International Journal of Water Resources and Environmental Engineering Vol. 4(5), pp. 139-143, May 2012
Available online at http://www.academicjournals.org/IJWREE
DOI: 10.5897/IJWREE11.152
ISSN 1991-637X ©2012 Academic Journals

Full Length Research Paper

EROD and PAH detection as biomonitoring tool in Golden Horn-Istanbul Turkey

Sebahat ŞEKER

Department of Environmental Engineering, Ardahan University, Ardahan, Turkey. E-mail: ebahatseker@ardahan.edu.tr. Tel: +90 478 211 38 22. Fax: +90 478 211 57 90.

Accepted 29 March, 2012

This investigation covers studies on the concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) in Golden Horn and their effects on *Ethoxyresorufin O-deethylase* EROD activities of human hepatocellular carcinoma cell line, HepG2. Water samples were collected from eight different stations around Golden Horn. Concentrations of total water PAHs ranged from 3.8 ng/L to about 259.7 ng/L. CYP1As enzymes were determined based on EROD Activity. A good correlation between total PAHs and EROD activity was observed. The analytical detection and biochemical EROD activity showed that the aquatic ecosystem around Golden Horn contained some hazardous PAHs. Possible sources for these chemicals could be industrial discharge and domestic wastewaters, transportation and shipping activities. Furthermore this study showed that EROD activity could be used as a bio-monitoring tool for the health effects of PAHs in aquatic ecosystems.

Key words: EROD, CYP1As, HepG2, Golden Horn, PAHs.

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are widespread persistent, carcinogenic and mutagenic contaminants, which could exist in water environments (Seker et al., 2005). PAHs are derived from benzene and exist as mixture compounds in contaminated areas (Vondráćek, 2001). They are by-products of all incomplete combustion of organic materials. While huge amounts of PAHs are coming from naturally occurring events, many come from anthropogenic sources (Dahle, 2003; Countway, 2003). They are attached to air particles and move from one place to another. Wastewaters and smoke from industrial areas contain significant amounts of PAHs. Owing to their hydrophobic property, PAHs tend to be adsorbed to the suspended particles while some quickly sink to the bottom of water in form of sediments. Sediments frequently contain high concentration of PAHs and it is important to assess contamination level by bioassays for ecological surveys (Walker and Dickhut, 2001). Sediment PAHs may disperse in turbulent waters only to be released in water column where they concentrate in aquatic biota (McCauley, 2000; Tolun, 2001).

If there is no definite point pollution, PAHs are usually uniformly distributed in remote marine environments. Sediment PAHs of near urbanized and industrialized sites show higher concentration because of their possible anthropogenic sources (De Luca, 2004). In our study, we selected active points which could be a good indicator between natural and anthropogenic variables. Owing to industrial wastewaters, street dust runoff, combustion of fossil products and shipping activities, coastal shores are contaminated by certain PAHs in Golden Horn.

While it is important to analyze the origin of the PAHs, it is also necessary to assess their pollution level and hazardous effects. Once PAHs are released into the water environments they could be genotoxic to the organisms, which are living in water column and/or have contact with sediment. PAHs can undergo biochemical reactions on living organisms when the quantity is sufficient enough.

Furthermore, they can find their way to the food chain and have genotoxic, mutagenic and carcinogenic effects in humans as well living organisms. Environmental contamination by PAHs could affect the bioactivation of

