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Sedimentary organic biomarkers suggest detrimental effects of PAHs on estuarine microbial biomass during the 20th century in San Francisco Bay, CA, USA

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HIGHLIGHTS

- Total PAHs in a sediment core were 20× higher after 1900 CE.
- PAH isomer ratios consistent with predominant petroleum combustion source.
- PAH concentrations exceed sediment quality screening values.
- High *trans*/*cis* ratios of unsaturated FA and PCA link PAHs to ecological affects.
- Petroleum contamination negatively impacted microbial and/or algal biomass.

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ABSTRACT

Hydrocarbon contaminants are ubiquitous in urban aquatic ecosystems, and the ability of some microbial strains to degrade certain polycyclic aromatic hydrocarbons (PAHs) is well established. However, detrimental effects of petroleum hydrocarbon contamination on nondegrader microbial populations and photosynthetic organisms have not often been considered. In the current study, fatty acid methyl ester (FAME) biomarkers in the sediment record were used to assess historical impacts of petroleum contamination on microbial and/or algal biomass in South San Francisco Bay, CA, USA. Profiles of saturated, branched, and monounsaturated fatty acids had similar concentrations and patterns downcore. Total PAHs in a sediment core were on average greater than 20× higher above ~200 cm than below, which corresponds roughly to the year 1900. Isomer ratios were consistent with a predominant petroleum combustion source for PAHs. Several individual PAHs exceeded sediment quality screening values. Negative correlations between petroleum contaminants and microbial and algal biomarkers – along with high *trans*/*cis* ratios of unsaturated FA, and principle component analysis of the PAH and fatty acid records – suggest a negative impacts of petroleum contamination, appearing early in the 20th century, on microbial and/or algal ecology at the site.

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1. Introduction

Coastal environments have suffered increasing pollution pressure as population and industrialization have increased rapidly over the past 150 years. San Francisco Bay is a highly impacted estuary on the West Coast of the USA. South San Francisco Bay provides important habitat for birds, fish, and many other organisms, and recreation and commercial benefits to human residents, despite heavy contamination via urban runoff, atmospheric deposition, and wastewater discharge. Hydrocarbon sources are ubiquitous near urban aquatic ecosystems such as South San Francisco Bay, and can potentially impact microbes, microalgae and higher organisms. Polycyclic aromatic hydrocarbons (PAHs) biomagnify through the food web (Twiss et al., 1999), and some can have acutely toxic effects (Phillips, 1983; Carman et al., 1997; Kanaly and Harayama, 2000; Ko and Day, 2004). The extent of these effects on resident organisms and food webs in coastal systems is largely

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unknown (Dickhut et al., 2000), but is of concern, especially since recent evidence shows an increase in the environmental occurrence of PAHs, a reversal of the decrease since the 1960s (Van Metre et al., 2000; Lima et al., 2003; Liu et al., 2012).

Some microbes can degrade certain PAHs (Shuttleworth and Cerniglia, 1995). Laboratory and *in situ* microcosm experiments have shown increased growth of hydrocarbon degraders in the presence of petroleum contaminants (Ghiorse et al., 1995) and shifts in community composition favoring degrader populations (Castle et al., 2006). Although bioavailability of PAHs in a sediment matrix can be limited by adsorption to carbonaceous materials such as black carbon (Cornelissen et al., 2005) exposure of PAHs to cells occurs by diffusive transport (Harms and Bosma, 1997; Johnsen et al., 2005). Low molecular weight (LMW) aliphatic contaminants can dramatically decrease microbial diversity by conferring a selective advantage to organisms that successfully metabolize these contaminants (Song and Bartha, 1990; Viñas et al., 2005). It has been suggested that sedimentary microbial communities may adapt to chronic, elevated PAH concentrations (Carman et al., 1995). However, detrimental impacts of petroleum hydrocarbon contamination on non-PAH degrading microbial populations have not often been considered, despite the fact that PAHs can be toxic to bacteria (Britton, 1984; Hyötyläinen and Olkari, 1999; El-Alawi et al., 2002; Samanta et al., 2002; Lee et al., 2003) and that microbial communities have shown rapid and profound changes in response to PAH contamination (Langworthy et al., 1998; Abed et al., 2002). Toxicity of these and other compounds to phytoplankton, algae, and aquatic plants has also not received thorough consideration (Lewis and Pryor, 2013).

Molecular biomarkers, such as fatty acid methyl esters (FAMEs), reflect changes in sources and amounts of organic matter present, including microbial and aquatic plant biomass. FAMEs are mainly components of cellular membranes and can help differentiate between higher plant, algal and bacterial sources of organic matter (Canuel et al., 1995; Zimmerman and Canuel, 2001; Waterson and Canuel, 2008). Individual fatty acids (FAs) are often grouped into chemical categories such as saturated FAs, branched (chain) FAs, monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs), dicarboxylic acids (DCAs), and hydroxy fatty acids to indicate different sources of organic matter.

In this study we analyzed a sediment core record for petroleum contaminants and FAME biomarkers to (1) assess the recent history of petroleum contaminants and their sources to the site over the period of record, (2) assess patterns in microbial and plant organic matter sources and accumulation over the period of record, and (3) test whether we can use FAME biomarkers in the sediment record to evaluate historical impacts of petroleum contamination on microbial and/or plant biomass.

2. Experimental section

2.1. Sample collection

A 2.7 m long, 8 cm diameter core was collected in South San Francisco Bay (Fig. 1) in September 2005 using a portable vibracore attached to an aluminum pipe 6 m in length. The core was sealed after the pipe was trimmed and was transported by truck upright to the laboratory in Menlo Park, CA the same day, then split in half lengthwise and sub-sampled in 2- to 5-cm intervals for 31 total subsamples. A portion of each sample was freeze dried, homogenized, crushed and sieved for organic carbon and radioisotope analyses; the remainder was frozen (−20 °C) until analyzed. Several other sediment cores were collected on earlier sampling dates, but were deemed unsuitable due to evidence of bioturbation or insufficient core length to resolve the historical period of interest. In future studies, analysis of replicate core records would be desirable.

2.2. Radionuclide analyses

Freeze-dried sediment samples were analyzed for ^{210}Pb, ^{226}Ra and ^{137}Cs to estimate sediment accumulation rates and for ^{7}Be (53.3 d half-life) and ^{234}Th (24.1 d half-life) as indicators of sediment mixing and recent deposition rate using a high-resolution intrinsic germanium detector gamma spectrometer based on ASTM methods C 1402-98 and E 181-98. These methods are similar to methodology previously described (Robbins and Edgington, 1976; Baskaran and Naidu, 1994; Fuller et al., 1999; Van Metre, 2004). The reported uncertainty in the measured activity was calculated from the random counting error of samples and background spectra at the one standard deviation level. Additional information on radioisotopes is provided in Appendix A.

2.3. Bathymetric reconstruction

The profile of sediment age was reconstructed based on the temporal sequence of bathymetric changes in the bay recorded via bathymetric surveys conducted in 1858, 1898, 1956, and 2005. A Geographic Information System (GIS) model called Bathychronology (Higgins et al., 2005, 2007) automates the reconstruction procedure using bathymetry grids (Foxgrover et al., 2004; Jaffe and Foxgrover, 2006) and allows for rapid and easily repeatable analyses. A decrease in water depth between surveys was interpreted as a depositional horizon and an increase in water depth between surveys was interpreted as erosion that has removed previously deposited sediments. The increased depth between surveys gives the erosion rate. Subsidence that occurred in South San Francisco Bay (Poland and Ireland, 1988) was accounted for in the algorithm. In subsiding areas, the deposition was increased by the amount of subsidence. Additional information on sediment chronology, radioisotopes, and bathymetric reconstruction is provided in Appendix A.

