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COMPLEMENTARY CHEMICAL AND BIOLOGICAL DETERMINATION OF DIOXIN-LIKE COMPOUNDS IN SEDIMENTS OF ISTANBUL STRAIT

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ABSTRACT

Bosphorus and Dardanelles form the Turkish Straits System together with the Marmara Sea. The system provides a connection between the Mediterranean and the Black Sea. Istanbul and its coastal environment have been strongly affected by wastewater discharges, high population and heavy ship traffic. The aim of this study is to provide a comparison between the chemical and biological analysis of surface sediment samples, which were collected from 17 different coastal stations of the Istanbul Strait, to determine the pollutants and comparable effect related data. For the chemical analysis, PAH, PCDD/F and PCB isotope dilution techniques, and for the biological analysis, Micro EROD (24-h and 72-h) assay were employed. Highest values were found at the most polluted sites due to ship traffic and wastewater discharges. Most of the results of chemical analysis were lower or close to biological analysis, except for the samples from two stations where antagonistic modulators are indicated to be present in the Micro EROD assay. This study suggests that the WWTP discharges into Bosphorus and the heavy ship traffic are important sources of pollution in the sediment, as evidenced by high concentrations of toxic and persistent organic chemicals. Local discharges of the megacity should be further decreased.

KEYWORDS: Micro EROD assay; PAH; PCB; PCDD/F; Sediment; Strait of Istanbul

INTRODUCTION

The hepatic cytochrome P450 monooxygenase system in animals has been shown to be functionally involved in the biotransformation of many xenobiotic compounds, such as polychlorinated dibenzo-p-dioxins, -furans (PCDD/F), polychlorinated biphenyls (PCB), and endogenous molecules, such as steroids. Induction of cytochrome P450 enzyme levels has been employed as a biochemical marker in a variety of monitoring studies over the past decade [1-3].

Judging from the levels of these enzyme activities, the impact of xenobiotics on biological systems can be determined; however, the chemical analyses of these chemicals do not provide sufficient information on the complete impact of dioxin-like pollutants on biological and ecosystems.

One of the most well used and standardized assays of P450 enzymes level is the measurement of the activity of an enzyme called 7-ethoxyresorufin o-deethylase [4]. This enzyme converts ethoxyresorufin (7-ethyl-resorufin, 7-ER) to resorufin by deethylation in the presence of NADPH and oxygen, and the assay is well-known as EROD assay. The amount of resorufin produced is measured fluorometrically by spectrophotometry at 535 nm after the addition of NADPH and the samples to be tested.

In addition to the EROD assay chemical analyses, which include analyses of polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs), has been executed complementary in this study. PCBs and PCDD/Fs are families of global persistent organic pollutants and they are widespread in the abiotic and biotic environmental compartments as complex mixtures of their congeners [5].

Istanbul Strait (Bosphorus), which is a narrow channel and Çanakkale Strait (Dardanelles) form the Turkish Straits System (TSS) together with the Marmara Sea. The system provides a connection between the Mediterranean Sea and the Black Sea. The TSS is also one of the most important routes for oil transportation in the world. Istanbul and its coastal environment (Istanbul Strait) have been strongly affected by wastewater discharges, high population and heavy ship traffic. The oceanographic characteristics of the Istanbul Strait have been studied earlier, however, the strait ecosystem and priority pollutants in the strait have not been studied in detail previously [6-8].

MATERIALS AND METHODS

Sampling and storage

Surface sediments (0-10 cm) were collected from the coastal stations (Fig. 1) of the Istanbul Strait and from the coast of an island in the Marmara Sea during the period of January-February 2007 (Fig. 1).