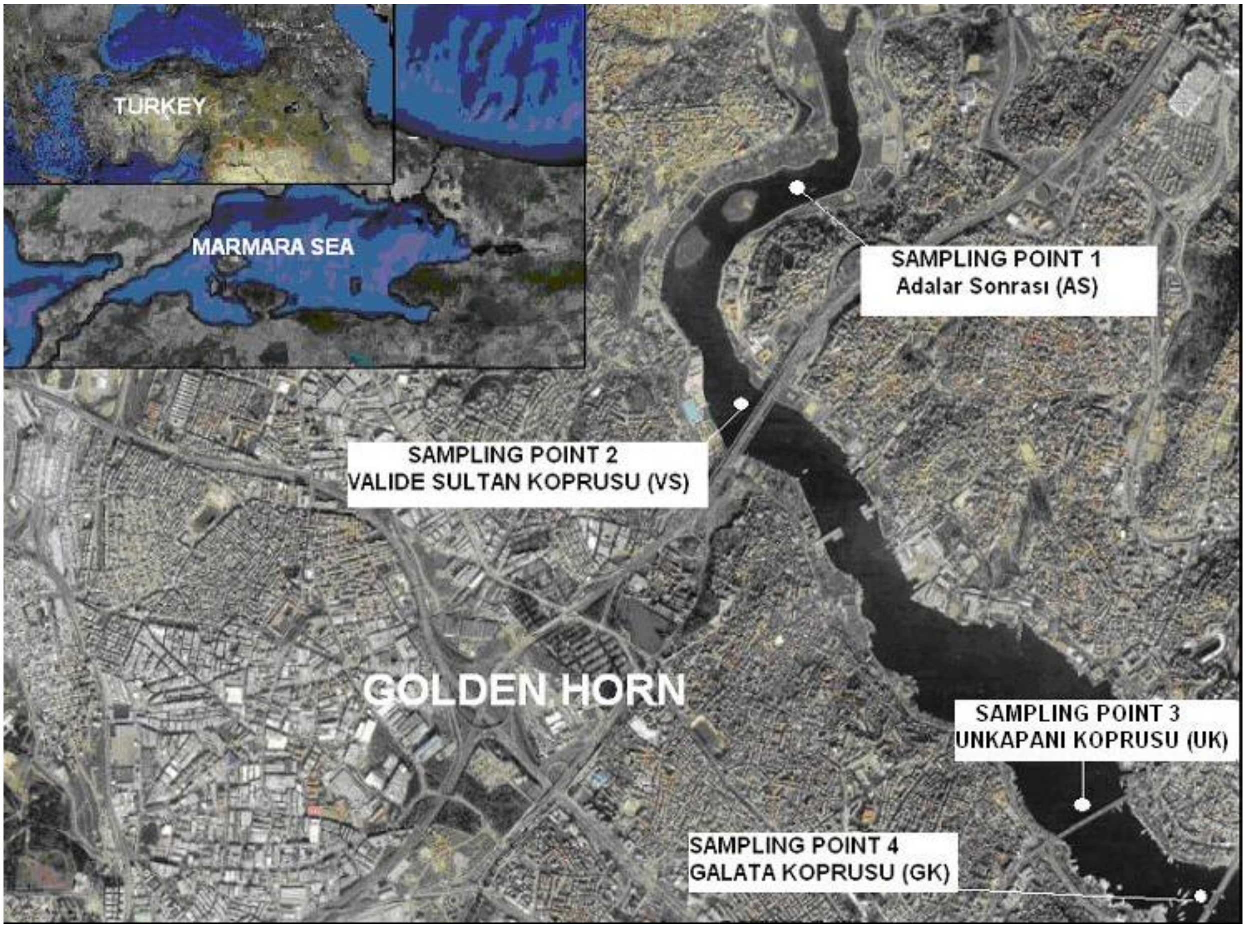

Figure 1. Map of the selected sampling stations.

cytochrome P450A (CYP1A) enzyme subfamily, such as CYP1A1 and CYP1A2. These enzymes are reported to be express with existence of PAHs by *in vitro* and *in vivo* bioassays (Shin'ichi, 2001). This activation can be evaluated by chemical analysis and biological tests as *Ethoxyresorufin O-deethylase* (EROD). Previously, some liquid and solid samples investigated as inducers of CYP1As, were collected between 1999 and 2001 at Okayama Prefecture, Japan by our laboratory. In this study, EROD Activity was used in order to clarify the effects of PAHs on expression of CYP1As at the base of cell defense in water environments. The objective of this study rely on the PAH Detection and using EROD Activity as a biomonitoring tool in Golden Horn, Istanbul.

MATERIALS AND METHODS

Sampling area

The Golden Horn is a heavily polluted water body in a large metropolitan area with a population of approximately more than 20 million. In this study sediment and water were sampled from four selected ports, which are the main shipping ways around Golden Horn. The samples were collected from Adalar Sonrası (AS), Galata Köprüsü (GK), Valide Sultan Koprusu (VS), Unkapani Koprusu (UK). Sampling map is shown in Figure 1.