2.4. Organic carbon

Total and inorganic carbon were measured by coulometric titration (UIC, Inc., Coulometrics Model 5012) and are reported as weight percent (dry weight). To determine carbonate carbon concentrations, ~5–10 mg of sample was reacted with dilute hydrochloric acid in a heated reaction vessel. The percentage of carbonate in each sample reflected the amount of inorganic carbon liberated as CO_2 with the assumption that all inorganic carbon was present as calcium carbonate. Total carbon concentrations reflected the amount of carbon released as CO_2 during combustion of a ~5–10-mg sample in oxygen at ~1000 °C. Total organic carbon (TOC) was determined by difference. Each sample was measured in duplicate and reported as an average. Relative standard deviation of the mean of multiple measures of a mixture of typical samples analyzed in replicate with each analytical run was 0.1%, and the detection limit for a typical sample size of 5–10 mg was 0.3 wt%. Splits of a portion of the intervals had organic carbon determined by Carlo Erba 1500 elemental analyzer attached to a Micromass Optima continuous-flow mass spectrometer. Values determined by coulometric technique and by elemental analyzer were comparable.

2.5. Contaminant analyses

Polycyclic aromatic and aliphatic hydrocarbons (Table 1) were extracted from wet aliquots of thawed and homogenized sediment (~5 g dry weight) using a microwave-assisted solvent extraction system (MARSX, CEM Corp). Deuterated surrogate standards were added to each sample just prior to extraction. Sediments were solvent-extracted twice in sequence, first with a mixture of hexane:acetone (1:1) then with a mixture of dichloromethane:acetone

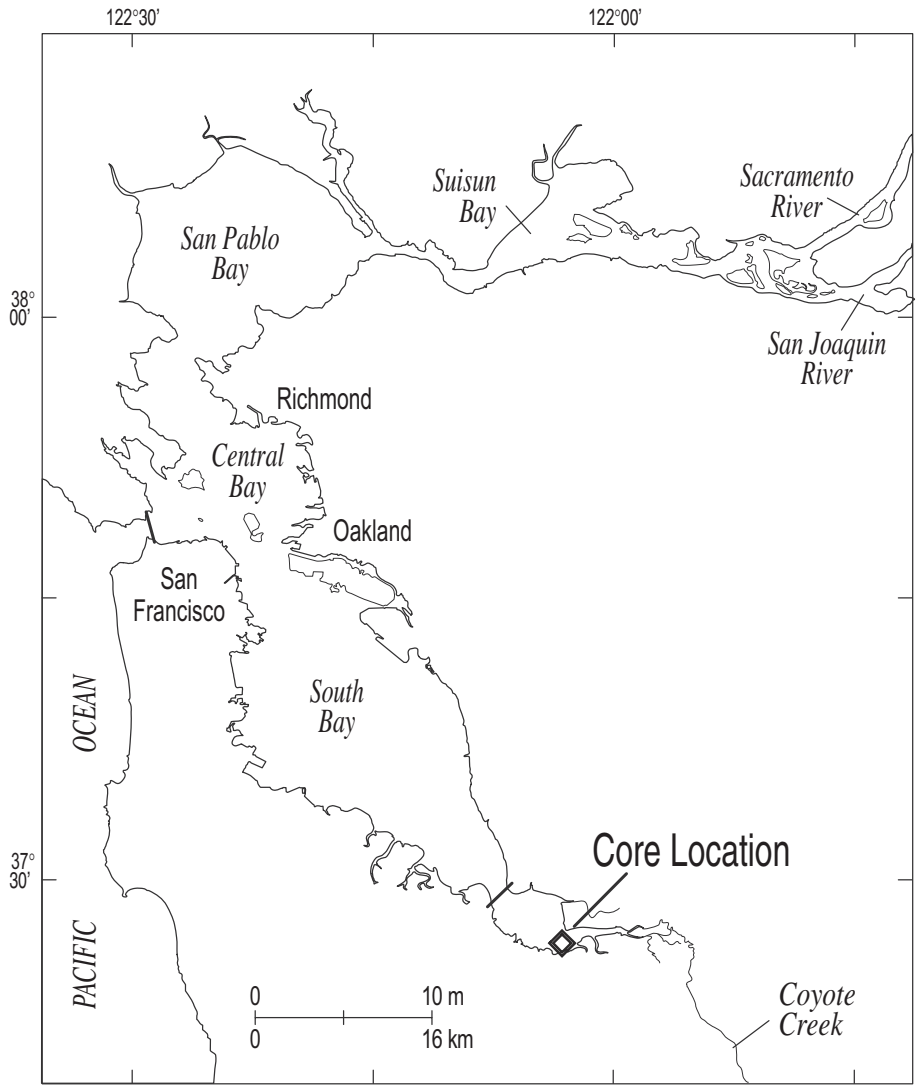

Fig. 1. Site map of the study area and core location (37.27038N, 122.02898W; collected in 1.2-m water depth).

(1:1). Extracts were filtered through $NaSO_4$ to remove water, treated with activated copper to remove sulfur compounds, and then concentrated to 1 mL in preparation for column chromatography to isolate specific compound classes and to remove interfering compounds.

Chromatographic columns were prepared with 2 g silica gel, wet-packed using hexane. Samples were loaded on to individual columns in hexane and the aliphatic fraction eluted with a hexane rinse. The aromatic fraction (including PAHs) was eluted using 25% toluene in hexane. The purified extracts were concentrated to a volume of 1 mL under a stream of nitrogen. An internal standard mixture was added to the aromatic fraction prior to final evaporation to a 1-ppm final concentration.

Aliphatic and aromatic fractions were identified and quantified on a gas chromatograph/mass spectrometer using Agilent Chemstation software. Compounds were separated on a fused silica capillary column. Aliphatic peaks were detected using scanning ion monitoring and quantified using an external calibration curve.

PAH peaks were detected using selected ion monitoring and quantified relative to internal standards added just prior to analysis. Standard curves of all target PAH compounds were analyzed at the beginning, middle and end of each GC–MS analysis.

The presence of monoaromatic steranes (MAS) and 17α,21β(H)-hopane (α,β-hopane) were qualitatively assessed where present. An ion profile of mass 253 was extracted from the total ion chromatograph of the aliphatic fraction. An area count of the MAS peaks was obtained for samples that had MAS present. The peak height of (α,β-hopane) was obtained where present from an extracted ion profile of mass 191. These data should be considered qualitative as they were based on peak heights and areas and not quantified using standard materials. Additional information on contaminant analysis procedures including chemical standard compositions and instrument specifications and conditions is provided in Appendix A.

2.6. Fatty acids

FAMEs were analyzed by MIDI, Inc. (Newark, DE) using the Sherlock® Microbial Identification System (Sasser, 1990). Approximately 3 g freeze-dried sediment were saponified using sodium hydroxide, methanol and water, then methylated with hydrochloric acid and methyl alcohol. The sample was then extracted using 1:1 hexane:methyl tert-butyl ether, concentrated and then analyzed by GC–MS using a 25 m × 0.2 mm i.d. phenyl methyl silicone fused silica capillary column. FAME peaks in the sample were compared to a stored database using the Sherlock pattern recognition software and an external calibration standard for peak naming.

