic inputs. Indeed, the community structure variability including bacteria and archaea could be explained statistically by a selection of biochemical variables (Misson et al. 2016). In this study, "Spearman correlation" was used in order to investigate the correlation between chemical parameters and microbial communities' structure. As observed in Table 5, the organic matter contribution to microbial diversity patterns appeared significant. Therefore, important links between TOC content and bacterial communities assigned within *Clostridia* class like *Acetobacterium*, *Fusibacter*, *Clostridiisalibacter*, *Clostridium*, *Halanaerobium* and *Romboutsia*, also *Cellulophaga* and *Bifidobacterium* genera were observed. These bacteria are implicated in hydrolysis, acidogenesis and degradation of complex organic compounds. In addition, *Seohaecicola* and *Calothrix* genera, with the ability to produce hydrogen sulfide, were related to the occurrence of the highest SO₄²⁻ concentration in PK4 sediment. Furthermore, the correlation underscored between *Thermocladium* and *Thermogladius* genera and sulfate content explained that PP and PK4 sediments are located in

Table 5 (continued)

Biotic variables		Abiotic variables																	
Domain	Species	TOC	SO ₄ ²⁻	13 PAHs	PY	PHE	ANT	FLU	CHR	BbF	BaP	BBaH	BghiP	IcdP	BOD ₅ /COD	Cum CH ₄ -MI	Cum CH ₄ -TI	BMP-TI	AB-TI
Archaea	<i>Methanobacterium</i>			1.000	- 1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	ns	ns	1.000	1.000	
	<i>Methanobrevibacter</i>	1.000													1.000	1.000	1.000		
	<i>Methanococcoides</i>	- 1.000													1.000	1.000	1.000		
	<i>Methanomethylovorans</i>	1.000													1.000	1.000	1.000		
	<i>Methanosarcina</i>	1.000													1.000	1.000	1.000		
	<i>Methanosphaera</i>	1.000													1.000	1.000	1.000		
	<i>Methanothermobacter</i>	- 1.000													1.000	1.000	1.000		
	<i>Methanothermobacter</i>	1.000													1.000	1.000	1.000		
	<i>Natronococcus</i>	1.000													1.000	1.000	1.000		
	<i>Staphylothermus</i>	- 1.000													1.000	1.000	1.000		
	<i>Thermocladium</i>		1.000												1.000	1.000	1.000		
	<i>Thermoglandius</i>		1.000												1.000	1.000	1.000		

TOC Total Organic Carbon, PY Pyrene, PHE Phenanthrene, ANT Anthracene, FLU Fluoranthene, CHR Chrysene, BbF Benzo(b)fluoranthene, BaP Benzo(a)pyrene, BBaH Dibenzo(ah)anthracene, BghiP Benzo(ghi)perylene, IcdP Indeno(123 cd)Pyrene, Cum cumulative, AB Anaerobic Biodegradability, BMP Biochemical Methane Potential

terrestrial acid–sulfate areas. The aerobic biodegradation potential showed that the best ultimate aerobic biodegradability (BOD_{42}/COD_T) observed in SS sediment correlated positively with the aerobic genus *Cellulophaga* and other genera including *Romboutsia*, *Halanaerobium*, *Fusibacter*, *Clostridium*, *Clostridiisalibacter*, *Bifidobacterium* and *Acetobacterium*. Moreover, a positive relationship was noticed between almost all the detected bacterial classes, except *Cyanobacteria*, *Alphaproteobacteria* and *Betaproteobacteria*, and the four-ring PAHs pyrene, which was mostly abundant in PP sediment. As shown in Table 5, the PAH contamination, including LMW-PAHs and HMW-PAHs and the highest PAH concentration ($981 \mu\text{g kg}^{-1} \text{dw}$), proved to be strongly related to *Brenneria* and *Thioalkalivibrio* genera which were mostly present in PP sediment. These PAHs were positively correlated with *Methanobacterium* genus. This can be explained by the abundance of *Methanobacterium petrolearium* species in SS sediment and *Methanobacterium ferruginis* species in PP sediment, which were in agreement with the presence of LMW-PAHs, having both petrogenic and combustion sources, and HMW-PAHs, which were predominantly of pyrogenic origin. The relative high contribution of low and moderate molecular weight PAHs in PP sediment (60% of total PAHs) may explain the occurrence of *Methanobacterium* genus, positively correlated with BMP-TI (highest value in PP sediment) and the anaerobic biodegradability-TI, and able to degrade bio-available molecules as shown in previous studies (Potter et al. 1999; Straube et al. 1999). Hence, decreases or very significant shifts in microbial diversity in response to PAHs contamination were reported (Correa et al. 2010; Guazzaroni et al. 2013). Therefore, organic contaminants correlated well with the highest cumulative CH_4 -MI recorded in SS sediment, with a rapid acclimation stage, and the cumulative CH_4 -TI, which was in accordance with the abundance of *Natronococcus*, *Methanothermococcus*, *Methanosphaera*, *Methanomethylovorans*, *Methanoseta* genera and also *Methanobrevibacter* genus, showing then the presence of industrial and municipal sources in SS site comparing to other studied sediments.

Overall, these findings suggest that anthropogenic inputs can deeply influence microbial communities' richness and diversity in surface coastal sediments. The anthropogenic activities across the coastal zone of the Gulf of Gabès, displaying sediments with heterogeneous contaminants, promote a high diversification of prokaryotic and archaeal communities, likely supposing the hypothesis of well-adapted communities to various contaminants mixtures. These results open perspectives for understanding the potential

contribution of communities to actions of contaminant bioremediation to elucidate the functioning of the sediment microbiome with the aim of evaluating the ecological and biochemical consequences of chemical pollution on coastal ecosystems.

Conclusion

The study indicated that superficial sediments from all sampling sites in the Sfax coastal area in the Gulf of Gabès received intensive anthropogenic inputs affecting the ecosystem health. Total concentrations of 13 PAHs in sediments reached $981 \mu\text{g kg}^{-1} \text{dw}$. This revealed a relationship between the contamination levels and the anthropogenic pressures on the coastline. The physicochemical characterization of sediments showed contamination by organic and inorganic compounds. The high organic matter reactivity resulting in the almost complete organic matter consumption and the little methane production indicated that industrial effluents were enriched with non-biodegradable substances. This was confirmed by the COD/BOD ratios showing the presence of recalcitrant chemical pollution. Thus, the contamination degree in the studied ecosystems allowed the selection of efficient microorganisms involved in the PAH removing and organic biodegradation process under anaerobic conditions. Then, a bioremediation strategy could be efficient knowing microbial communities and contaminant characterization of SS sediment in order to metabolize organic and inorganic compounds with the objective of preservation of aquatic environment and the maintain of the sustainability of its resources.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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