



Continuous surface seawater surveillance on poly aromatic hydrocarbons (PAHs) and mutagenicity of East and South China Seas

Huifeng Ren^a, Tsubasa Kawagoe^a, Huijuan Jia^{a,*}, Hideaki Endo^a, Akira Kitazawa^b, Sumio Goto^c, Tetsuhito Hayashi^a

^a Department of Ocean Science, Tokyo University of Marine Science and Technology (TUMST), Konan-4, Minato-ku, Tokyo 108-8477 Japan

^b Umitaka-Marui, Research and Training Ship, TUMST, Konan-4, Minato-ku, Tokyo 108-8477 Japan

^c Department Environmental Health, Azabu University, Fuchinobe, Sagami-hara-shi, Kanagawa 229-8501 Japan

ARTICLE INFO

Article history:

Received 30 January 2009

Accepted 28 September 2009

Available online 12 October 2009

Keywords:

poly aromatic hydrocarbons (PAHs)

mutagenicity

surface seawater

oceanic current

depth

East China Sea

South China Sea

ABSTRACT

Poly aromatic hydrocarbons (PAHs) and mutagenic compounds were adsorbed on Blue Rayon (BR) to monitor the pollution level of the surface seawater of the East and South China Seas by continuous sampling of seawater along the ship lane of our research and training vessel Umitaka-Marui. Eluted PAHs from BR were quantitatively determined by HPLC and their total amount in each sample ranged between 30.40 and 120.29 ng per liter of seawater, showing a difference of about 4 times among 8 samples. Acenaphthylene, naphthalene, and benz(a)anthracene were the most common PAHs found in the East and South China Seas, however, no big difference was observed in the concentrations of PAHs distributed throughout those two closed seas. Mutagenicity was detected as pseudo-positive from three samples. Among them, one sample collected along the east edge of the East China Sea showed a strong direct cytotoxicity which interrupted the mutagenicity test.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

In and around the East and South China Seas, there are many international shipping lanes, such as the Bashi Channel and the Strait of Malacca, through which a large number of tankers and cargo liners come and go on a daily basis. We are concerned about the chronic pollution of petroleum related compounds (Davenport and Davenport, 2006) discharged from ships and vessels in these sea areas, in addition to chemical contaminants generated by industrial activities of nearby coastal areas. Other than PAHs, we are interested in many types of chemical pollutants that are known to exist in the water environment, some of which show mutagenic or carcinogenic activities (Levi, 1995; Valavanidis et al., 2008). Studies on the environmental contaminants, in general, proceed as follows. We first try to identify the object substance using the authentic compound as a standard, and then analyze it quantitatively as the first step of risk evaluation. In this study, we measured 16 PAHs by HPLC, found in the USEPA watch list, however, this type of study might overlook the presence of hazardous substances which were excluded from the target list. We then employed the mutagenicity

test as a comprehensive pollution index (Sakamoto and Hayatsu, 1990) to monitor the presence of undetermined hazardous compounds by HPLC. Very few studies of quantitative determination of PAHs and measurement of mutagenicity were carried out in the past through the continuous surface water of broad oceans, such as the East and South China Seas. In this study we came on board the research and training vessel “Umitaka-Marui” of the Tokyo University of Marine Science and Technology on her voyage from Tokyo to the Antarctic sea between November 2005 and February 2006 during which time we continuously sampled the surface seawater at the rate of one liter per minute from Tokyo to Penang, Malaysia. This paper deals with the result of this continuous surface seawater surveillance on PAHs and mutagenicity of the East and South China Seas.

2. Materials and methods

2.1. Sampling of seawater and extraction of pollutants

Adsorption method using Blue Rayon (BR, commercially available from Funakoshi, Tokyo) has been commonly used to monitor PAHs and other pollutants consisting of more than two ring-structures, being found in very diluted concentrations, and distributed in an aquatic environment such as rivers or seas (Kira

* Corresponding author.

E-mail address: ginajhj@kaiyodai.ac.jp (H. Jia).

Table 1
Date and time (Nov. 2005, GMT), latitude and longitude, navigational distance (nautical mile), and total volume of seawater (L) of each sampling.

