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ScienceDirect
Marine Pollution Bulletin 52 (2006) 1090–1117
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

Baseline

Edited by Bruce J. Richardson

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Polycyclic aromatic hydrocarbons (PAHs) in small island coastal environments: A case study from harbours in Guam, Micronesia

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants derived mainly from anthropogenic activities with minor contributions from natural sources (Law et al., 1997). Most PAHs enter the marine environment in urban runoff, municipal and industrial waste discharges, in bilge and fuel oil leaks associated with day-to-day shipping operations, and in oil spills from maritime accidents, and they are rapidly scavenged out of the water column by sediments (McElroy et al., 1989). Harbours, because of the associated shipping activities, are often sites of such marine pollution. Recent concern about the potential carcinogenic properties of some PAHs has led to an increased need to determine the extent of pollution caused by this group of compounds (Cerniglia and Heitkamp, 1989).

Guam (13°28′N, 144°45′E) is the largest and most densely populated island in Micronesia and has served as the major shipping centre for the region for over 400 years. In spite of this, the impact of human activities on the island's coastal environment has received little attention. This paper reports on the first major study of PAHs in the sediments and organisms in four local harbours (Fig. 1): Agana Boat Basin (small boat harbour located in the business centre of the island – 5 sites); the Outer Apra Harbour (heavily used commercial and military port for over 50 years – 30 sites); Agat Marina (newly constructed suburban facility – 4 sites); and Merizo Pier (small boat harbour and ferry terminal located in a rural residential area – 5 sites).

The site locations and sampling collection details for sediments are given in Denton et al. (1997, 2005). PAH analyses were based on USEPA SW 846 methods (USEPA, 1996). All reagents used were analytical grade and all glassware was acid-washed and deionized water rinsed prior to use. Standard stock solutions were purchased from a commercial supplier. All analyses were performed in duplicate and were accompanied by appropriate method blanks and matrix spikes.

Air-dried, sieved (1 mm) sediment samples (1–1.5 g) were weighed into a 10 mL Teflon centrifuge tube and extracted with methylene chloride (3 mL) in a commercial microwave oven (700 W) with a rotating turntable for sequential periods of 30, 15 and 15 s (Ganzler et al., 1986). Each tube was touched against a vortex mixer for 5 s between heating cycles to ensure thorough mixing. Upon standing overnight, the samples were further vortexed and centrifuged at 2500 rpm for 5 min. After decanting into 10 mL graduated glass centrifuge tubes, the samples were reduced in volume (~0.75 mL) under a gentle stream of nitrogen in a water bath (~45 °C). Solvent exchange into hexane (~1.25 mL) and further reduction in volume (~0.5 mL) was necessary prior to clean up on silica gel (0.5 g) microcolumns (see Denton et al., 1997

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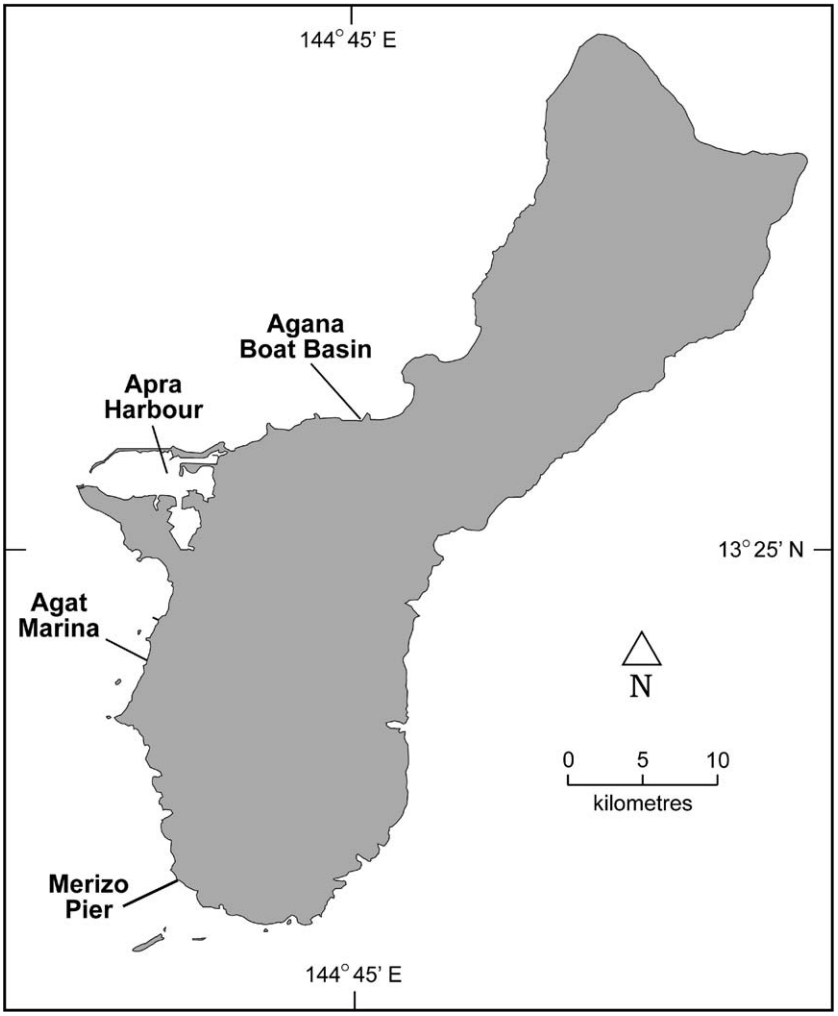

Fig. 1. Locations of harbours studied in Guam.

for additional details). Following elution with methylene chloride, and solvent exchange with acetonitrile, final volumes were adjusted to 0.1 mL prior to analysis.

Biota sampling sites, collection procedures and a list of species selected for study are given elsewhere (Denton et al., 1999, 2006). Tissue homogenates (~3 g) were extracted with 20 mL of methylene chloride in 50 mL Teflon centrifuge tubes containing 10 g of anhydrous sodium sulfate. Following solvent extraction into hexane the final volume was reduced to 0.2 mL on a water bath as described above.