PAH extraction and GC-MS detection

PAHs were extracted by acetone-hexane method from one-liter of water and 5 g (dry weight) sediment as per the procedure according to Seker et al. (2005). The extracted samples were detected by GC-MS spectrometry which maintained in selected ion monitoring (SIM) mode according to Nagashima et al. (2002). A QP5050 (Shimadzu, Japan) equipped with injector (splitless time 10 min; flow 1ml/min) was used. Anthracene, Benzo [a] Anthracene (BaA), Benzo [b] Fluoranthene (BbF), Benzo [a] Pyrene (BaP), Benzo [g,h,i] perylene (Bg,h,ip), Benzophenone (BP), BkF, Chrysene, Dibenz [a, h] Anthracene, Fluorene, Fluoranthene, Indeno [1,2,3-cd] Pyrene (I123cdP), Phenanthrene (P), Pyrene (Py), and 9-Methylphenanthrene (9MP) were analyzed by GC-MS.

EROD activity

Hepatocellular epithelial, adherent carcinoma cell line, HepG2, which was from the liver of a 15 years old Caucasian male (HB-8065), was purchased from Dainippon, Japan. Expressed CYP1A enzymes were detected by flourometric methods (Nagashima,

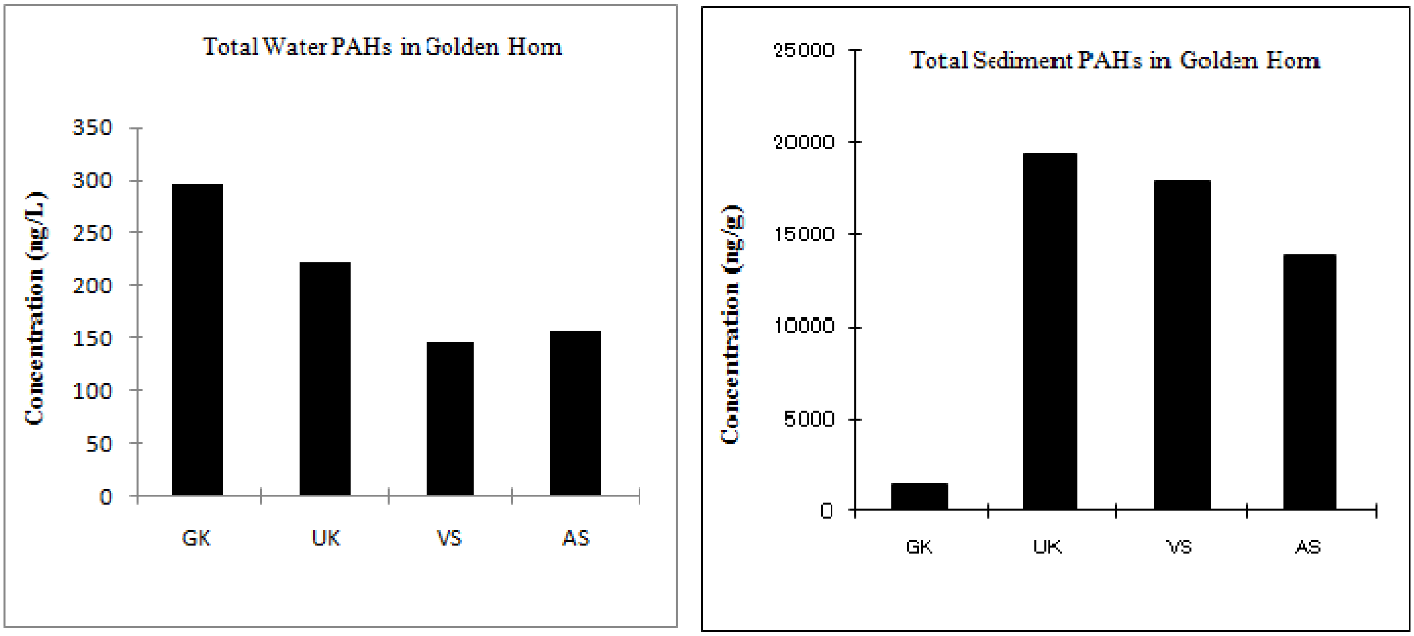

Figure 2. Total water and sediment PAHs in Golden Horn GK; Galata Köprüsü, UK; Unkapani Köprüsü, VS; Valide Sultan Köprüsü, AS; Adalar Sonrasi.