Sample number #	Start time (dd/hh/mm)	Ending time (dd/hh/mm)	Starting positions	Ending positions	Nautical mile	Seawater sampled (L)
1	22, 5:15	23, 5:15	31°59.27 N, 135°15.73 E	28°4.62 N, 130°36.03 E	346.68	794.0
2	23, 6:31	24, 6:31	27°37.87 N, 130°19.19 E	24°19.51 N, 125°04.24 E	359.00	776.1
3	24, 6:32	25, 6:32	24°19.51 N, 125°04.24 E	20°28.11 N, 120°29.35 E	356.70	765.3
4	25, 6:57	26, 6:57	20°24.29 N, 120°24.59 E	16°17.12 N, 116°17.42 E	338.92	833.7
5	26, 7:01	27, 7:01	16°15.89 N, 116°16.20 E	13°35.60 N, 113°36.20 E	279.95	1079.5
6	27, 7:10	28, 7:10	13°35.60 N, 113°36.20 E	09°31.71 N, 109°54.95 E	271.79	913.0
7	28, 7:11	29, 7:11	09°31.71 N, 109°54.95 E	04°58.84 N, 106°22.12 E	345.88	803.9
8	29, 7:12	30, 7:12	04°58.84 N, 106°22.12 E	01°47.07 N, 102°37.59 E	371.20	1025.6

et al., 1997). The process of extraction using a liquid–liquid partition method after concentrating a large water sample is thought impractical for our study, which is based on continuous surveillance of chemical pollutants along the sea lane extending over 5000 km (2670 mile). Consequently we employed the BR for consecutive PAHs sampling from surface seawater. BR is made of rayon fibers, to which copper phthalocyanine (blue pigment) is covalently bonded, which has a high affinity for aromatic compounds with three or more fused rings in their structure. Since a very small amount of BR, through which several hundred liters of seawater was passed, is necessary to adsorb and to elute target compounds, BR extraction is very efficient in comparison with a liquid–liquid or a solid–liquid partition method after concentration of the whole environmental water sample.

Researchers on “Umitaka-Maru” can collect surface seawater 24 h a day from a faucet located in a dry laboratory through a surface seawater continuous sampling system. The system pulls seawater up from a depth of 3 m by “noncontact” type pump. The sample is taken through a titan made pipe, connected to an inlet located at the bottom of bow of the ship. We attached an eddy current type flowmeter to the faucet and connected a plastic tube, in which 5 g of BR wrapped in nylon mesh was inserted to the outlet of the meter. Flow rate was set to about 1.0 L/min, and the BR was replaced for sample collection once a day. Table 1 shows sampling date and time (Greenwich Mean Time), positional information (latitude and longitude), travel distance (miles), and total sampling volume (L) for the eight samples we collected between Tokyo and Penang, Malaysia.

The amount of BR used in this procedure was determined based on our preliminary examination, carried out at Tokyo Bay. This suggested that less than 0.5 g of BR can adsorb whole contaminants in the water sample from the deepest part of Tokyo Bay, where the water is most polluted. As a result, we decided to use 5 g of BR for our main experiment per safety coefficient.

2.2. Extraction from BR

The BR removed from the outlet of the flowmeter was washed with pure water and then wiped dry with a Kim-towel. The dried BR was put into a glass vial with a silicon liner cap and a 150 mL mixture of methanol and ammonium water (50:1, v/v) for the

Table 2
Elution gradient program for HPLC (%).

Min	Methanol	Acetonitrile	Water
0–4	72	10	18
4–7	72 → 0	10 → 100	18 → 0
7–25	0	100	0
25–50	72	10	18
50 (Stop)	–	–	–

extraction of the PAHs. The vial was vigorously shaken 30 times vertically by hand, and this was repeated 6 times at 5 min intervals. This extraction procedure was repeated three times for each BR and all eluate (450 mL) were combined and concentrated at about 2 mL under low vacuum using a rotary evaporator at 20 degree C. This concentrate was diluted to 10 mL with methanol using a volumetric flask. 1 mL each was stored in two vials for HPLC analysis and the remainder (about 8 mL) for mutagenicity test. All these samples were kept frozen at –30 °C until analyzed.