Tissue cleanup was achieved on small columns of silica gel (2 g) over alumina (1 g) using methylene chloride as the packing solvent. Each column was eluted with 5 mL of pentane (discarded) followed by 10 mL of 50% methylene chloride in pentane. The latter fraction containing the PAHs was collected in a 10 mL graduated, glass centrifuge tube, evaporated to 5 mL and solvent exchanged with acetonitrile. The extract was reduced to a final volume of 0.1 mL prior to chromatographic analysis.

Deuterated acenaphthene and benzo(*a*)pyrene were used as surrogates in the tissue homogenate, and deuterated naphthalene was used as an internal standard (50 ng/μL) in the final volume presented for analysis. The extraction and cleanup procedures outlined above were customarily performed on sets of five wet tissue samples with an accompanying method blank.

All analyses was carried out by high performance liquid chromatography (HPLC) using a fluorescence/UV (diode array) detector system and a 10 cm × 4.6 cm i.d., stainless steel LC-PAH column (Supelco), containing a porous silica stationary phase (3 μm particle size). Following sample injection, isocratic elution with acetonitrile/water (4:6, v/v) occurred for the first 0.3 min, followed by a linear gradient to 100% acetonitrile over the next 10 min. Elution with 100% acetonitrile continued for a further 5 min before the run was terminated. The solvent flow rate through the column was held constant at 2 mL/min. Quantification with the more sensitive fluorescence detector was achieved with excitation at 280 nm and emission at 380 nm. The diode array provided a synchronous absorption scan from 190 to 357 nm, with a wavelength difference of 4 nm, and was used primarily for confirmatory analysis at the higher levels of detection.

The calibration standards were made up containing 16 PAHs ranging in size from 2 to 6 rings. These included a number of compounds known to be carcinogenic and/or genotoxic. Method detection limits with the fluorescence detector were as follows: naphthalene (34 ng/g), acenaphthene (4 ng/g), fluorene (8 ng/g), phenanthrene (3 ng/g), anthracene (2 ng/g), fluoranthene (5 ng/g), pyrene (3 ng/g), benzo(*a*)anthracene (1 ng/g), chrysene (1 ng/g), benzo(*b*)-fluoranthene (5 ng/g), benzo(*k*)fluoranthene (4 ng/g), benzo(*a*)pyrene (3 ng/g), dibenzo(*a*,*h*)anthracene (8 ng/g), and benzo(*ghi*)perylene (13 ng/g). Detection limits for the non-fluorescing PAHs, acenaphthylene and indenol(1,2,3-*cd*)pyrene, were 3 ng/g and 6 ng/g respectively, using the UV diode array detector. All calculations were based on peak area comparisons of components sharing identical retention times in both sample and standard. From these data, the "total" PAH (Σ_{16}PAH) content of the sample was calculated. Non-detectable residues were set to zero during the summing process. PAH recoveries from a soil standard reference material were within acceptable limits of the certified means (Table 1). In contrast, recoveries from spiked oyster tissues were disappointingly low (22–42%); nevertheless, we believe the biota data provide a useful preliminary screening.

The PAH data for sediments are presented in Table 2, in which the total PAH (Σ_{16}PAH) concentrations and rank order of congener abundance for each harbour site are listed. Total PAH concentrations in uncontaminated sediments are generally less that 5 ng/g (Pierce et al., 1986; Van Vleet et al., 1986), although values of 10–15 ng/g have been reported for some unimpacted deep-sea sediments (Hites et al., 1980). In this study, Σ_{16}PAH concentrations ranged from below detection to about 11 ng/g. The highest mean values of 6.14 and 8.14 μg/g were recorded in Apra Harbour sediments (sites 1 and 6 respectively). Relatively high concentrations were also found at Sites 8, 9 and 25 in Apra Harbour, and from site 2 in Agana Boat Basin. The values found for Guam harbours are generally towards the lower end of the range reported for harbours elsewhere in the world (McCarry, 1977).

The PAH assemblages in Guam harbour sediments show substantial differences between sites, suggesting multiple sources of both petrogenic and pyrogenic origin. There was a tendency, however, for some of the lighter PAHs, particularly acenaphthylene and anthracene, to dominate the sediments from relatively clean sites (e.g., Agana Boat Basin Sites 3–5; Apra Harbour sites 15, 18, 23, 29; Agat Marina all sites). This may be due to the greater environmental mobility of these compounds rather than source, being related to their relatively high water solubility and vapour pressure. Benlahcen et al. (1997) suggested that phenanthrene/anthracene ratios of less than 10 and/or fluoranthene/pyrene ratios greater than 1 were indicative of combustion sources of PAHs. Examination of these ratios for the present study data showed fluoranthene/pyrene ratios >1, suggesting that fossil fuel combustion was the primary source of local PAHs in the majority of sediment samples analysed. The notable exceptions were in Apra Harbour sites 1, 6, and 21 where the fluoranthene/pyrene ratios were <1, indicative of petrogenic hydrocarbon spillages. These latter sites are close to fuel loading and storage facilities.