2002) as well as EROD Activity.

RESULTS AND DISCUSSION

The total water PAHs ranged from 2.5 to 132.3 ng/L, which is less than those in water samples of Mankyung River (Seung Min, 2003) and Izmit Bay (T-Karakoc, 2002). The total PAH concentrations were less than 1.00 µg/l, which is implying that there is no alarming oil input in water samples from selected areas as well those in Admiralty Bay (Bicego, 1996). Similarly to T-Karakoc (2002) and Bicego (1996) 3 rings- PAHs were dominant compared to those of 4, 5 and 6-rings in all stations.

The GC-MS results showed that huge amounts of PAHs were accumulated in dried sediment samples. The PAH concentrations of surface sediment ranged from 296.3 to 3992.9 ng/g which less than those in Tokyo (Shin'ichi, 2000) and Izmit Bay (T-Karakoc, 2002) (Figure 2).

The isomer ratios also showed that the origin of PAHs could be pyrogenic (P/A<10 and F/P>1 (De Luca, 2004). The individual PAHs are shown at the Figure 3.

EROD Activities of the samples, highest and lowest concentrations, were detected. The measured EROD Activities for highest concentrations were obtained at the BkF and used as the standard EROD inducer. The CYP1As were induced by low concentration of sediment extracts (5 ppm) for all stations (Figure 4). The correlation between concentration of PAHs and EROD Activity was well observed for these all stations. Relationship between PAHs and EROD activities clearly showed the capability of the PAHs on the expression of CYP1As in the water environments. Correlation between total molecular weight of PAHs and EROD activity was more pronounced in case of sediment samples.

It was also observed that the EROD inducing potential of liquid samples were less than those in solid samples of environmental sources; a confirmation of previous results from our laboratory. Relatively, water samples from our selected stations had less induction potential of EROD than those in sediment samples.

Conclusion

PAHs are hazardous chemicals in water environments that were detected around Golden Horn in Istanbul, Turkey. Origin plot and molecular weight ratios showed that these PAHs originated mainly from anthropogenic sources in Golden Horn. Determination of sources of PAHs could be helpful in proffering possible preventive measures in selected area. As a conclusion EROD Activity could be an important biomonitoring technique in water environments from the deep sediment to surface water in Golden Horn, Istanbul. The correlation between concentration of PAHs and EROD Activity was well observed for these stations. Relationship between PAHs and EROD activities clearly showed that the capability of the PAHs on the expression of CYP1As in the water environments. Correlation between total molecular weight of PAHs and EROD activity was more pronounced in case of sediment. This study suggests that biomonitoring