Table 1
Polycyclic aromatic hydrocarbon (PAH) names and abbreviations.

PAH	Abbreviation
Naphthalene	Na
Acenaphthylene	Ay
Acenaphthene	Ac
Phenanthrene	Ph
Anthracene	An
Fluoranthene	Fl
Pyrene	Py
Benzo[a]anthracene	BaA
Chrysene	Ch
Benzo[b]fluoranthene	BbF
Benzo[k]fluoranthene	BkF
Benzo[a]pyrene	BaP
Indendo[1,2,3-cd]pyrene	IP
Dibenzo[a,h]anthracene	DBaA
Benzo[g,h,I]perylene	BgP
2-Methylnaphthalene	2-Na
1-Methylnaphthalene	1-Na
1,5-Dimethylnaphthalene	1,5-DMNa
2-Methylphenanthrene	2-Ph
3,6-Dimethylphenanthrene	3,6-DMPh
7,12-Dimethylbenz[a]anthracene	7,12-DMBaA
Benzo[e]pyrene	BeP
Perylene	Pe
Fluorene	n/a

2.7. Statistics

Statistical analyses were performed using JMP™ software (version 5.0.1a, by SAS Institute, Inc.). Pairwise correlations and principal component analysis (PCA) were performed using the multivariate analysis platform. Before PCA was performed, any variable that was undetectable was set to a value of 1. Data were log transformed after being standardized by subtracting the mean and then dividing by the standard deviation for each constituent (Yunker et al., 2005).

3. Results and discussion

3.1. Sediment chronology from radioisotopes and bathymetric reconstruction

Total ^{210}Pb activity decreased from the core top to the supported activity (defined by ^{226}Ra) at depths greater than 67 cm. Very low levels of unsupported ^{210}Pb in this core were consistent with other cores from San Francisco Bay and result from the low atmospheric ^{210}Pb fallout rates and dilution of suspended sediments by older sediments (Fuller and Hammond, 1983; Fuller et al., 1999). Below 67 cm, unsupported ^{210}Pb was not detectable. The detection limit for unsupported ^{210}Pb (0.6 dpm g^{-1}) corresponds to an age of about 45 years (or 1962) using a mass balance model (Fuller et al., 1999) and assuming the depositing sediments have an unsupported ^{210}Pb activity (2.7 dpm g^{-1}) equal to the average for South San Francisco Bay suspended sediments (Fuller, 1982).

A sediment MAR of 1.0 ± 0.2 g cm^{-2} year^{-1} was estimated using the Constant Flux–Constant Supply (CF–CS) method (Appleby and Oldfield, 1992) by linear regression of the natural log (ln) of the unsupported ^{210}Pb activity to a depth of 67 cm versus cumulative dry sediment mass (g cm^{-2}) to account for sediment compaction. This method assumes a constant MAR over time, which is reasonable given the near exponential decrease inferred by the linear relationship of the ln(unsupported ^{210}Pb) with cumulative dry mass (Appendix Fig. A1). Sediment deposition date (i.e., age) was determined as a function of cumulative dry mass from the resulting MAR and plotted as a function of depth (Fig. 2). This ^{210}Pb chronology was valid only through the maximum depth (67 cm) of measurable unsupported ^{210}Pb used to define the MAR, but was extrapolated to 100 cm for comparison to the historical bathymetric reconstruction at this site.

Activities of ^{137}Cs were relatively constant in the upper 70 cm of the sediment profile and then decreased sharply to nondetectable below 79 cm. The ^{137}Cs profile was likely not the result of post-depositional mixing (e.g. bioturbation) since no measurable activity of ^{7}Be or unsupported ^{234}Th were observed in the upper 10 cm of a box core collected at this site, indicating negligible sediment mixing, in contrast to other coring studies in San Francisco Bay (Fuller, 1982; Fuller et al., 1999). Instead, the lack of structure in the ^{137}Cs profile may result from mixing of sediments during transport prior to deposition at the core site at the southern-most end of the bay. The maximum depth of measurable ^{137}Cs, 79 cm, has a ^{210}Pb date of 1953, consistent with the first occurrence of measurable ^{137}Cs fallout in 1952 from large-scale atomic weapons testing (Van Metre, 2004).

Reconstruction of sediment ages from historical bathymetry records for this site (Foxgrover et al., 2004; Higgins et al., 2007) yielded apparent dates for depths based on local changes in water depth derived from baywide bathymetric surveys conducted over the past 150 years. Sediment MARs of 1.7 and 1.1 g cm^{-2} year^{-1} were calculated from adjacent bathymetric reconstruction model depth horizon dates for the 1983–2005 and 1956–1983 periods, respectively. Comparison of bathymetric reconstructed chronology to the ^{210}Pb dates illustrates the higher apparent accumulation rate of the former (Fig. 2). The agreement in sediment dates between the two methods was reasonable given the level of uncertainty in each method. More detailed discussion of both the radionuclide profiles and bathymetric reconstruction is provided in Appendix A.

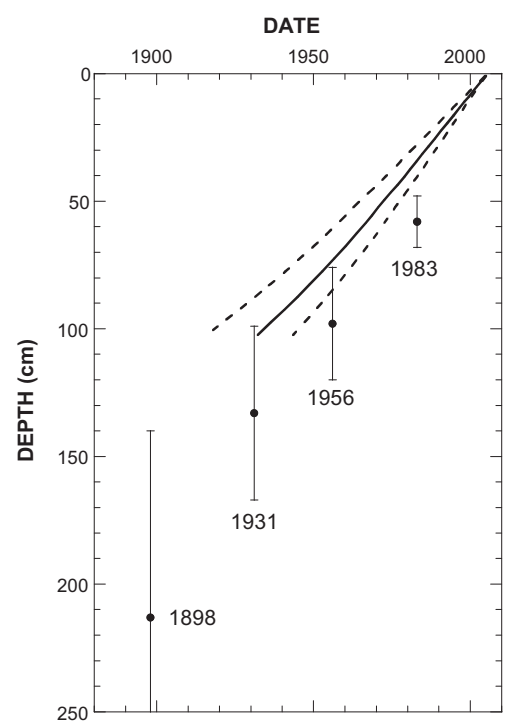

Fig. 2. Sediment deposition date versus depth. Solid line depicts age defined by ^{210}Pb mass accumulation rate; dashed lines depict age range for each interval based on the uncertainty in slope of ln(unsupported ^{210}Pb) versus cumulative dry mass. Solid symbols depict sediment age from bathymetric reconstruction of core with error bars representing the uncertainty in depth range for the dated horizons.

3.2. Bulk organic matter and fatty acid composition

Sediment core intervals were screened for 257 biomarker compounds (Appendix B). Branched FAs generally indicate a bacterial source, while PUFAs and some MUFAs are more labile organic compounds often derived from algal sources, although many MUFAs are also bacterial in origin (Waterson and Canuel, 2008 and references therein). In fact, MUFAs have been shown to reflect Gram-negative bacteria, cynobacteria and microalgae; branched FAs have been shown to indicate Gram-positive bacteria, Gram-negative anaerobes and sulfate reducing bacteria (Harji et al., 2010). Organic-carbon-normalized profiles of saturated, branched, and MUFA compounds have similar concentrations and patterns downcore (Fig. 3). One exception was a large peak in MUFAs at ~200 cm, which was not present in the saturated and branched FAs, but occurs in the OC record. This peak corresponds to an interval of laminated sediments in the core, probably reflecting tidal marsh production. Labile PUFAs are highly susceptible to degradation and were present in very few intervals. There was insufficient material to analyze for FAs in the surface (0–2 cm) intervals. In general, the DCAs and hydroxy fatty acids show similar downcore patterns to those of the saturated and branched FAs. All hydroxy fatty acids detected were short chain (<C20), probably reflecting an aquatic phytoplankton (Mudge and Norris, 1997; Treignier et al., 2006) and/or bacterial (Rieley et al., 1991; Waterson and Canuel, 2008) source.