Frozen samples were thawed at room temperature just before chemical analysis, filtered through a membrane filter (pore size 0.2 μm), pored individually into washed vials, and stored in a refrigerator until injection. Whole quantities of the mutagenicity test samples (8 mL each) were transferred to a pear-shaped flask, concentrated using a rotary evaporator under low vacuum, and finally dried under the quiet gas flow of dry nitrogen. Dried residue, thus obtained, was dissolved with 75 μL dimethylsulfoxide (DMSO) and 1425 μL pure water to make a final concentration of DMSO at 5%. The sample solution was then filtered through sterilized membrane filter (pore size 0.2 μm) and kept frozen until just before microbiological assay.

2.3. HPLC analysis

The low-pressure mobile phase mixing system (Shimadzu Co. Kyoto) used in this study consisted of a LC-9A pump unit with helium degasser, a SPD-6A ultraviolet spectrophotometric detector, and a C-R6A data processor. All analyses were carried out on a Wakosil-PAHs column (Wako Pure Chemical, Tokyo, 25 cm x 4.6 mm), with a sample injection volume of 20 μL, at a mobile phase flow rate of 1.0 mL/min, by the liner gradient elution program (complete in 50 min for one analysis) shown in Table 2. Before quantitative analysis, blank running was repeated twice for mobile phase stabilization in the column. Sixteen authentic PAHs standards, purchased from Wako, were individually dissolved into HPLC grade methanol and used for confirmation of their retention time. The mixture of these solutions was prepared to make their final concentration shown in Table 3. Identification of each compound in actual samples was performed by a recovery test after the addition of specific amounts of authentic standards. Differences in retention time of these additions and subsequent recovery tests were confirmed within ±0.2 min. Methanol and acetonitrile, both HPLC grade, were purchased from Wako, and freshly deionized water was distilled with a glass distillation apparatus for mobile phase water.

2.4. Mutagenicity test

All reagents (reagent grade) prepared for buffer solution and culture medium for the mutagenicity test in this study, such as magnesium sulfate, ammonium sodium hydrogenphosphate,