The findings of the biota analyses are summarized in Table 3, for those organisms where one or more PAH were detected. One hundred and seventeen additional organisms (5 seaweed, 3 sponges, 18 sea cucumbers, 8 oysters, 2 chamids, 1 spondylid, 1 octopus, 1 mantis shrimp, 3 ascidians and 75 fish) were analysed with no detectable PAHs found. The data are briefly reviewed in the context of previously published information. Unfortunately, little or no compar-

Table 1
Analysis of standard reference material (μg/g dry wt.) (RTC PAH contaminated soil/sediment [Catalog No. CRM104-100; Lot No. CR12])

Analyte	Certified value		This study		*n*
	Mean	Range	Mean	Range	
Naphthalene	0.77	0.0–1.57[a]	NC	<0.65–<0.70	6
Acenaphthylene	1.21	0.0–2.98	0.06	0.04–0.08	6
Acenaphthene	0.77	0.27–1.2	0.16	0.11–0.22	6
Fluorene	0.65	0.25–1.05	0.37	0.25–0.54	6
Phenanthrene	5.79	2.11–9.48	4.95	4.00–6.45	6
Anthracene	1.44	0.08–2.80	1.38	1.14–1.81	6
Fluoranthene	24.6	4.53–44.6	23.8	20.5–30.8	6
Pyrene	15.0	0.0–30.7	11.0	9.38–14.4	6
Benzo(*a*)anthracene	7.98	2.09–13.9	3.60	3.04–4.66	6
Chrysene	8.60	3.39–13.8	5.85	4.94–7.47	6
Benzo(*b*)fluoranthene	(9.69)	None given	5.33	4.56–6.89	6
Benzo(*k*)fluoranthene	(5.10)	None given	2.92	2.64–3.79	6
Benzo(*a*)pyrene	5.09	1.56–8.63	5.11	4.20–6.52	6
Benzo(*ghi*)perylene	3.58	0.0–8.08	2.84	2.50–3.68	6
Indenol(1,2,3,-*cd*)pyrene	4.46	0.0–9.09	3.38	2.77–4.46	6
Dibenzo(*a*,*h*)anthracene	(1.55)	None given	2.72	2.29–3.64	6

[a] Certificate of analysis for PAH standard reference material gives only the 95% prediction interval about the certified mean. Values in parenthesis are not certified and are listed for information only; NC = not calculable.

Table 2
PAHs in sediments from Guam Harbours (May–June 1997)

Site	Σ_{16}PAH concentration (μg/g dry wt.)			Overall order of abundance of detectable PAH congeners
	Mean	Median	Range	
Agana Boat Basin				
1 (a–c)	0.21	0.21	0.18–0.23	BBF > BPE > INP > PYR > BKF > ANT > FLU > CHR > BAP > PHE > BAA > ACE
2 (a–c)	1.90	0.64	0.64–4.50	BBF > PYR > FLU > BAP > INP > CHR > BPE > BAA > BKF > PHE > ANT > DBA > ACE
3 (a–c)	0.02	0.02	0.02–0.03	ANT > ACE > BPE
4 (a–c)	0.05	0.05	0.03–0.05	ANT > ACE > BPE
5 (a–c)	0.02	0.02	0.02–0.03	ANT > ACE
Apra Harbour				
1 (a–c)	6.14	4.19	3.79–10.4	BBF > PYR > BAP > BKF > BPE > INP > PHE > FLU > CHR > BAA > ANT > DBA > FLR > ACE
2 (a–c)	0.07	0.09	0.03–0.09	BPE > ANT > BKF > BBF
3 (a–c)	0.18	0.15	0.13–0.25	BPE > ANT > BBF > PYR > BKF > CHR > INP > BAP > BAA
4 (a–c)	0.25	0.25	0.22–0.28	BKF > BBF > INP > BPE > BAP > ANT > BAA > PHE
5 (a–c)	0.21	0.17	0.16–0.30	BBF > BKF > INP > BPE > CHR > BAP > BAA > PYR > ANT > FLU > DBA > PHE
6 (a–c)	8.14	9.12	4.57–10.7	BBF > BAP > BPE > INP > CHR > BKF > BAA > DBA > FLU > PYR > PHE > ANT > ACE
7 (a–c)	0.48	0.48	0.35–0.61	BKF > BBF > INP > BPE > PYR > BAP > FLU > CHR > ANT > BAA > PHE > DBA
8 (a–c)	2.07	1.32	0.32–4.57	PYR > FLU > BBF > CHR > BAA > BKF > INP > ANT > BPE > BAP > DBA > PHE > ACE
9 (a–c)	1.12	1.45	0.45–1.45	BBF > BAP > PYR > BPE > INP > BKF > CHR > BAA > FLU > PHE > DBA > ANT
10 (a–c)	0.15	0.11	0.06–0.27	BPE > ANT > BBF > BKF > PYR > BAP > BAA
11 (a–c)	0.35	0.35	0.18–0.53	BBF > BPE > INP > BAP > BKF > PYR > BAA > FLU > ANT > CHR > PHE > DBA
12 (a–c)	0.14	0.15	0.09–0.18	BBF > ANT > BPE > INP > CHR > BKF > PYR > FLU > BAA > PHE > BAP > BPE
13 (a–c)	0.20	0.22	0.13–0.25	BBF > BPE > CHR > BKF > BAP > ANT > FLU > PYR > BAA > INP > PHE > DBA
14 (a–c)	0.22	0.23	0.15–0.23	BBF > CHR > BPE > BAP > BKF > BAA > FLU > ANT > PYR > PHE > DBA
15 (a–c)	0.02	0.02	0.01–0.03	ANT > CHR
16 (a–c)	0.46	0.29	0.25–0.83	BBF > FLU > PYR > BPE > CHR > INP > BKF > BAA > BAP > PHE > ANT > DBA
17 (a–c)	0.35	0.35	0.27–0.44	BKF > BBF > BPE > INP > PYR > CHR > BAP > FLU > ANT > BAA > PHE > DBA
18 (a–c)	0.02	0.03	0.02–0.03	ANT > PYR > BAA
19 (a–c)	0.11	0.08	0.06–0.18	BPE > ANT > BBF > PYR > PHE > FLU > CHR > BAP > BKF > BAA > ACE
20 (a–c)	0.16	0.09	0.07–0.32	BPE > ANT > PHE > CHR > FLU > BBF > PYR > INP > BAP > BKF > BAA
21 (a–c)	0.55	0.49	0.18–0.98	PYR > BPE > FLU > BBF > BAA > INP > CHR > BAP > PHE > BKF > ANT > DBA
22 (a–c)	0.16	0.15	0.10–0.24	PYR > FLU > CHR > BBF > BAA > BAP > ANT > BKF > INP > BPE > PHE
23 (a–c)	0.07	0.06	0.01–0.13	ACE > ANT > BPE > BBF > PYR
24 (a–c)	0.82	0.53	0.29–1.64	BBF > BAP > BPE > BKF > INP > CHR > FLU > PYR > BAA > DBA > ANT > ACE > ACY > PHE
25 (a–c)	2.47	0.82	0.75–5.85	FLU > PYR > BBF > BAP > BAA > BPE > CHR > INP > PHE > BKF > DBA > ACE
26 (a–c)	0.13	0.13	0.10–0.16	BPE > BBF > PHE > ANT > CHR > BAA > BKF > FLU > ACE
27 (a–c)	0.28	0.27	0.21–0.36	BBF > BPE > PHE > CHR > BKF > INP > PYR > FLU > BAA > BAP > ANT > DBA
28 (a–c)	0.12	0.15	0.06–0.16	BPE > CHR > ANT > BBF
29 (a–c)	NC	BDL	BDL–0.02	ANT
30 (a–c)	0.05	0.05	0.03–0.08	BPE > ANT
Agat Marina				
1 (a–c)	0.01	0.01	0.01–0.02	ANT
2 (a–c)	NC	BDL	BDL–0.05	FLR > INP > ANT
3 (a–c)	NC	BDL	BDL–0.02	PYR > ANT
4 (a–c)	NC	BDL	BDL–0.03	PYR > ANT
5 (a–c)	NC	BDL	BDL–0.01	ANT > ACE
6 (a–c)	NC	BDL	BDL–0.02	PYR > ANT
Merizo Pier				
1 (a–c)	NC	BDL	BDL	
2 (a–c)	0.52	0.43	0.30–0.83	PYR > FLU > BBF > CHR > BKF > BPE > BAA > BAP > INP > PHE > ANT > DBA
3 (a–c)	0.08	0.06	0.06–0.11	BPE > CHR > FLU > ANT
4 (a–c)	0.04	0.03	0.03–0.06	BPE > ANT > CHR > FLU
5 (a–c)	0.36	0.35	0.26–0.48	BPE > CHR > BBF > BAP > BAA > BKF > INP > PYR > FLU > ANT > PHE