Most of the FA categories were positively correlated and had statistically significant relationships (Table 2). Only MUFAs and hydroxy fatty acids did not have a statistically significant correlation (not shown), suggesting that these groups of compounds had distinct sources. MUFAs and to a lesser extent total FAs and DCAs were positively correlated with OC. Hydroxy fatty acids and branched and saturated FAs had no statistical relationship to the OC record. Although samples were screened for several PUFAs, only 18:3ω6c was detected. Aside from the PUFAs, the FA profiles likely reflect changes in sources rather than diagenesis since ratios of compound classes having different reactivities were not consistent with a pattern expected for diagenesis (Zimmerman and Canuel, 2002) where concentrations would be higher at the surface and decrease over time assuming steady state inputs (Appendix C Fig. C1).

Table 2
Pairwise correlations of San Francisco Bay contaminants, organic carbon (OC) and fatty acids (FA).

Variable		r^a	n^b	p^c
FA categories				
∑HFA	∑Branched	0.66	29	***
∑HFA	∑Saturated	0.64	29	***
∑Branched	∑Saturated	0.80	29	***
∑DCA	∑Alcohols	0.46	29	**
∑DCA	∑Branched	0.79	29	***
∑DCA	∑MUFA	0.69	29	***
∑DCA	∑Saturated	0.74	29	***
∑MUFA	∑Branched	0.64	29	***
∑MUFA	∑Saturated	0.38	29	*
OC and FA categories				
OC	Total FA	0.37	29	*
OC	∑DCA	0.40	29	*
OC	∑MUFA	0.58	29	***
Contaminants				
Total PAH	Total FA	−0.37	29	*
Total PAH	∑Branched	−0.41	29	*
Total PAH	∑DCA	−0.41	29	*
Total PAH	OC	−0.64	29	***
α,β-Hopane	OC	−0.50	31	***
MAS	OC	−0.55	31	***

HFA = hydroxyl fatty acids, DCA = dicarboxylic acids, MUFA = monounsaturated fatty acids, OC = organic carbon, FA = fatty acids, MAS = monoaromatic steranes.
[a] Correlation coefficient.
[b] Sample number.
[c] Significance probability.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

3.3. Petroleum contaminants

Total PAHs (∑PAHs) ranged from ~120 to 9560 ng g^{-1} dry sediment, comparable to recently studied sediment cores collected from urbanized areas in Sweden (Sánchez-García et al., 2010) and southwest China (Liu et al., 2012). The downcore profile of ∑PAHs displayed three distinct segments (Fig. 4a). ∑PAHs were

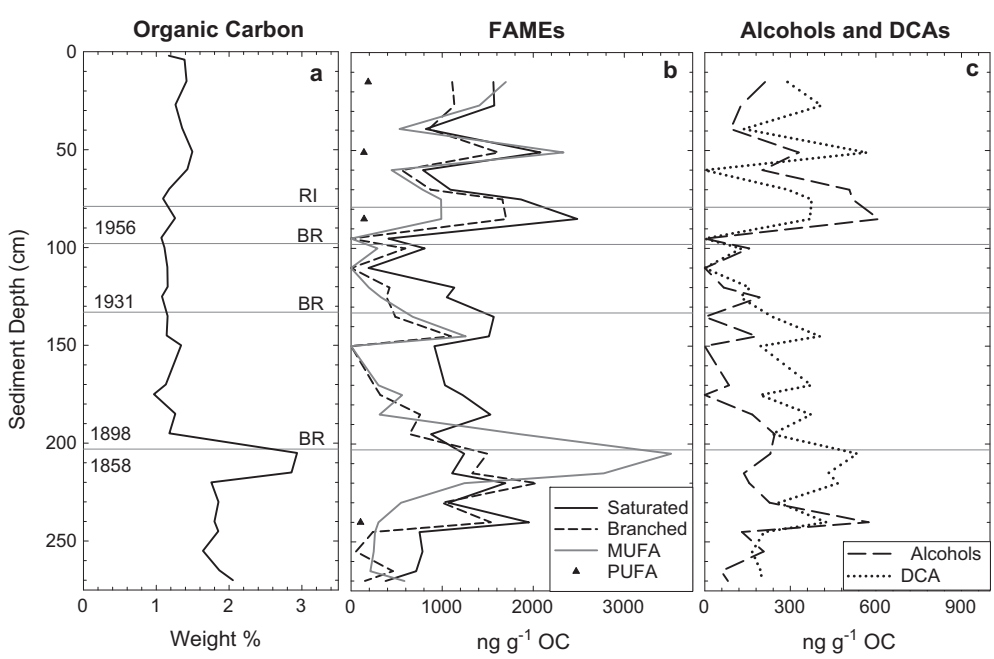

Fig. 3. Concentrations of total organic carbon (a), saturated FAs, branched FAs, MUFAs and PUFAs (b), and hydroxy fatty acids and DCAs (c) relative to total organic carbon and versus depth (cm). The horizontal lines are age-depth horizon centers derived from radioisotope data (RI) and bathymetric reconstruction (BR).

uniform and lower than 320 ng g^{-1} in the interval deeper than 200 cm. At ~200 cm ∑PAHs increased sharply and between ~200 and 75 cm ∑PAHs oscillated between ~2400 and >9500 ng g^{-1} with maxima at 110 cm and 170–190 cm. From ~75 cm to the sediment surface, ∑PAHs were generally uniform and ranged between ~1800 and 2600 ng g^{-1}. ∑PAHs were on average greater than 20× higher between ~200 cm and 75 cm than deeper than 200 cm. The ∑PAH profile we observed (Fig. 4) was similar to those observed in other urban estuaries (Latimer and Quinn, 1996; Lima et al., 2003). The PAH increase at ~200 cm likely reflects the advent of industrialization in the San Francisco Bay Area beginning in the late 1800s. The subsequent decrease has been attributed to increased petroleum and natural gas usage compared to coal burning beginning after the Great Depression, and to the implementation of stricter fuel emissions standards in the 1960s (Lima et al., 2003). The peak at ~110 cm may reflect emissions during World War II. The ∑PAH decrease toward the present in our record begins somewhat earlier than that observed in previous studies. This was perhaps unexpected since the period between 1950 and 1970s was characterized by rapid development in the San Francisco Bay Area. The PAH increase in the early 1900s also appears to occur at a faster rate in South San Francisco Bay than observed elsewhere, possibly owing to a faster rate of population growth associated with the gold rush in the preceding decades. It is also possible that the PAH increase only appears relatively rapid due to a hiatus in the sediment record between 1858 and 1898.