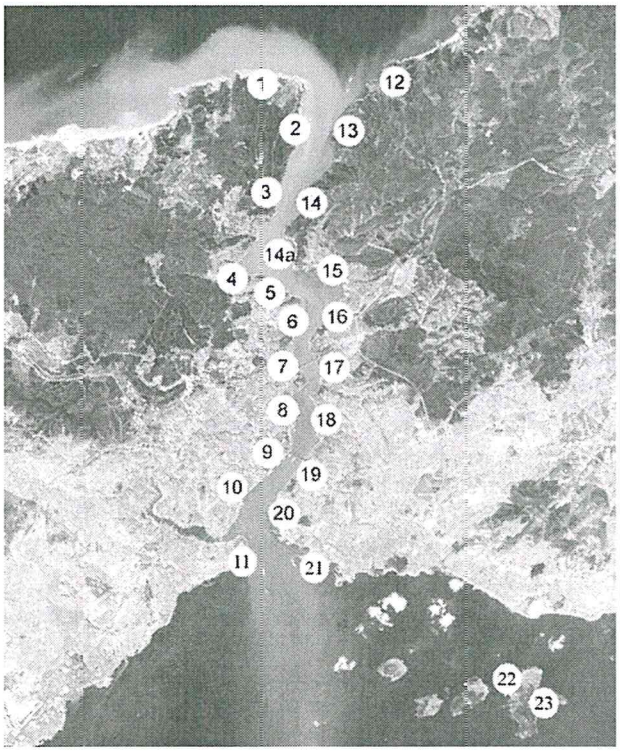

FIGURE 1 - The coastal stations of the Istanbul Strait [5].

Due to the rocky structure of the sampling stations on the Strait coastline, core and grab sampling was not possible. Divers carefully collect the surface sediment ensured to avoid the creation of "bow wave" by using glass beakers. At some stations, sediment sampling was not possible, even by diving because of the current and rocky seafloor. Samples were collected by SCUBA and/or free diving, and the range of water depth was recorded as 1-5 m. The names of the stations and their coordinates are given in Table 1. The samples were stored at -20 °C until analysis.

Test materials

In the extracts of 17 sediment samples (16 are from the strait and 1 from the island in Marmara Sea), 7-ethoxyresorufin-O-deethylase (EROD)–activity of intact cells grown in 96-well microtiter plates was analyzed following a standardized bio-analytical protocol.

Analytical methods

Chemical analyses were performed in the laboratory which are compliant with EN 17025 standards.

Extraction and clean-up of samples for the Micro-EROD assay

20 g of wet sediment sample, mixed with hydromatrix (Isolute HM-N, Biotage) for water adsorption, was extracted for 24 h in a Soxhlet apparatus using 250 ml toluene. After that, the extracts were concentrated up to 4 ml in a rotary evaporator for the execution of a clean-up step through a multilayer chromatography column. This column consisted of 5 g active silica gel, 10 g silica gel (44% H_2SO_4), 20 g silica gel (4% H_2O) and 5 g Na_2SO_4, from bottom to top. The column was eluted with 450 ml solvent mixture of n-hexane/dichloromethane (99:1, v:v). The eluate was concentrated and transferred to 500 µl DMSO/ isopropanol 4:1 (v:v) solution under a gentle N_2 stream at 45 °C.

Cell culture for the micro-EROD-assay

Rat hepatoma cells H4IIEC3/T (H4IIE) were grown as described earlier [9, 10]. Cells were seeded at a density of $1x10^5$/60 mm circular culture plate. Upon attaining a den-

TABLE 1 - Station names and their coordinates

Sampling Sites Name	Sampling Sites No	Coordinate	Specifications
Rumeli Feneri ilerisi	1	41° 14.455 N - 029° 05.855 E	Small bay
Garipçe Köyü	2	41° 12.818 N - 029° 06.594 E	Small fishing village
Rumeli Kavağı	3	41° 11.022 N - 029° 04.574 E	Touristic place with a small fishing port
Büyükdere	4	41° 09.207 N - 029° 02.324 E	Residential area with a small river mouth
Tarabya	5	41° 08.232 N - 029° 03.444 E	Small bay used as a port
İstinye	6	41° 06.633 N - 029° 03.515 E	River mouth and old shipyard
Balta Limanı	7	41° 05.963 N - 029° 03.256 E	River mouth connected with a treatment plant
Bebek	8	41° 04.803 N - 029° 03.084 E	Crowded residential area and cost line used as a port
Ortaköy	9	41° 02.829 N - 029° 01.639 E	Historical touristic place connected with a small sewage
Beşiktaş	10	41° 02.429 N - 029° 00.343 E	Crowded city center with sea bus port
Anadolu Feneri	12	41° 12.907 N - 029° 09.109 E	Small fishing village
Poyraz	13	41° 12.334 N - 029° 07.596 E	Harbor
Kandilli	18	41° 04.451 N - 029° 03.541 E	Near two river mouth connected with industry area
Kuzguncuk	19	41° 02.380 N - 029° 02.144 E	Residential area
Üsküdar	20	41° 01.285 N - 029° 00.411 E	Residential area with high current and ship traffic
Moda iskelesi	21	40° 58.786 N - 029° 01.488 E	Small port
Büyükada plaj	23	40° 51.550 N - 029° 06.769 E	Small touristic bay on the island

sity of about 70% confluency, cells were exposed to known amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, 0.5-40 pg TCDD/well). All TCDD standards or samples were dissolved in DMSO/isopropanol (4:1, v:v) and added to the cell cultures. The final volume of TCDD standards and samples was 0.5% of the total volume per well. After 72-h exposure, the medium was removed and cells were harvested by scraping with a rubber spatula. Cell suspensions were transferred with 1 ml phosphate buffered saline (PBS) into Eppendorf vials and kept at -80 °C until use.