NC = not calculable; BDL = below detection limits.
PAH Abbreviations (in order of molar mass):
NAP: naphthalene; ACY: acenaphthylene; ACE: acenaphthene; FLR: fluorene; PHE: phenanthrene; ANT: anthracene; FLU: fluoranthene; PYR: pyrene; BAA: benzo(*a*)anthracene; CHR: chrysene; BBF: benzo(*b*)fluoranthene; BKF: benzo(*k*)fluoranthene; BAP: benzo(*a*)pyrene; BPE: benzo(*ghi*)perylene; INP: indenol(1,2,3-*cd*)pyrene; DBA: dibenzo(*a*,*h*)anthracene.

ative data exist for several of the invertebrate groups considered here. All referenced data included in the following discussions are expressed on a wet weight basis unless indicated otherwise.

Table 3
PAHs in organisms from Guam Harbours (June–December 1998)

Species	Location and (closest sediment site)	PAH congener concentration (μg/g wet wt.)																
		NAP	ACY	ACE	FLR	PHE	ANT	FLU	PYR	BAA	CHR	BBF	BKF	BAP	DBA	BPE	INP	Σ_{16}PAH
Seaweed																		
Padina sp.	Apra Harbour (8)	BDL	BDL	BDL	BDL	BDL	BDL	0.016	BDL	BDL	BDL	BDL	0.010	0.018	BDL	BDL	BDL	0.037
Padina sp.	Apra Harbour (25)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.005	BDL	BDL	BDL	0.036	BDL	BDL	0.041
Padina sp.	Apra Harbour (14)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.030	BDL	BDL	BDL	0.030
Sponges																		
Callyspongia diffusa	Agat Marina	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.002	BDL	BDL	BDL	BDL	BDL	BDL	0.074	0.075
Dysidea sp.	Apra Harbour (6)	BDL	BDL	BDL	BDL	0.005	BDL	0.042	BDL	BDL	0.083	BDL	BDL	0.035	0.449	0.084	0.024	0.722
Dysidea sp.	Apta Harbour (8)	BDL	BDL	BDL	BDL	0.022	0.015	0.026	BDL	BDL	0.228	BDL	BDL	BDL	BDL	BDL	BDL	0.291
Dysidea sp.	Apra Harbour (14)	BDL	BDL	BDL	BDL	BDL	0.006	BDL	BDL	BDL	BDL	0.103	BDL	BDL	0.201	0.025	0.008	0.343
Liosina cf. granularis	Apra Harbour (1)	BDL	BDL	BDL	BDL	BDL	0.007	BDL	0.006	0.006	0.028	0.041	0.011	BDL	0.330	0.165	BDL	0.595
Liosina cf. granularis	Apra Harbour (25)	BDL	BDL	BDL	BDL	BDL	0.004	0.008	0.012	0.008	0.014	0.016	0.007	BDL	0.274	0.046	BDL	0.387
Stylotella aurantium	Apra Harbour (1)	BDL	BDL	BDL	BDL	BDL	BDL	0.006	0.003	BDL	0.008	0.007	BDL	BDL	0.180	BDL	BDL	0.204
Stylotella aurantium	Apra Harbour (25)	BDL	BDL	BDL	BDL	0.016	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.151	0.044	BDL	0.211
Stylotella aurantium	Merizo Pier	BDL	BDL	BDL	BDL	BDL	BDL	0.006	BDL	0.030	0.546	BDL	BDL	BDL	BDL	BDL	BDL	0.582
Brown wart sponge	Apra Harbour (25)	BDL	BDL	BDL	BDL	BDL	0.013	BDL	BDL	0.001	0.015	BDL	BDL	BDL	0.061	BDL	BDL	0.091
Brown wart sponge	Apra Harbour (14)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.003	BDL	BDL	BDL	0.141	BDL	BDL	0.143
Orange wart sponge	Apra Harbour (25)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.009	BDL	BDL	0.037	BDL	BDL	0.046
Yellow sponge	Apra Harbour (6)	BDL	BDL	BDL	BDL	BDL	BDL	0.017	0.010	0.001	BDL	0.047	0.023	0.030	0.144	0.020	0.020	0.312
Soft corals																		
Sinularia sp.	Apra Harbour (6)	BDL	BDL	BDL	BDL	BDL	0.003	0.014	BDL	BDL	0.101	BDL	BDL	BDL	BDL	BDL	BDL	0.117
Sinularia sp.	Apra Harbour (25)	BDL	BDL	BDL	BDL	BDL	BDL	0.007	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.007
Sinularia sp.	Agana Boat Basin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.024	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.024
Sinularia sp.	Merizo Pier	BDL	BDL	BDL	BDL	BDL	BDL	0.041	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.041
Sea cucumbers (tissue)																		
Bohadschia argus (M)	Apra Harbour (6)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.006	0.002	BDL	BDL	BDL	BDL	0.059	BDL	BDL	0.067
Holothuria atra (H)	Apra Harbour (15)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.016	BDL	0.008	BDL	BDL	0.058	BDL	BDL	BDL	0.083
Holothuria atra (H)	Merizo Pier	BDL	BDL	BDL	BDL	0.015	BDL	0.011	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.026
Holothuria atra (M)	Apra Harbour (25)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.035	BDL	BDL	0.035
Oysters (pool size)																		
Saccostrea cucculata (10)	Apra Harbour (6)	BDL	BDL	BDL	BDL	0.022	0.003	0.036	BDL	BDL	BDL	BDL	0.011	BDL	BDL	BDL	BDL	0.073
S. cucculata (7 juv.)	Merizo Pier	BDL	BDL	BDL	BDL	0.013	BDL	0.021	BDL	BDL	0.007	BDL	BDL	BDL	BDL	BDL	BDL	0.041
Striostrea mytiloides (4)	Agana Boat Basin	BDL	BDL	BDL	BDL	0.004	BDL	0.021	BDL	BDL	BDL	BDL	0.012	0.010	BDL	BDL	BDL	0.048