The parent PAHs dominate the alkyl homolog series for Na and Ph (PAH abbreviations defined in Table 1) with the methylated to parent ratio less than 2 (Fig. 4b), indicating a petroleum combustion rather than a petrogenic source for the PAHs (Yunker and Macdonald, 1997). Ph/An ratios, which had a maximum value of 4, were also well below the value of ⩾50 indicative of fresh petroleum (Lake et al., 1979). A combustion source is consistent with previous results for surface sediments from the region (Pereira et al., 1999; Oros and Ross, 2004). Lower ratios of BaP/BgP below ~200 cm indicate that biomass burning was a likely source of PAHs during that time period; however, this ratio was also lower in the more recent portion of the record. Additional isomer ratios indicate a mixture of PAH sources among petroleum, petroleum combustion, and biomass burning (Fig. 5). Although using these kinds of ratios to assign sources can be problematic in some cases (Lima et al., 2005), this is still a widely used tool to help shed light on PAH sources and processes (e.g., Boonyatumanond et al., 2006; Arias et al., 2010).

Monoaromatic steranes (MAS), hopanes (α,β-hopane and tricyclic hopanes) and an unresolved complex mixture (UCM) were present above ~200 cm, indicating residual petroleum (Hostettler et al., 1999) likely sourced from urban runoff and/or spillage of petroleum and its refined products containing PAHs (Pereira et al., 1999). Peak areas of MAS and peak heights of α,β-hopane have maxima at ~65 cm and were not present below ~200 cm (Fig. 6). The only documented major oil spill during the time period studied was the 1971 San Francisco Bay Oil Spill, which may be consistent with the timing of the maxima at ~65 cm. UCM was visible in chromatograms (not shown), but areas could not be quantified because of interfering peaks. MAS were positively correlated to ∑PAH ($r = 0.47$; $p < 0.01$) and α,β-hopane ($r = 0.86$; $p < 0.001$). The PAH and α,β-hopane records were not statistically related. This lack of correlation could be due to differences in recovery since the latter data are qualitative.

3.4. Potential effects of petroleum contaminants on ecology

The ∑PAH and the total FA records were significantly negatively correlated (Table 2). ∑PAHs were also significantly negatively correlated to some of the individual biomarker compound classes, i.e., branched FAs and DCAs. ∑PAHs, MAS, and α,β-hopane were all significantly negatively correlated to OC. Correlation does not equate to causality; however, the data suggest a negative relationship between petroleum contamination (and/or some other associated contaminant that was not measured) beginning early in the 20th century and microbial and/or algal biomass.

The *trans* to *cis* ratio of unsaturated FA has previously been used to indicate starvation or other stress within bacterial communities. *Trans*/*cis* ratios were calculated for this study (Appendix F) and

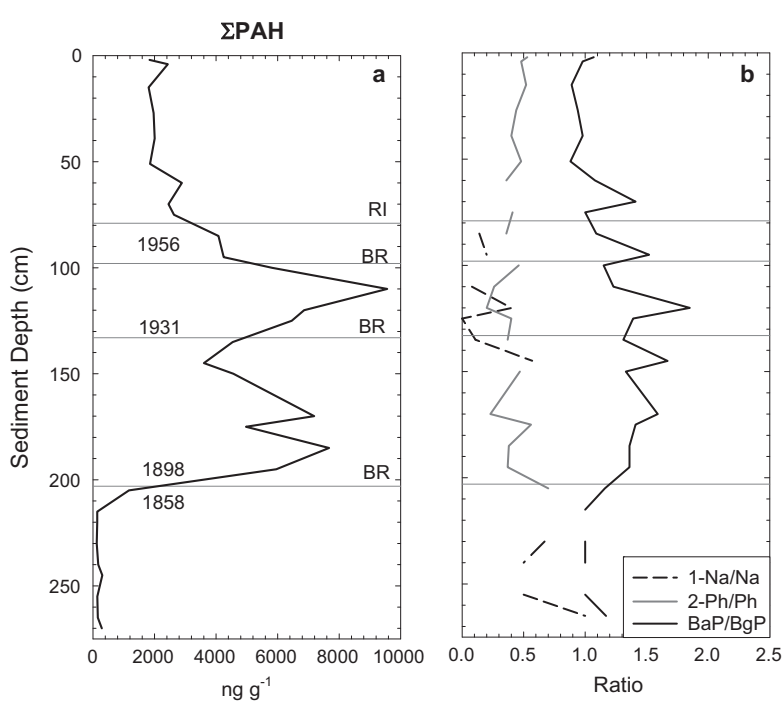

Fig. 4. Concentration of total PAHs (a) and 1-Na/Na, 2-Ph/Ph, and BaP/BgP isomer ratios (b) versus depth (cm). Horizontal lines as in Fig. 2.

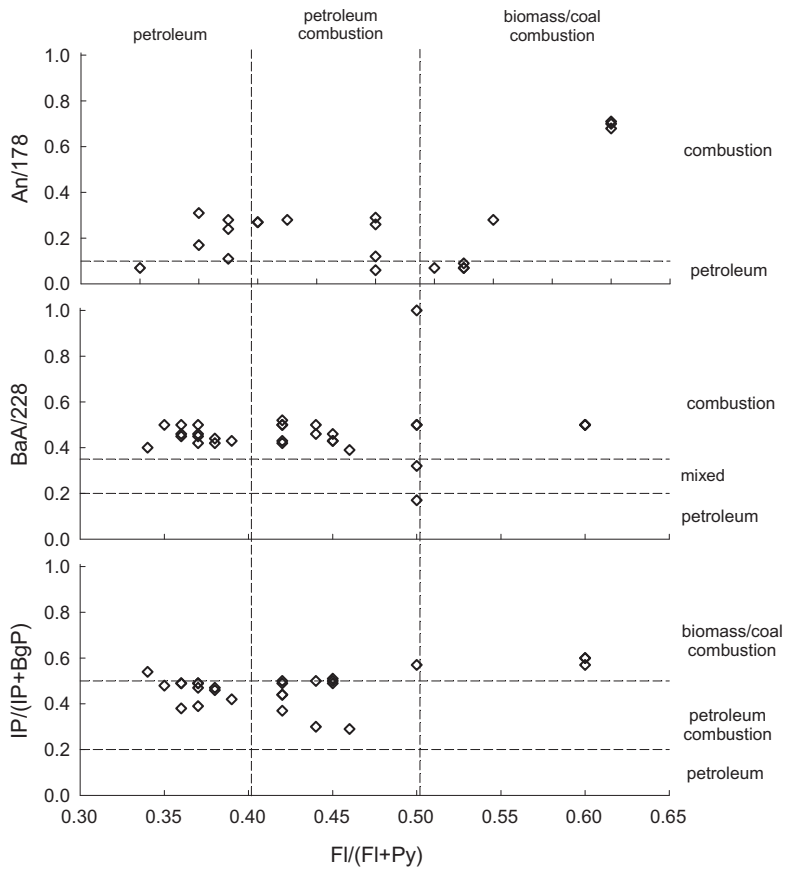

Fig. 5. Plots of PAH isomer ratios. An/178 = An/(An + Ph), BaP/228 = BaP/(BaP + Ch). Dashed lines denote ranges for different sources. Figure after Oros and Ross (2004); source ranges based on Yunker et al. (2002).