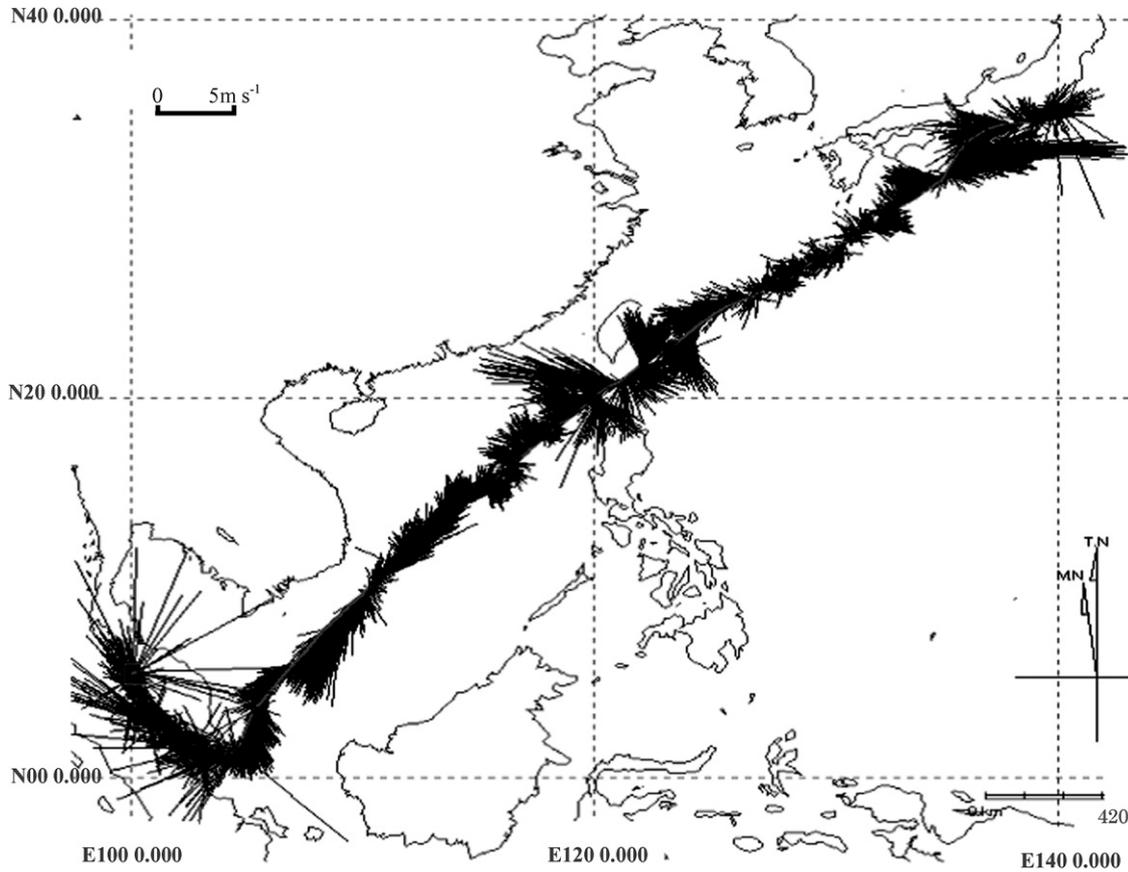


Fig. 1. Regional oceanic current at the depth of 30 m, along with the sea lane where sample surface seawater was collected, and the velocity and direction of current continuously monitored by acoustic Doppler current profiler (ADCP). Length and direction of each line, derived from sea lane of Umitaka-Mar, indicates the velocity and direction of the oceanic current, respectively.

tetracyclic compounds (Zhou and Maskaoui, 2003). In the same bay area, Gao and Chen (2008) reported the chemical composition of petroleum related aliphatic and alicyclic hydrocarbons in surface sediment. The PAHs detected in our study were also aromatic compounds, consisting of two to four aromatic rings. Moreover, in

the same report, the number of the rings of PAHs distributed in Daya Bay differed completely even though the sampling locations were within the comparatively small bay. These differences in PAHs compositions might be caused by variant sources, such as an oil spill, swage water, sedimentation from polluted air, or industrial

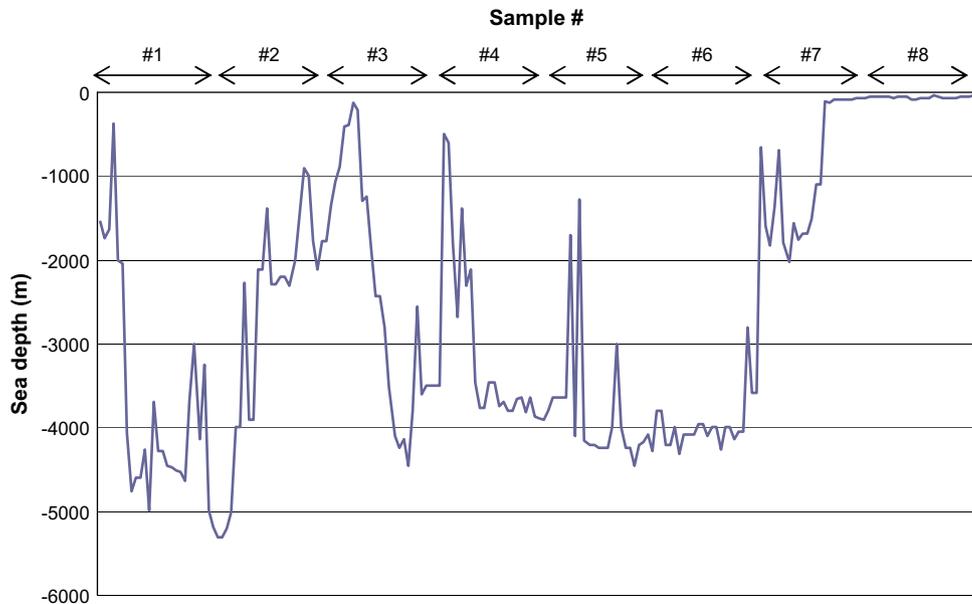


Fig. 2. Sea depth was continuously monitored along the ship lane of this voyage. Vertical axis indicates the sea depth (m), and numbers side-by-side on horizontal axis indicate the sea areas corresponding to each sample number (#1–8).