Striostrea mytiloides (2)	Apra Harbour (1)	BDL	BDL	BDL	0.022	0.014	BDL	0.013	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.049
Striostrea mytiloides (2)	Apra Harbour (1)	BDL	BDL	BDL	BDL	BDL	BDL	0.007	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.007
Striostrea mytiloides (2)	Apra Harbour (1)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.015	BDL	BDL	BDL	BDL	BDL	0.015
Striostrea mytiloides (5)	Apra Harbour (1)	BDL	BDL	BDL	BDL	BDL	BDL	0.009	BDL	BDL	BDL	0.008	BDL	BDL	BDL	BDL	BDL	0.017
Striostrea mytiloides (1)	Apra Harbour (6)	BDL	BDL	BDL	BDL	0.037	BDL	0.041	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.078
Striostrea mytiloides (1)	Apra Harbour (14)	BDL	BDL	BDL	BDL	BDL	BDL	0.005	0.005	BDL	BDL	0.009	BDL	BDL	BDL	BDL	BDL	0.019
Chamids (pool size)																		
Chama brassica (2)	Apra Harbour (8)	BDL	BDL	BDL	BDL	0.049	0.035	0.430	BDL	BDL	0.030	BDL	0.052	0.050	BDL	BDL	BDL	0.259
Chama brassica (2)	Apra Harbour (14)	BDL	BDL	BDL	BDL	BDL	0.020	BDL	0.005	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.024
Chama lazarus (3)	Apra Harbour (1)	BDL	BDL	BDL	BDL	BDL	BDL	0.014	BDL	BDL	BDL	0.008	BDL	BDL	0.026	BDL	BDL	0.048
Chama lazarus (3)	Apra Harbour (1)	BDL	BDL	BDL	BDL	0.005	BDL	0.025	BDL	0.004	BDL	0.008	0.007	BDL	0.073	BDL	BDL	0.122
Chama lazarus (1)	Apra Harbour (6)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.003	BDL	BDL	0.007	BDL	BDL	BDL	BDL	BDL	0.010
Chama lazarus (2)	Apra Harbour (8)	BDL	BDL	BDL	BDL	0.027	0.004	BDL	BDL	BDL	BDL	BDL	0.019	0.013	BDL	BDL	BDL	0.063
Chama lazarus (2)	Apra Harbour (8)	BDL	BDL	BDL	BDL	0.071	0.013	0.025	BDL	BDL	0.021	BDL	0.030	0.028	0.021	0.028	BDL	0.238
Chama lazarus (2)	Apra Harbour (8)	BDL	BDL	BDL	BDL	0.259	BDL	0.315	BDL	BDL	0.044	BDL	0.071	0.047	BDL	0.047	BDL	0.783
Chama lazarus (2)	Apra Harbour (25)	BDL	BDL	BDL	BDL	BDL	BDL	0.007	0.005	0.001	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.012
Chama lazarus (2)	Apra Harbour (14)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.009	BDL	BDL	BDL	BDL	BDL	0.009
Chama lazarus (2)	Apra Harbour (14)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.153	BDL	BDL	BDL	0.153
Chama lazarus (1)	Merizo Pier	BDL	BDL	BDL	BDL	0.004	BDL	0.012	0.009	BDL	0.003	BDL	BDL	BDL	BDL	BDL	BDL	0.028
Spondylids (pool size)																		
Spondylus? multimuricatus (1)	Apra Harbour (25)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.011	BDL	BDL	BDL	0.011
Spondylus? multimuricatus (1)	Apra Harbour (25)	BDL	BDL	BDL	BDL	BDL	BDL	0.008	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.008
Spondylus? multimuricatus (4)	Agat Marina	BDL	BDL	BDL	BDL	0.014	0.003	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.016
Ascidians																		
Rhopalea (whole)	Apra Harbour (8)	BDL	BDL	BDL	BDL	BDL	0.003	BDL	BDL	BDL	BDL	BDL	0.009	BDL	BDL	BDL	BDL	0.012
Fish (axial muscle)																		
Acanthurus xanthopterus	Apra Harbour (14)	BDL	BDL	0.008	0.009	0.044	0.003	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.064
Ctenochaetus binotatus	Apra Harbour (8)	BDL	BDL	BDL	BDL	0.005	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.005
Monodactylus argenteus	Apra Harbour (8)	BDL	BDL	BDL	BDL	0.007	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.007
Monodactylus argenteus	Apra Harbour (8)	BDL	BDL	BDL	BDL	0.004	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.004
Monodactylus argenteus	Apra Harbour (8)	BDL	BDL	BDL	BDL	0.009	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.010	0.019	BDL	0.037
Monodactylus argenteus	Apra Harbour (8)	BDL	BDL	BDL	BDL	0.006	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.006
Monodactylus argenteus	Apra Harbour (8)	BDL	BDL	BDL	BDL	0.009	0.003	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.012
Naso unicornis	Apra Harbour (1)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.030	BDL	0.030
Parupeneus cyclostomus	Merizo Pier	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.012	0.024	BDL	0.036
Parupeneus multifasciatus	Merizo Pier	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.015	0.046	BDL	0.061