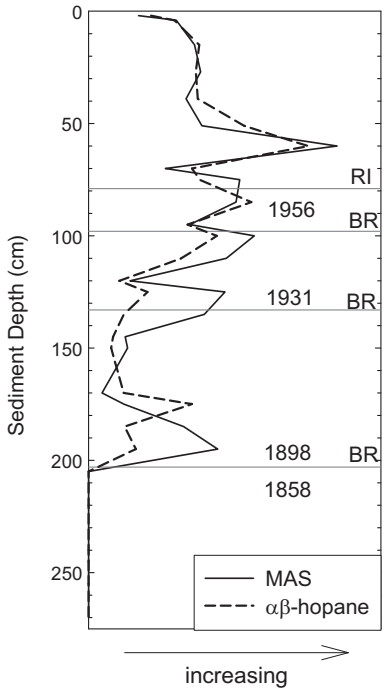

Fig. 6. Depth profiles of petroleum indicators: monoaromatic steranes (MAS) and α,β-hopane. Horizontal lines as in previous figures.

were found to be 2–320× greater than the normal index of 0.1 reported for most environmental samples (Guckert et al., 1986). Because *cis*/*trans* isomerization has been identified as an assessment tool for toxicity of organic compounds to *Pseudomonas* and *Vibrio* genera (Heipieper et al., 1995, 2003) the high ratios calculated for this study could be another indication of detrimental effects of petroleum contamination on bacteria.

Principal component analysis (PCA) was used to further examine the relationships between biomarkers and contaminant concentrations. The first principal component (PC1) of all of the individual FA records accounts for 25% of the variance in the records (Table 3). Although PC1 loadings were significantly positively correlated to the total FAs, they had a stronger correlation to the branched FAs ($r = 0.97$, $p < 0.001$), which are typically markers for microbial biomass. PC2 accounts for 16% of the variance in all of the FA records and was only positively correlated to the saturated fatty acids ($r = 0.41$, $p < 0.05$). Observing principal component loading values showed that PC1 loadings were predominantly positive while PC2 loadings were predominantly negative. The largest PC1 loadings (those >0.18) comprised several saturated FA, lower molecular weight branched FA and DCAs, and 1 MUFA. Whereas the largest absolute value PC2 loadings (those >|0.17|) were made up of higher molecular weight branched FA, 2 DCAs, and 2 MUFAs (Appendix D). These observations were consistent with PC1 and PC2 possibly reflecting different microbial communities.

The first principal component of the PAH records accounts for 60% of the data (Table 3) and was strongly influenced by 16 out of the 24 PAHs (Appendix D), including many of the four- to six-ring nonvolatile PAHs, while PC2 accounts for 17% of the variance and

Table 3
Principal component scores for fatty acid (FA) and PAH records.

Principal component	Eigenvalue	Percent	Cum Percent
FA PC1	14.09	24.72	24.72
FA PC2	8.84	15.50	40.22
FA PC3	6.70	11.76	51.98
PAH PC1	14.50	60.40	60.40
PAH PC2	4.17	17.36	77.76

was most strongly influenced by several volatile low molecular weight PAHs and lighter methylated PAHs (Appendix E; Fig. E1a). The record was dominated by the larger, more persistent PAH compounds that resist degradation. These have also been shown to have greater potential for harmful ecological effects (Kanaly and Harayama, 2000). PAH fingerprints captured by PCA show depth-dependent clustering of the sediment depth intervals that had the highest ∑PAH concentrations, between ~80 and 200 cm (Appendix E; Fig. E1b). A loading plot of the first two principal components of the fatty acid records showed clustering of the highest PAH intervals (Fig. 7) further indicating potential effects of PAH contamination on microbial community composition, although there was some overlap with mid- and low-PAH intervals.

Past studies have shown a shift in the microbial community composition in petroleum-contaminated surface sediments favoring hydrocarbon degrading microbial populations, but not showing an overall decrease in microbial biomass (Langworthy et al., 1998). The current study is the first examination of historical changes in these parameters in the sediment record and suggests that overall microbial and/or algal biomass decreased in response to petroleum contamination. The vast majority of the individual FAs were either negatively correlated to the contaminant records or had no relationship. However, the FA 13:0 ISO 3OH and 15:1 ISO F were strongly positively correlated to the MAS (0.54, $p < 0.002$; 0.61, $p < 0.001$, respectively) and α,β-hopanes (0.54, $p < 0.003$; 0.61, $p < 0.001$, respectively). Some evidence exists suggesting that these two FAs represent the hydrocarbon-degrader microbial populations that received a selective benefit from exposure to petroleum contaminants. 13:0 iso 3OH is characteristic for most members of the *Xanthomonas* genus (Yang et al., 1993), which is known to include petroleum hydrocarbon degrading species (Li et al., 2000).

Individual PAHs that were significantly negatively correlated to FAs were BaA, BaP, BbF, BeP, BgP, BkF, Ch, Fl, IP, DBaA, Pe, and Py.

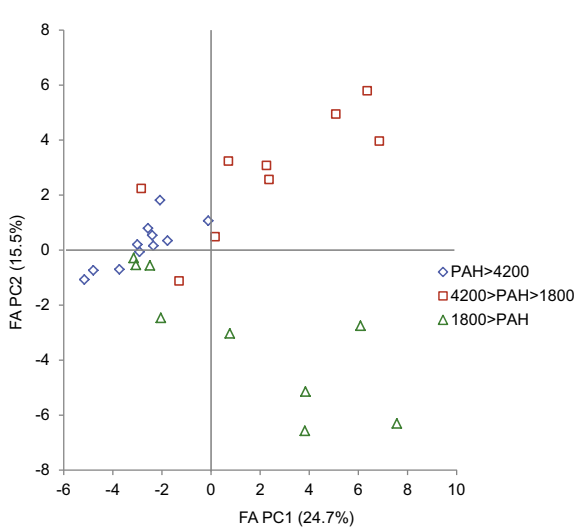

Fig. 7. Loading plot of the first two principal components of the fatty acid records. Symbols reflect the PAH concentration ranges (ng g^{-1}) in corresponding intervals.

The specific mechanism of toxicity of these compounds to bacteria or plants is unknown (Lewis and Pryor, 2013), but likely involves growth inhibition (El-Alawi et al., 2002), cellular toxicity (Lee et al., 2003), alteration to cell permeability, and/or damage to cellular lipids, membranes, and/or cellular integrity (Britton, 1984). Johnsen et al. (2006) suggested that in areas receiving chronic petroleum contamination, high molecular weight PAHs that have mutagenic, genotoxic and carcinogenic properties are concentrated over time, while low molecular weight PAHs are rapidly degraded. Sediment quality screening values have been established with respect to toxicity to benthic macroinvertebrates for Na, Ay, Ac, fluorene, Ph, An, Fl, Py, BaA, Ch, BbF, BkF, BgP, 2-Na, 1-Na, BeP, and Pe (USEPA, 2004). Relevance of these criteria (designed for protection of benthic macro organisms) to single-celled prokaryotes and algae is unknown. However, screening values predicting sediment toxicity in 25–50% of cases based on logistic regression modeling (USEPA, 2004) were exceeded in multiple intervals for Ph, An, Fl, Py, BaA, Ch, BbF, BkF, BgP, BeP, and Pe (Appendix F), of which Ph, An, Fl, Py, and BgP are U.S. EPA priority pollutants.

4. Implications

Petroleum contaminants increased rapidly in South San Francisco Bay beginning in the early 20th century. Negative correlations between petroleum contaminants and microbial and algal biomarkers – along with high *trans*/*cis* ratios of unsaturated FA and clustering of high PAH interval fatty acid PCA loadings – suggests impacts of contamination on ecosystem ecology. Further study in additional locations, including less impacted sites and replicate core records, is needed before we can move toward understanding relevance to toxicity evaluations or effects thresholds for microbes and algae.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.08.053.