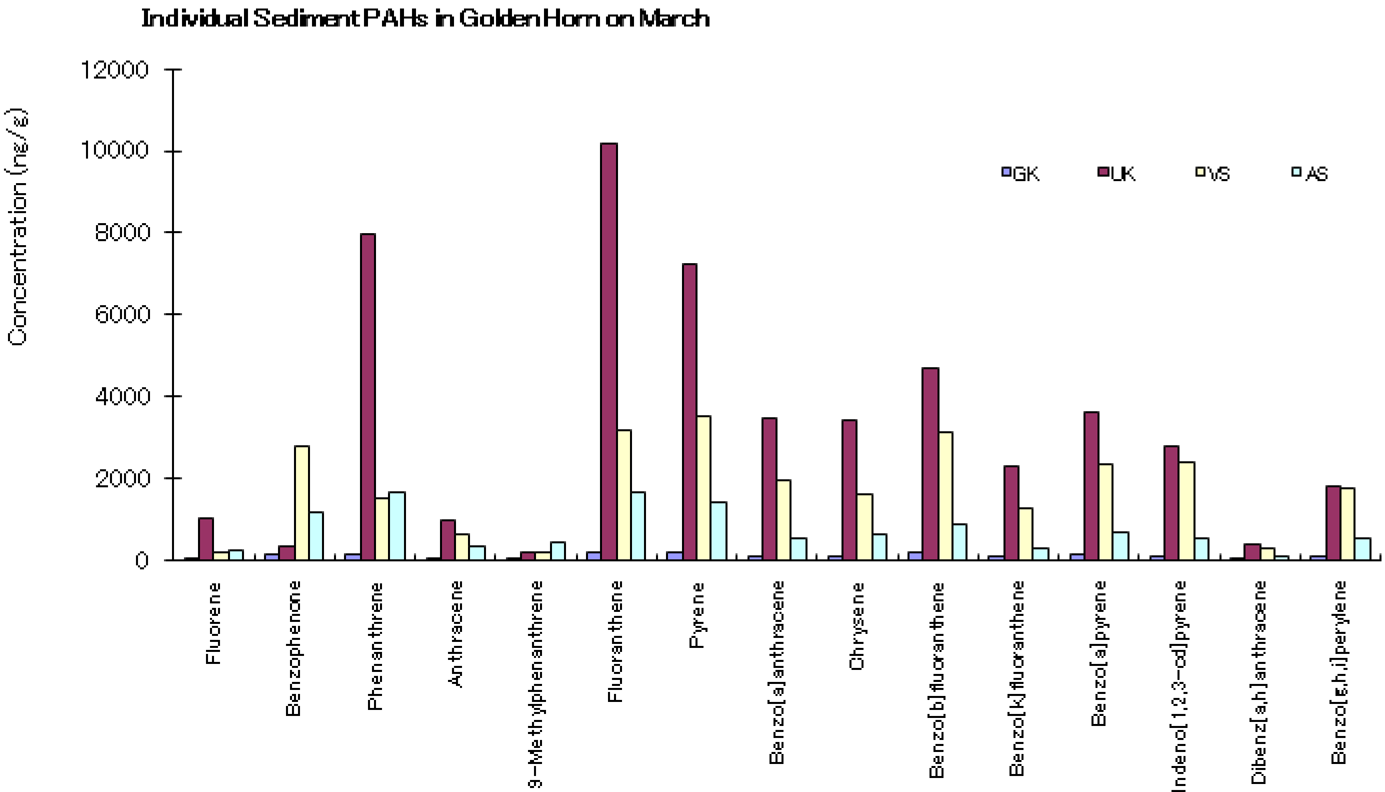

Figure 3. The individual sediment PAHs in sampling stations. GK; Galata Köprüsü, UK;Unkapani Köprüsü, VS; Valide Sultan Köprüsü, AS; Adalar Sonrasi.