BDL = below detection limits; see Table 2 for key to PAH abbreviations used.

For algae, only very low levels of some of the higher molar mass PAHs were detected in *Padina* sp. from Commercial Port (site 8), Dry Dock Island (site 25), and Echo Wharf (site 14) in Apra Harbour. Σ_{16}PAH concentrations ranged from 30 to 41 ng/g and are presumably a reflection of pyrogenic PAH contributions from the engine exhaust streams of watercraft in the area. The absence of detectable 2- and 3-ring PAHs indicated that significant fuel spills had not occurred at these sites in the recent past. Harrison et al. (1975) published a maximum value of 60 ng/g for total PAHs in marine algae from Greenland. This value is not too far removed from the maximum Σ_{16}PAH concentration reported here for *Padina* sp. In an earlier series of studies, Mallet and co-workers found benzo(*a*)pyrene levels in marine algae from Greenland and French Mediterranean coastal waters to range from undetectable to 60 ng/g dry weight (Mallet, 1961; Mallet et al., 1963; Perdriau, 1964). The highest value reported by these researchers translates to ~15 ng/g on a wet weight basis and is approximately half the maximum benzo(*a*)pyrene concentration determined in *Padina* sp. during the present study.

Σ_{16}PAH concentrations in the Guam harbour sponges were at least an order of magnitude higher than in *Padina* sp. Presumably, this reflects the relatively high lipid content of the various representatives looked at within this group. The fact that sponges have very limited PAH metabolizing capabilities may also be a contributing factor here (Kurulec et al., 1985). PAH profiles were largely dominated by 4–6 ring compounds of pyrogenic origin (Table 3). Σ_{16}PAH concentrations in the soft coral *Sinularia* sp. were of the same order as determined in *Padina* sp., apart from one sample taken from underneath the Shell Fox-1 Fuel Pier, in Apra Harbor (site 6). This particular specimen had a total quantifiable PAH concentration of 117 ng/g. Its PAH profiles were dominated by anthracene, fluorene and chrysene, three common constituents of fossil fuel combustion.

A limited number of PAHs were detected in sea cucumbers from Apra Harbor and the Merizo Pier area, although there was no consistency in residue patterns between sites. Total quantifiable concentrations were relatively low and ranged from 26 to 83 ng/g. Aquatic organisms can acquire PAHs from water, food and sediments. Direct uptake from water is generally considered to be more efficient than from food or sediment. Sediment bound PAHs have only limited biological availability, and so, benthic organisms, like sea cucumbers, rarely contain higher levels of PAHs than the sediment in their immediate surroundings, even in highly polluted waters (Neff, 1979). Moreover, there is now evidence to suggest that higher invertebrates like echinoderms, arthropods and annelids, can metabolize PAHs, whereas lower invertebrates like coelenterates and sponges generally cannot (James, 1989). The fact that we were unable to detect any PAHs in the majority of sea cucumbers analysed is, therefore, not surprising.

Remarkably little attention has been directed towards the PAH assimilating capacity of echinoderms considering the intimate contact these organisms have with marine sediments. Mallet et al. (1963) were unable to detect benzo(*a*)pyrene in an unidentified sea cucumber from the west coast of Greenland, but a maximum value of 126 ng/g dry weight was found for this PAH in an unidentified starfish from the North Sea coast of France. In the present study, detectable levels of benzo(*a*)pyrene were only found in the hemal system of *Holothuria atra* from the Port Authority Beach area (58 ng/g). This equates to ~387 ng/g when recalculated on a dry weight basis and is relatively high for an aquatic organism.

PAHs were detected in 53% of the oyster samples analysed. Total quantifiable levels ranged from 15 to 78 ng/g and were highest in samples collected from underneath the Shell Fox-1 Fuel Pier (site 6) in Apra Harbour. Phenanthrene and fluoranthene were the most commonly detected congeners. Benzo(*a*)pyrene was identified only once, in oysters from Agana Boat Basin, and at a relatively low concentration of 10 ng/g. The data were recalculated on a dry weight basis and ranged from ~100 to 520 ng/g, values very close to the annual median ranges for oysters and mussels in USA coastal waters (O'Connor, 1998; Sericiano et al., 1995). Total PAH levels in oysters from clean environments are usually less than 10 ng/g on a fresh weight (Pendoley, 1992). Michel and Zengel (1998) measured 14 pure and 20 alkylated PAHs in the oysters from Acajutla, El Salvador, following two oil spill incidences. They reported total PAH concentrations ranging from 37 ng/g dry weight (~6 ng/g wet weight) in specimens from clean areas, and up to 18,000 ng/g dry weight at the most heavily impacted sites. Thus, PAH levels in oysters from Guam harbours are not representative of pristine conditions, but they fall far short of those encountered in bivalves from heavily polluted waters.