Micro-EROD-assay

7-Ethoxyresorufin-O-deethylase (EROD)–activity of intact cells grown in 96-well microtiter plates was determined [9, 10]. H4IIE cells were incubated in culture flasks at 37 °C and 7.5 % CO_2. The hepatic cells were trypsinized, then counted with a Neubauer cell counter and diluted in supplemented medium (DMEM) in order to achieve a cell concentration of $1x10^5$ cells ml^{-1}. Cells were seeded into 96-well plates. After 1 day of growth, the medium was replaced by a 100 µl medium containing the sediments (each sample with 1/1, 1/2, 1/4 and 1/8 concentrations) and cells were exposed for 24 and 72-h. The medium was then removed and 100 µl fresh medium containing 8 µM 7-ethoxyresorufin and 10 µM dicuramol were added, and after incubation at 37 °C for 30 min, the medium was transferred to another 96-well plate containing 200 µl ethanol. Resorufin-associated flurorescence was measured at 535 nm excitation and 590 nm emission using a multi-well fluorescence reader. All samples, blanks and standards were prepared in duplicate on well plates as quadruplicates. One of the duplicates was used for the 24-h incubation, and the other one for the 72-h incubation.

After measurement of EROD activity, the 96-well plates were used for determining the protein amounts [11]. Finally, cytotoxicity of the test materials were assayed employing the Resazurin test [9, 10].

Calculation of biological TEQ values

The biological TEQ values were calculated according to Hanberg *et al.* [12]. Comparing the induction or inhibition of enzyme activity by environmental sample extracts with series concentrations of TCDD standard, the average deviation between the quadruplicate measurements was 25% [2].

PCDD/F and PCB analysis

Extraction and clean-up of samples

Extraction of PCB and PCDD/F was carried out with 20 g of wet sediment sample, mixed with hydromatrix (Isolute HM-N, Biotage) for water adsorption, using an Accelerated Solvent Extractor (ASE 200) device (Dionex, Sunnyvatem CA, USA) [13].

After spiking with the 13C-labelled standard mixture for quantification, the extraction was performed by using a mixture of n-hexane:acetone (75:25, v/v) at 120 °C and a pressure of 12 MPa. Two static cycles of 10 min were applied for a complete extraction. To remove interferences, the concentrated crude extracts (ca. 1 ml) were cleaned-up by several sequential liquid chromatography steps as follows: A multilayer chromatography column was filled with 5 g anhydrous Na_2SO_4, 2 g activated silica gel, 4 g silica gel treated with 10% $AgNO_3$, 2 g silica gel, 2 g silica gel treated with 30% NaOH, 2 g silica gel, 10 g of silica gel treated with 44% H_2SO_4, 2 g silica gel and 5 g anhydrous Na_2SO_4 (top), and washed with 50 ml n-hexane.

A reversible carbon column (100 mg Carboxen 1016, Supelco) was rinsed with 25 ml toluene and 25 ml n-hexane, respectively, and directly connected to the outlet of the multilayer column. The concentrated extract was added onto the top of the multilayer column and eluted with 200 ml n-hexane. The PCDD/F and coplanar PCB compounds were retained on the carbon column, whereas all other PCB congeners passed both columns. After disconnection, the carbon column was further eluted with 30 ml of n-hexane/ dichloromethane (9:1; v:v). Both eluates were combined, concentrated and further cleaned on a C18-modified silica column (1 g Isolute C18, Biotage). The column was eluted with 4 ml acetonitrile, and the eluate was reduced to a final volume of 20 µl for GC/MS analysis.

The PCDD/F and coplanar PCB compounds were backwashed from the carbon column with 100 ml toluene. This fraction was further cleaned on a chromatography column filled with 5 g aluminium oxide (Alumina B super I, ICN) [14]. The first fraction with 35 ml n-hexane/chloroform (88:12, v:v) was discarded; the second fraction with 50 ml dichloromethane was reduced to a final volume of 10 µl and analyzed by GC/MS.

Determination

PCDD/F and PCB analysis was performed by gas chromatography-high resolution mass spectrometry; the instrumental parameters are listed in Table 2. The MS was operated in SIM mode and the two most intense ions of the molecular ion cluster were monitored for unlabelled and labeled isomers [15, 16].