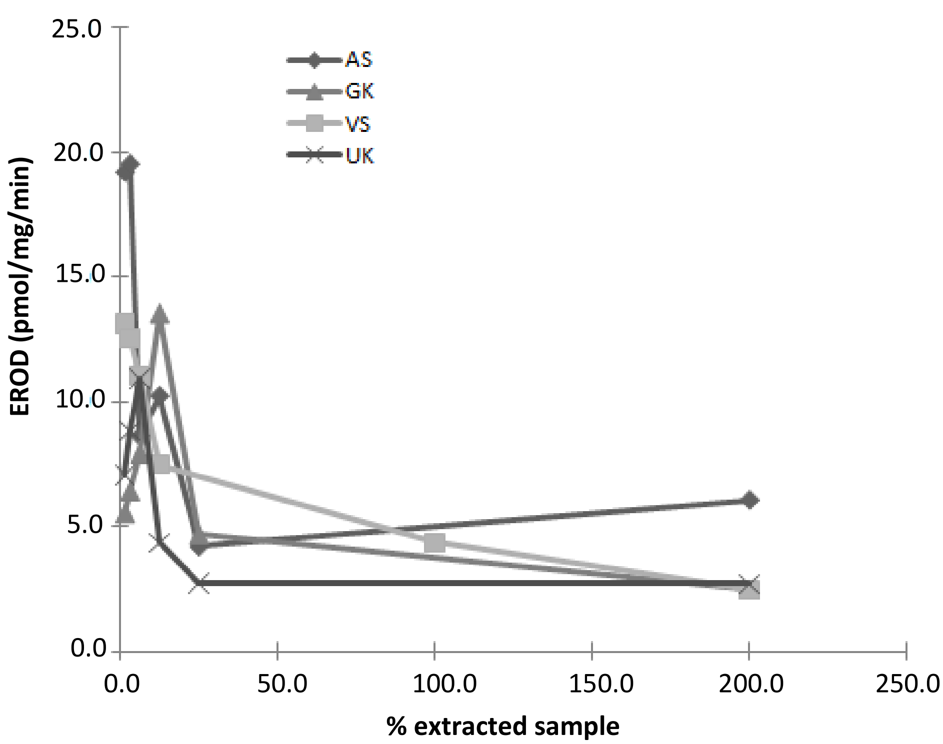

Figure 4. The EROD Activity of sediment samples (pmol/mg/min).

by expression of CYP1As is the necessary tool for understanding the hazardous effects of PAHs.

REFERENCES

Bicego MC, Roland R, Ito RG (1996). Aromatic hydrocarbons on surface waters of Admiralty Bay, King George Island, Antarctica. Marine Pollution Bulletin., 32 (7) : 549-553.

Countway RE, Dickhout RM, Canuel EA (2003). Polycyclic aromatic hydrocarbon (PAH) distributions and associations with organic matter in surface waters of the York River, VAEstuary. Organic Chemistry. 34: 209-234.

Dahle S, Savinov MS, Matishov GG, Evenset A, Næes K (2003). Polycyclic aromatic hydrocarbons (PAHs) in bottom sediments of the Kara Sea shelf, Gulf of Ob and Yenisei Bay. Sci. Total Environ., 306: 57-71.

De Luca G, Furesi A, Leardi R, Micera G, Panzanelli A, Piu PC, Sanna G (2004). Polycyclic aromatic hydrocarbons assessment in the sediments of the Porto Torres Harbor (Northern Sardinia Italy). Mar. Chem., 86: 15-32.

McCauley DJ, DeGraeve GM, Linton TK (2000). Sediment quality guidelines and assessment: overview and research needs. Environ. Sci. Policy, 3: 133-144.

Nagashima H, Ono Y, Sekiguchi M (2002). Polycyclic aromatic hydrocarbon concentration and 7-Ethoxyresorufin O-deethylase activity in environment. Environ. Sci., 10(1): 37-50.

Seker S, Arakawa K, Sekiguchi M, Ono Y (2005). “Biomonitoring of polycyclic aromatic hydrocarbons on hepatocellular carcinoma cell line. Water Sci. Technol., 52(9): 219-224.

Seung MO, Byung WH, Jeong HK, Kyu HC (2003). Novel quantitive assessment for the toxic effect of polycyclic aromatic hydrocarbon-like compounds in a water environment using the ethoxyresorufin O–deethylase microbioassay. J. Health Sci., 49(1): 59-64.

Shin’ichi N, Natsumi T, Miyako M, Takayuki N, Yoshio I (2001). Biological evaluation of the pollution of rivers flowing into Tokyo Bay with the 7- Ethoxycoumarin O-Deethylase (ECOD) Activity Induced by River Sediment extracts in HepG2 Cells. J. Health Sci., 47(2): 118-122.

T-Karakoc F, Tolun L, Henkelmann B, Klimm C, Okay O, Schramm KW (2002). Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in the bay of Marmara Sea: Izmit Bay. Environ. Pollut., 119: 383-397.

Vondráćek J, Machala M, Minksová K, Bláha AJM, Kozubík A, Hofmanová J, Hilscherova K, Ulrich R, Ciganek M, Neča J, Švrčkova D, Holoubek I (2001). Monitoring river sediments contaminated predominantly with polyaromatic hydrocarbons by chemical and *in vitro* bioassay techniques. Environ. Toxicol. Chem., 20(7): 1499-1506.

Walker SE, Dickhut RM (2001). Sources of PAHs to Sediments of the Elizabeth River, VA. 10(6): 611-632.