No comparative data exist to evaluate the PAH levels found in chamids and spondylids during the present investigation. The highest PAH values recorded for chamids were in specimens from the western end of Commercial Port in Apra Harbour (site 8), where total quantifiable concentrations ranged from 63 to 783 ng/g with an overall geometric mean value of 235 ng/g. Such high sample variability may reflect individual differences in size and/or physiological condition related to gonad development and spawning. Tissue PAH profiles in chamids from site 8 were dominated by phenanthrene, anthracene, fluoranthene, chrysene, benzo(*k*)fluoranthene and benzo(*a*)pyrene. The absence of the low molecular weight homologues, in addition to the fact that phenanthrene/anthracene ratios were less than 10, indicates that residues were primarily of pyrogenic origin (Benlahcen et al., 1997).

No PAH residues were detectable in the tail muscle of the stomatopod, *Gonodactylus* sp. from Apra Harbour. This burrowing predatory species might be expected to reflect the PAH loading of the bottom sediments in which it lives, although, as mentioned above, sediment-sorbed PAHs have limited bioavailability. Nevertheless, the stark absence of PAHs in the tail muscle of this specimen

deserves further investigation to determine possible links between habitat and/or effective PAH metabolism. Similarly, no PAH residues were found in the ascidians analysed, apart from very low levels of anthracene (3 ng/g) and benzo(*k*)fluoranthene (9 ng/g) in *Rhopalaea* sp. from site 8 in Apra Harbour. The fact that ascidians are approximately 95% water could possibly account for their apparent lack of sensitivity to environmental PAHs although metabolic process cannot be discounted.

Out of 85 fish analysed, quantifiable levels of PAHs were detected in the axial muscle of only 10 specimens. Levels ranged from 4 to 64 ng/g with a median value of 20 ng/g. Tissue PAH profiles varied between species but, in general, were dominated by phenanthrene, followed in decreasing frequency of detection by: benzo(*g*,*h*,*i*)perylene > dibenz(*a*,*h*)anthracene > anthracene > acenaphthene and fluorene. This ranking suggests exposure to PAHs of predominantly pyrogenic origin, with minor contribution from petrogenic sources. PAHs were not detected in any of the fish livers examined.

This preliminary survey generally indicates low-level movement of PAHs into the biota of each harbour studied. The biota from Apra Harbour are particularly clean when compared with levels found in related species from similar sized ports elsewhere in the world. This is somewhat surprising considering the intensity of military and commercial shipping activities occurring there on a day-to-day basis. Current harbour policies aimed at preventing petroleum spillage and oil/water discharges from boats and ships in the area probably contribute to this situation. In addition, PAH degradation and volatilization rates are higher in Guam compared with cooler regions, and, in all probability, are paralleled by higher PAH turnover rates in the local biota. Thus, the impact of a small spill on tissue PAH residues will very likely be short-lived, as will the telltale PAH signatures in the bioindicators of choice, once conditions return to normal.

Acknowledgements

Our thanks to Vance Eflin, Danzel Narcis and Greg Pangelinan (Guam Environmental Protection Agency) for assistance with the sample collection. This work was funded, in part, by the National Oceanographic and Atmospheric Administration, Office of Ocean and Coastal Resource Management, and the Guam Coastal Management Program, Bureau of Planning, Government of Guam, through NOAA Grant Awards #NA67OZ0365 and NA77OZ0184.

References

Benlahcen, K.T., Chaoiu, A., Budzinski, H., Bellocq, J., Garrigues, P., 1997. Distribution and sources of polycyclic aromatic hydrocarbons in some Mediterranean coastal sediments. Marine Pollution Bulletin 34, 298–305.

Cerniglia, C.E., Heitkamp, M.A., 1989. Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment. In: Varanasi, U. (Ed.), Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. CRC Press Inc., Boca Raton, pp. 41–68.

Denton, G.R.W., Wood, H.R., Concepcion, L.P., Siegrist, H.G., Eflin, V.S., Narcis, D.K., Pangelinan, G.T., 1997. Analysis of in-place contaminants in marine sediments from four harbor locations on Guam. A pilot study. Technical Report No. 81, Water and Environmental Research Institute of the Western Pacific, University of Guam, Mangilao, 120p. Available from: <http://www.weriguam.org/reports/>.

Denton, G.R.W., Concepcion, L.P., Wood, H.R., Eflin, V.S., Pangelinan, G.T., 1999. Heavy metals, PCBs and PAHs in marine organisms from four harbour locations on Guam. Technical Report No. 87, Water and Environmental Research Institute of the Western Pacific, University of Guam Mangilao, 158p. Available from: <http://www.weriguam.org/reports/>.

Denton, G.R.W., Concepcion, L.P., Wood, H.R., Morrison, R.J., 2005. Trace metals in sediments in four harbours in Guam. Marine Pollution Bulletin 50, 1133–1141.

Denton, G.R.W., Concepcion, L.P., Wood, H.R., Morrison, R.J., 2006. Polychlorinated biphenyls (PCBs) in marine organisms from four harbours in Guam. Marine Pollution Bulletin 52, 214–226.

Ganzler, K., Salgo, A., Valko, K., 1986. Microwave extraction. A novel preparation method for chromatography. Journal of Chromatography 371, 299–306.

Harrison, R.M., Perry, R., Wellings, R.A., 1975. Polynuclear aromatic hydrocarbons in raw, potable and waste waters. Water Research 9, 334–346.

Hites, R.A., Laflamme, R.E., Windsor Jr., J.G., 1980. Polycyclic aromatic hydrocarbons in marine/aquatic sediments: their ubiquity. In: Petroleum in the Marine Environment. American Chemical Society, Washington, DC, pp. 289–311.

James, M., 1989. Biotransformation and disposition of PAH in aquatic environments. In: Varanasi, U. (Ed.), Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. CRC Press Inc., Boca Raton, pp. 69–91.