References

Abed, R.M.M., Safi, N.M.D., Koster, J., de Beer, D., El-Nahhal, Y., Rullkotter, J., Garcia-Pichel, F., 2002. Microbial diversity of a heavily polluted microbial mat and its community changes following degradation of petroleum compounds. Appl. Environ. Microbiol. 68, 1674–1683.

Appleby, P.G., Oldfield, F., 1992. Application of lead-210 to sedimentation studies. In: Ivanovich, M., Harmon, S. (Eds.), Uranium-series Disequilibrium – Applications to Earth, Marine, and Environmental Sciences. Clarendon Press, Oxford, pp. 731–778.

Arias, A., Vazquez-Botello, A., Tombesi, N., Ponce-Vélez, G., Freije, H., Marcovecchio, J., 2010. Presence, distribution, and origins of polycyclic aromatic hydrocarbons (PAHs) in sediments from Bahía Blanca estuary, Argentina. Environ. Monit. Assess. 160, 301–314.

Baskaran, M., Naidu, A.S., 1994. ^{210}Pb-derived chronology and the fluxes of ^{210}Pb and ^{137}Cs isotopes into continental shelf sediments, East Chukchi Sea, Alaskan Arctic. Geochim. Cosmochim. Acta 59, 4435–4448.

Boonyatumanond, R., Wattayakorn, G., Togo, A., Takada, H., 2006. Distribution and origins of polycyclic aromatic hydrocarbons (PAHs) in riverine, estuarine, and marine sediments in Thailand. Mar. Pollut. Bull. 52, 942–956.

Britton, L.H., 1984. Microbial Degradation of Organic Compounds. Marcel Dekker, New York.

Canuel, E.A., Cloern, J.E., Ringelberg, D.B., Guckert, J.B., Rau, G.H., 1995. Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay. Limnol. Oceanogr. 40, 67–81.

Carman, K.R., Fleeger, J.W., Means, J.C., Pomarico, S.M., McMillin, D.J., 1995. Experimental investigation of the effects of polynuclear aromatic hydrocarbons on an estuarine sediment food web. Mar. Environ. Res. 40, 289–318.

Carman, K.R., Fleeger, J.W., Pomarico, S.M., 1997. Response of a benthic foodweb to hydrocarbon contamination. Limnol. Oceanogr. 42, 561–571.

Castle, D.M., Montgomery, M.T., Kirchman, D.L., 2006. Effects of naphthalene on microbial community composition in the Delaware estuary. FEMS Microbiol. Ecol. 56, 55–63.

Cornelissen, G., Gustafsson, O., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., Van Noort, P.C.M., 2005. Critical review: extensive sorption of organic compounds to black carbon, coal and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation and biodegradation. Environ. Sci. Technol. 39, 6881–6895.

Dickhut, R.M., Canuel, E.A., Gustafson, K.E., Liu, K., Arzayus, K.M., Walker, S.E., Edgecombe, G., Gaylor, M.O., MacDonald, E.H., 2000. Automotive sources of carcinogenic polycyclic aromatic hydrocarbons associated with particulate matter in the Chesapeake Bay region. Environ. Sci. Technol. 34, 4635–4640.

El-Alawi, Y.S., McConkey, B.J., George Dixon, D., Greenberg, B.M., 2002. Measurement of short- and long-term toxicity of polycyclic aromatic hydrocarbons using luminescent bacteria. Ecotoxicol. Environ. Saf. 51, 12–21.

Foxgrover, A.C., Higgins, S.A., Ingraca, M.K., Jaffe, B.E., Smith, R.E., 2004. Deposition, erosion, and bathymetric change in South San Francisco Bay; 1858–1983. U.S. Geological Survey Open-File Report 2004-1192, pp. 1–25.

Fuller, C.C., 1982. The Use of ^{210}Pb, ^{234}Th and ^{137}Cs as Tracers of Sedimentary Processes in San Francisco Bay. University of Southern California.

Fuller, C.C., Hammond, D.E., 1983. The fallout rate of Pb 210 on the western coast of the United States. Geophys. Res. Lett. 10, 1164–1167.

Fuller, C.C., van Geen, A., Baskaran, M., Anima, R., 1999. Sediment chronology in San Francisco Bay, California, defined by ^{210}Pb, ^{234}Th, ^{137}Cs, and ^{239,240}Pu. Mar. Chem. 64, 7–27.

Ghiorse, W.C., Herrick, J.B., Sandoli, R.L., Madsen, E.L., 1995. Natural selection of PAH-degrading bacterial guilds at coal–tar disposal sites. Environ. Health Perspect. 103, 107–111.

Guckert, J.B., Hood, M.A., White, D.C., 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the *trans*/*cis* ratio and proportions of cyclopropyl fatty acids. Appl. Environ. Microbiol. 52, 794–801.

Harji, R.R., Bhosle, N.B., Garg, A., Sawant, S.S., Venkat, K., 2010. Sources of organic matter and microbial community structure in the sediments of the Visakhapatnam Harbour, east coat of India. Chem. Geol. 276, 309–317.

Harms, H., Bosma, T.N.P., 1997. Mass transfer limitation of microbial growth and pollutant degradation. J. Ind. Microbiol., 97–105.

Heipieper, H.J., Loffeld, B., Keweloh, H., de Bont, J.A.M., 1995. The cis/trans isomerization of unsaturated fatty acids in *Pseudomonas putida* S12: an indicator for environmental stress due to organic compounds. Chemosphere 30, 1041–1051.

Heipieper, H., Meinhardt, F., Segura, A., 2003. The cis-trans isomerase of unsaturated fatty acids in *Pseudomonas* and *Vibrio*: biochemistry, molecular biology and physiological function of a unique stress adaptive mechanism. FEMS Microbiol. Lett. 229, 1–7.

Higgins, S.A., Jaffe, B.E., Smith, R.E., 2005. Bathychronology: Reconstructing historical sedimentation from bathymetric data in a GIS. U.S. Geological Survey Open-File Report 2005-1284, p. 19.

Higgins, S.A., Jaffe, B.E., Fuller, C.C., 2007. Reconstructing sediment age profiles from historical bathymetry changes in San Pablo Bay, California. Estuar. Coast. Shelf Sci. 73, 165–174.

Hostettler, F.D., Pereira, W.E., Kvenvolden, K.A., van Geen, A., Luoma, S.N., Fuller, C.C., Anima, R., 1999. A record of hydrocarbon input to San Francisco Bay as traced by biomarker profiles in surface sediment and sediment cores. Mar. Chem. 64, 115–127.

Hyötyläinen, T., Olkari, A., 1999. The toxicity and concentrations of PAHs in creosote-contaminated lake sediment. Chemosphere 38, 1135–1144.

Jaffe, B.E., Foxgrover, A.C., 2006. Sediment Deposition and Erosion in South San Francisco Bay, California from 1956 to 2005. U.S. Geological Survey Open-File Report 2006-1262, p. 24.

Johnsen, A.R., Wick, L.Y., Harms, H., 2005. Principles of microbial PAH-degradation in soil. Environ. Pollut. 133, 71–84.

Johnsen, A.R., Lipthay, J.R.D., Reichenberg, F., Sørensen, S.J., Andersen, O., Christensen, P., Binderup, M.L., Jacobsen, C., 2006. Biodegradation, bioaccessibility, and genotoxicity of diffuse polycyclic aromatic hydrocarbon (PAH) pollution at a motorway site. Environ. Sci. Technol. 40, 3293–3298.