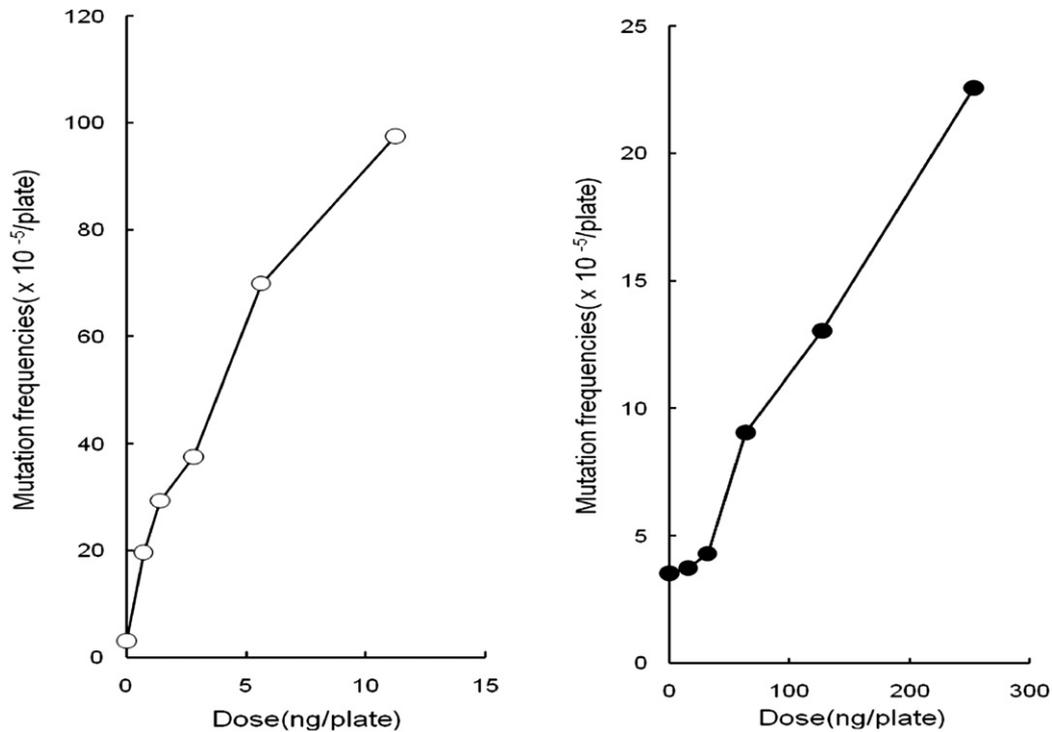


Fig. 3. Dose-response curve for 4-nitorquinoline (○) and benzo(a)pyrene (●).

drainage, it is difficult to identify each source of pollution solely by the basis of the PAHs regional and compositional data. Since our study investigated geographical and chemical distributions of PAHs in a much wider sea area than previous reports, it is impossible to pin-point the source for specific chemical contaminants at this moment. However, like a computer assisted back trajectory analysis of atmospheric wet deposition for acid rain, the invention of new technology is expected, in the near future, to retrospectively trace back the contaminating sources for oceanic pollution.

3.3. Correlation with oceanic current and sea depth along the sea lane and the distribution of PAHs

Oceanic currents at the depth of 30 m along the sea lane where we collected the surface seawater samples were continuously monitored by an acoustic Doppler current profiler (ADCP) mounted to the Umitaka-Maru. ADCP is a continuous measuring instrument to read the direction and velocity of oceanic currents at a certain sea depth by observing the Doppler shifts of reflex acoustic waves after shooting off ultrasound toward the bottom of the sea. In Fig. 1,