Kurulec, B., Britvic, S., Zahn, R.K., 1985. The activation of aromatic amines in some marine invertebrates. Marine Environmental Research 17, 141–144.

Law, R.J., Dawes, V.J., Woodhead, R.J., Matthiessen, P., 1997. Polycyclic aromatic hydrocarbons (PAH) in seawater around England and Wales. Marine Pollution Bulletin 34, 306–322.

Mallet, L., 1961. Investigation of benzo-3,4-pyrene-type polycyclic aromatic hydrocarbons in the fauna of marine environments (the Channel, Atlantic and Mediterranean). Comptes Rendus de L'Academie des Sciences (Paris) Series D 253, 168–170.

Mallet, L., Perdriau, V., Perdriau, S., 1963. Etendue des pollutions par les hydrocarbures polybenziques du type benzo-3,4-pyrene en Mer du Nord et dans L'Ocean Glacial Arctique. Bulletin de L'Academie National de Medicine (Paris) 147, 320–325.

McCarry, B.E., 1977. A chemico-biological study of sediments in Hamilton harbour. SETAC News 17, 18–19.

McElroy, A.E., Farrington, J.W., Teal, J.M., 1989. Bioavailability of polycyclic aromatic hydrocarbons in the aquatic environment. In: Varanasi, U. (Ed.), Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. CRC Press Inc., Boca Raton, pp. 1–39.

Michel, J., Zengel, S., 1998. Monitoring of oysters and sediments in Acajutla, El Salvador. Marine Pollution Bulletin 36, 256–266.

Neff, J.M., 1979. Polycyclic Aromatic Hydrocarbons in the Marine Environment – Sources, Fates and Biological Effects. Applied Science Publishers, London.

O'Connor, T.P., 1998. Mussel Watch results from 1986 to 1996. Marine Pollution Bulletin 37, 14–19.

Pendoley, K., 1992. Hydrocarbons in Rowley Shelf (Western Australia) oysters and sediments. Marine Pollution Bulletin 24, 210–215.

Perdriau, J., 1964. Marine pollution by carcinogenic benzo-3,4-pyrene-type hydrocarbons – biological incidences. Part II. PAH. Oceanography 16, 205–229.

Pierce, R.H., Brown, R.C., Van Vleet, E.S., Joyce, R.M., 1986. Hydrocarbon contamination and coastal development. In: Sohn, M.L. (Ed.),

Organic Marine Geochemistry. ACS Symposium Series No. 305. American Chemical Society, New York, pp. 229–246.
Sericiano, J.L., Wade, T.J., Jackson, T.J., Brooks, J.M., Trip, B.W., Farrington, J.W., Mee, L.D., Readmann, J.W., Villeneuve, J-P., Goldberg, E.D., 1995. Trace organic contamination in the Americas: An overview of the US national status and trends in the International 'Mussel Watch' Programmes. Marine Pollution Bulletin 31, 214–225.
USEPA, 1996. SW-846 test methods for evaluating solid waste physical/chemical methods. US Environmental Protection Agency, Office of Solid Waste and the National Technical Information Service, Springfield, Virginia, CD-ROM.
Van Vleet, E.S., Joyce, R.M., Sherwin, M.R., 1986. Comparison of anthropogenic hydrocarbon inputs to two subtropical marine estuaries. The Science of the Total Environment 56, 221–230.

0025-326X/$ - see front matter
doi:10.1016/j.marpolbul.2006.05.002

Trace metals and organic compounds in the benthic environment of a subtropical embayment (Ubatuba Bay, Brazil)

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Contamination of the aquatic environment has become a serious problem in many parts of the world, with rivers and bays often seriously affected. Urbanised littoral areas receive the impact of effluent discharges resulting, in general, in the contamination of water, sediments and biota that can affect human health by direct contact or through the food chain. Almost all marine coastal ecosystems have complex structural and dynamic characteristics that can be easily modified by human influence. The north coast of the São Paulo State (Brazil), the most populated state of the country, has several enclosed bays strongly affected by increasing tourism and urbanisation. Ubatuba Bay is located in the northern coast of São Paulo State (Fig. 1), and is protected from south and southwest waves from the open sea. Water circulation is clock-wise with the inflow from the south. The input of fluvial sediments is strongly dependent on the rainfall regime leading to a higher contribution during the summer season (Mahiques et al., 1998). Four rivers flow into the bay and greatly influence its water quality (CETESB, 2000; Burone et al., 2003) especially, during summer and rainy periods when large amounts of untreated sewage are introduced from Ubatuba City, due to the rapid increase in the city's population (five times greater).

There are no previous studies concerning the accumulation of contaminants in bottom sediments of this bay; however previous investigations showed alterations of benthic macrofauna (Santos and Pires-Vanin, 2004) and benthic foraminifera communities (Burone and Pires-Vanin, 2006). In view of these studies and the absence of industrial activities in the area, it is possible that contamination derived from domestic river-borne sewage accumulates in the inner portion of the bay. The aim of this study was to identify heavy metal content, chemical composition and sources of the organic material, and quantify them in the sediments in order to assess the environmental quality of this benthic environment.

During April of 2001, sediments from nine stations of the Ubatuba region were sampled. Six of them (Sts. 1–6) were located in the inner region of Ubatuba Bay, one (St. 7) in the outer region and two (Sts. 8 and 9) were located in the neighbouring Picinguaba Bay (Fig. 1). The rationale of the sample selection was based on their similar depth, sediment type and possible degree of anthropogenic impact. Sts. 1–6 are under the direct riverine influence, St. 7 without the river influence in Ubatuba Bay, and Sts. 8 and 9 in the pristine Picinguaba Bay (CETESB, 2000; Paiva, 2001).

At each station, seven sediment samples were collected with a manual Kajac corer (8.0 cm internal diameter) for the following analyses: granulometric parameters, photosynthetic pigments, total organic matter (TOM), total organic carbon (TOC), total nitrogen (N), sulphur (S), chromium, zinc, copper, hydrocarbons and sterols. The

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