Kanaly, R.A., Harayama, S., 2000. Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. J. Bacteriol. 182, 2059–2067.

Ko, J.-Y., Day, J.W., 2004. A review of ecological impacts of oil and gas development on coastal ecosystems in the Mississippi Delta. Ocean Coast. Manage. 47, 597–623.

Lake, J.L., Norwood, C., Dimock, C., Bowen, R., 1979. Origins of polycyclic aromatic hydrocarbons in estuarine sediments. Geochim. Cosmochim. Acta 34, 1847–1854.

Langworthy, D., Stapleton, R., Sayler, G., Findlay, R., 1998. Genotypic and phenotypic responses of a riverine microbial community to polycyclic aromatic hydrocarbon contamination. Appl. Environ. Microbiol. 64, 3422–3428.

Latimer, J.S., Quinn, J.G., 1996. Historical trends and current inputs of hydrophobic organic compounds in an urban estuary: the sedimentary record. Environ. Sci. Technol. 30, 623–633.

Lee, H.J., Villaume, J., Cullen, D.C., Kim, B.C., Gu, M.B., 2003. Monitoring and classification of PAH toxicity using an immobilized bioluminescent bacteria. Biosens. Bioelectron. 18, 571–577.

Lewis, M., Pryor, R., 2013. Toxicities of oils, dispersants and dispersed oils to algae and aquatic plants: review and database value to resource sustainability. Environ. Pollut. 180, 345–367.

Li, G., Zhang, X., Huang, W., 2000. Enhanced biodegradation of petroleum hydrocarbons in polluted soil. J. Environ. Sci. Health 35, 177–188.

Lima, A.L.C., Eglinton, T.I., Reddy, C.M., 2003. High-resolution record of pyrogenic polycyclic aromatic hydrocarbon deposition during the 20th Century. Environ. Sci. Technol. 37, 53–61.

Lima, A.L.C., Farrington, J.W., Reddy, C.M., 2005. Combustion-derived polycyclic aromatic hydrocarbons in the environment—a review. Environ. Forensics 6, 109–131.

Liu, Y., Yu, N., Li, Z., Wei, Y., Ma, L., Zhao, J., 2012. Sedimentary record of PAHs in the Liangtan River and its relation to socioeconomic development of Chongqing, Southwest China. Chemosphere 89, 893–899.

Mudge, S.M., Norris, C.E., 1997. Lipid biomarkers in the Conwy Estuary (North Wales, U.K.): a comparison between fatty alcohols and sterols. Mar. Chem. 57, 61–84.

Oros, D.R., Ross, J.R.M., 2004. Polycyclic aromatic hydrocarbons in San Francisco Estuary sediments. Mar. Chem. 86, 169–184.

Pereira, W.E., Hostettler, F.D., Luoma, S.N., Geen, A.V., Fuller, C.C., Anima, R.J., 1999. Sedimentary record of anthropogenic and biogenic polycyclic aromatic hydrocarbons in San Francisco Bay, California. Mar. Chem. 64, 99–113.

Phillips, D.L., 1983. Fifty years of benzo[a]pyrene. Nature 303, 468–472.

Poland, J.F., Ireland, R.L., 1988. Land Subsidence in the Santa Clara Valley, California, as of 1982. U.S. Geological Survey Professional Paper 497-F, p. 61.

Rieley, G., Collier, R.J., Jones, D.M., Eglinton, G., 1991. The biogeochemistry of Ellesmere Lake, U.K.—I: source correlation of leaf wax inputs to the sedimentary lipid record. Org. Geochem. 17, 901–912.

Robbins, J.A., Edgington, D.N., 1976. Determination of recent sedimentation rates in Lake Michigan using ^{210}Pb and ^{137}Cs. Geochim. Cosmochim. Acta 39, 285–304.

Samanta, S.K., Singh, O.V., Jain, R.K., 2002. Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. Trends Biotechnol. 20, 243–248.

Sánchez-García, L., Cato, I., Gustafsson, Ö., 2010. Evaluation of the influence of black carbon on the distribution of PAHs in sediments from along the entire Swedish continental shelf. Mar. Chem. 119, 44–51.

Sasser, M., 1990. Identification of bacteria by gas chromatography of cellular fatty acids. Technical Note. MIDI, Inc.

Shuttleworth, K.L., Cerniglia, C.E., 1995. Environmental aspects of PAH biodegradation. Appl. Biochem. Biotechnol. 54, 291–302.

Song, H., Bartha, R., 1990. Effects of jet fuel spills on the microbial community of soil. Appl. Environ. Microbiol. 56, 646–651.

Treignier, C., Derenne, S., Saliot, A., 2006. Terrestrial and marine n-alcohol inputs and degradation processes relating to a sudden turbidity current in the Zaire canyon. Org. Geochem. 37, 1170–1184.

Twiss, M.R., Granier, L., Lafrance, P., Campbell, P.G.C., 1999. Accumulation of 2,2′,5,5′-tetrachlorobiphenyl and pyrene by picoplankton (Synechococcus leopoliensis, Cyanophyceae): Influence of variable humic acid concentrations and pH. Environ. Toxicol. Chem. 18, 2063–2069.

USEPA, 2004. The incidence and severity of sediment contamination in surface waters of the United States. National Sediment Quality Survey, second ed., EPA-823-R-04-007. United States Environmental Protection Agency Office of Science and Technology, p. 142 p.

Van Metre, P., 2004. Collection, analysis, and age-dating of sediment cores from 56 U.S. lakes and reservoirs sampled by the U.S. Geological Survey, 1992–2001. Scientific Investigations Report. U.S. Geological Survey, Reston, VA, p. 180.

Van Metre, P.C., Mahler, B.J., Furlong, E.T., 2000. Urban sprawl leaves its PAH signature. Environ. Sci. Technol. 34, 4064–4070.

Viñas, M., Sabate, J., Espuny, M.J., Solanas, A.M., 2005. Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediation of heavily creosote-contaminated soil. Appl. Environ. Microbiol. 71, 7008–7018.

Waterson, E.J., Canuel, E.A., 2008. Sources of sedimentary organic matter in the Mississippi River and adjacent Gulf of Mexico as revealed by lipid biomarker and δ13CTOC analyses. Org. Geochem. 39, 422–439.

Yang, P., Vauterin, L., Vancanneyt, M., Swings, J., Kersters, K., 1993. Application of fatty acid methyl esters for the taxonomic analysis of the genus *Xanthomonas*. Syst. Appl. Microbiol., 47–71.

Yunker, M.B., Macdonald, R.W., 1997. Preliminary assessment of alkane and PAH data for sediment cores from six lakes in the Fraser River basin. DOE FRAP, p. 93.

Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D., Sylvestre, S., 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Org. Geochem. 33, 489–515.

Yunker, M.B., Belicka, L.L., Harvey, H.R., Macdonald, R.W., 2005. Tracing the inputs and fate of marine and terrigenous organic matter in Arctic Ocean sediments: a multivariate analysis of lipid biomarkers. Deep-Sea Res. II 52, 3478–3508.

Zimmerman, A.R., Canuel, E.A., 2001. Bulk organic matter and lipid biomarker composition of Chesapeake Bay surficial sediments as indicators of environmental processes. Estuar. Coast. Shelf Sci. 53, 319–341.

Zimmerman, A.R., Canuel, E.A., 2002. Sediment geochemical records of eutrophication in the mesohaline Chesapeake Bay. Limnol. Oceanogr. 47, 1084–1093.