length and direction of each line, derived from sea lane of the Umitaka-Maru, indicates the velocity and course of the oceanic current, respectively. From this ADCP data, we can trace that the ship crossed over the *Kuroshio* (Black Tide) running eastward at speed of 4 knots (7.4 km) per hour, at about 200 km southeast of Shikoku Island, Japan. The figure also shows that the direction of the regional oceanic current frequently reversed its direction within a short period of time, at 150–200 km east of Taiwan and at the north border of the South China Sea. This suggests the existence of intricate oceanic currents from Taiwan to the Pacific Ocean and from the Pacific to the Asian Continent, in the sea areas #3–#4. Taking such an alternating oceanic currents into consideration, our analysis found that B[b]F1 was present only in sample #3, and was not detected at all in samples #2 and #4, both of which were collected in sea areas adjacent to #3 (Table 5). This characteristic of the chemical distribution in surface seawater strongly suggests the presence of a point of origin for B[b]F1, located upstream of the regional oceanic current where sample #3 collected. NAP, the second most commonly found PAHs in the East and South China Seas in our study, was below detection limit in seawater collected at the north edge (#4) and near the center of the South China Sea. This noncontiguous NAP distribution might also support our speculation that PAHs composition in surface seawater is easily influenced by multiple regional small oceanic currents. Fig. 2 shows the sea depth, continuously monitored by sonar and nautical charts, along the ship lane of this voyage.

While our samples were collected from the surface are of the seas, the data from ADCP clearly shows the intricacy of regional and small oceanic currents in the area at various depths, and this suggests that more complex factors, aside from the distance from the coastal line and depth of water, exist and influence how PAHs are distributed into wider areas of ocean. This experiment was not designed to collect all of those data, and further sampling using different methods and technologies is necessary. For example, collecting samples at different depths at each location would provide us other insight, and cross-examining the vertical and

Table 6
Mutagenicity detected in each sample by forward mutation assay using *Salmonella typhimurium* TA677 with or without the presence of S9.

Sample #	–S9	+S9
1	*±	*–
2	–	±
3	–	–
4	–	–
5	–	–
6	–	–
7	±	±
8	–	–

+: positive, ±: pseudo-positive, -: negative, * “killing” caused by some direct cytotoxic compounds, number of mutant colony was compensated by the ratio of [(viable cell count of sample)/(negative control)] for final judgment of mutagenicity.

horizontal distributions will guide us to a better understanding of true distribution of PAHs in the ocean.

3.4. Mutagenicity determined in surface seawater collected in the East and South China Seas

For quantitative discussion about the mutagenicity in actual samples, we need to indicate that the strength of mutagenicity that we observed is equivalent to a certain concentration of authentic mutagenic compounds. In this study, we employed 4NQO and B[a]P as the direct and indirect mutagenic standards, respectively. The dose-response curves shown in Fig. 3 suggest that this microbiological assay system we employed in our study was able to measure both direct and indirect mutagenicity within a specified range.

Among 8 samples collected from the East and South China Seas, samples #1, #2 and #7 were judged to be positive by the mutation assay with or without the presence of S9. Some of these samples were estimated to be pseudo-positive, as listed in Table 6. Remarkable decreases in the viable cell count, by the phenomenon called “killing”, observed in sample #1 suggested the presence of cytotoxic pollutants in the sample, collected from outside of the north edge of the East China Sea. Since the decreasing rate of viable cell numbers was less than 50% compared to that of the negative control test, the number of mutants was compensated in proportion of these two parameters and was judged to be pseudo-positive under the presence of S9, and to be negative without S9. On the other hand, sample #2 collected from sea area adjacent to #1 was pseudo-positive only in the presence of S9, and sample #7, obtained from the southern most part of the South China Sea, was pseudo-positive regardless of the presence of S9.

Since BR specifically adsorbs condensed ring compounds, mutagenic substances, such as PAHs or heterocyclic amines, are easily extracted from enormous amounts of very diluted water (Umbuzeiro et al., 2004; Kummrow and Rech, 2006). However, there are many more mutagenic substances, having no condensed ring in their structure, such as trihalomethane and methyl methanesulfonate, in a natural environment. Those hazardous pollutants are supposed to be stable in general, and distributed in seawater all over the globe, but unfortunately they cannot be trapped by BR. No clear relationship was observed between the concentration of PAHs and strength of mutagenicity in surface seawaters from the East and South China Seas. This could be due to very low concentrations of those mutagenic substances in the seawater of the investigated areas, in addition to the lack of BR's capability to catch such non-cyclic compounds, as mentioned above.

4. Conclusion

A group of PAHs was detected in all sea areas, ranging between 30 and 120 ng L⁻¹, though not every sample collected from adjacent areas show similar concentration levels of PAHs. Acenaphthylene,

naphthalene, and benz(a)anthracene were the three major PAHs. Species and concentrations of PAHs found in the samples collected in the East China Sea and South China Sea showed no significant difference from one another.

Oceanic current data at the depth of 30 m showed that the direction of the regional current frequently reversed itself, at east of Taiwan and at the northern border of the South China Sea. PAHs composition seemed to be influenced greatly by multiple regional, small oceanic currents. Among eight samples collected, samples #1, #2 and #7 were judged to be pseudo-positive by the mutation assay.

Acknowledgement

We would like to thank Mr. Aaron Brock for his time to read the manuscript and to discuss the contents in depth.

References

- Davenport, J., Davenport, J.L., 2006. The impact of tourism and personal leisure transport on coastal environment: a review. *Estuarine, Coastal and Shelf Science* 67, 280–292.
- Gao, X., Chen, S., 2008. Petroleum pollution in surface sediments of Daya Bay, South China, revealed by chemical fingerprinting of aliphatic and alicyclic hydrocarbons. *Estuarine, Coastal and Shelf Science* 80, 95–102.
- Kira, S., Horiguchi, H., Nogami, Y., Komatsu, T., Fujisawa, K., Ito, T., Hayatsu, H., 1997. Improved blue rayon hanging technique that can measure a time-weighted average concentration of benzo(a)pyrene in sea water. *Bulletin of Environmental Contamination and Toxicology* 59, 941–947.
- Kummrow, F., Rech, C.M., 2006. Blue rayon-anchored technique/*Salmonella* microsome micro-suspension assay as a tool to monitor for genotoxic polycyclic compounds in Santos estuary. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 609, 60–67.
- Levi, P.E., 1995. Chemical Pollutions in the Soil and Water. In: Hodgson, E., Levi, P.E. (Eds.), *A Textbook of Modern Toxicology*. Appteton & Lange, Connecticut, pp. 192–195.
- Ren, H., Hayashi, T., Ohkubo, T., Goto, S., Endo, H., Watanabe, E., 1997. Mutagenicity test suitable for marine natural seasoning containing free histidine. *Nippon Suisan Gakkaishi (Bulletin of the Japanese Society of Scientific Fisheries)* 63, 90–96.
- Sakamoto, H., Hayatsu, H., 1990. A simple method for monitoring mutagenicity of river water. Mutagens in Yodo river system, Kyoto-Osaka. *Bulletin of Environmental Contamination and Toxicology* 44, 521–528.
- Skopek, T.R., Liber, H.L., Kaden, D.A., Thilly, W.G., 1978. Relative sensitivities of forward and reverse mutation assays in *Salmonella typhimurium*. *Proceedings of the National Academy of Sciences of the United States of America* 75, 4465–4467.
- Takagi, Y., Goto, S., Murata, M., Matsushita, H., Lewtas, J., 1988. Application of Micro-forward mutation method to measure mutagenicity of air born particles. *Journal of Japan Society of Air Pollution* 23, 9–19.
- Umbuzeiro, G.A., Roubicek, D.A., Rech, C.M., Sato, M.I.Z., Claxton, L.D., 2004. Investigating the sources of the mutagenic activity found in a river using the *Salmonella* assay and different water extraction procedures. *Chemosphere* 54, 1589–1597.
- Valavanidis, A., Vlachogianni, Th., Triantafyllaki, S., Dassenakis, M., Androutsos, F., Scoullou, M., 2008. Polycyclic aromatic hydrocarbons in surface seawater and in indigenous mussel (*Mytilus galloprovincialis*) from coastal area of the Saronikos Gulf (Greece). *Estuarine, Coastal and Shelf Science* 79, 733–739.
- Zhou, J.L., Maskouki, K., 2003. Distribution of polycyclic aromatic hydrocarbons in water and surface sediments from Daya Bay, China. *Environmental Pollution* 121, 269–281.