PAH analysis

Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)-anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(ghi)-perylene and dibenzo(a,h)anthracene were analyzed in a previous study [17] by using a high resolution gas chromatography/high resolution mass-spectrometry (HRGC/HRMS) system with the same samples. The instrumental analytical details can be found in Wang *et al.* [18].

RESULTS AND DISCUSSION

Comparison of PAH concentrations in sediments from different coastal areas of Turkey and other countries

TABLE 2 - GC/MS parameters for the isomer specific detection of PCDD/F and PCB compounds.

	PCDD/F	PCB
GC:	Type: Agilent 6890; Column: Rtx-Dioxin2, 60 m, 0.25 mm ID, 0.25 µm film thicknes (Restek); Temperature program: 130 °C, 1.5 min, 45 °C min^{-1}, 205 °C, 5 min, 9 °C min^{-1}, 305 °C, 20 °C min^{-1}, 310 °C, 15 min; Carrier gas: helium, constant flow: 1.5 ml min^{-1}; Injector: Cold injection system CIS 4 (Gerstel); Temperature program injector: 120 °C, 12 °C s^{-1}, 280 °C, 5 min; Temperature transferline: 300 °C; Autosampler: A200S (CTC); Injection volume: 1 µl pulsed splitless	Type: Agilent 5890 Series II; Column: Rtx-CLPesticides2, 30 m, 0.25 mm ID, 0.2 µm film thickness (Restek); Temperature program: 100°C, 1.5 min, 3 °C min^{-1}, 270 °C, 15 °C min^{-1}, 300 °C, 10 min; Carrier gas: helium, head pressure: 16 psi; Injector: Cold injection system CIS 3 (Gerstel); Temperature program injector: 120 °C, 12 °C s^{-1}, 280 °C, 5 min; Temperature transferline: 300 °C; Autosampler: MPS2 (Gerstel); Injection volume: 1 µl splitless
MS:	Type: MAT 95S (Thermo); Ionisation mode: EI, 50 eV, 260 °C; Resolution: > 9000; Detection: SIM mode	Type: MAT 95 (Thermo); Ionisation mode: EI, 47 eV, 260 °C; Resolution: > 8000; Detection: SIM mode

Table 3. Comparison of PAH concentrations in sediments (pg g^{-1}, dry weight) from different coastal areas of Turkey and other countries.

Sites	Concentration range ($x10^3$)	References
Istanbul Strait	1.1–3152	[17]
Marmara Sea	144	[17]
Istanbul Strait entrance, Black Sea	14–531	[23]
Russian Federation, Black Sea	61–368	[23]
Ukraine, Black Sea	7.2–638	[23]
Izmit Bay, Marmara Sea, Turkey	120–11400	[24]
Gemlik Bay, Marmara Sea, Turkey	51–13482	[25]
Cretan Sea (South Aegean Sea)	15–159	[26]
South Turkey, Eastern Mediterranean	550–18700	[27]
Italy, North Adriatic	18–577	[28]
Croatia, North Adriatic	32–13681	[29]
Italy, Mar Piccolo, Taranto, Ionian Sea	380–12750	[30]
Baltic Sea	5–22100	[31]
Arcachon Bay	32–4120	[31]
Western Mediterranean	1.2–20400	[31]

are as are shown in Table 3. There are also some studies including chemical analyses and/or EROD assays on the marine environment, which were mostly focused on fish species and/or mussels [19-22]. However, there is no comparison between chemical analysis and bioassay on sediment published. This study exemplifies this approach on the Istanbul Strait, which has a unique geographical formation with different currents, and also sediments of Istanbul Strait, having a very busy watercourse with annually 50,000 ships.

The pressures over the Istanbul Strait ecosystem may result from intentional or accidental discharges from shipping activities, atmospheric emissions, Black Sea inflow, other non-point sources and disposal of mainly domestic wastes, including river effluents [5]. The discharges of Istanbul city have been injected into the lower layer of Marmara Sea, and carried by the lower current into the Black Sea since the beginning of the 90s [32]. The wastewaters have been discharged directly into the surface waters until the sewage outfalls installed by "The Istanbul Water and Sewage Authority (ISKI)" [33]. ISKI wastewater discharges along the Strait coastline are shown in Fig. 2. There are also several uncontrolled discharges along the strait coast-line [6].

The currents are also a very important factor for the dispersion of pollution. In Istanbul Strait, they are surface, deep, counter and Orkoz currents [32, 33]. Because of their curvy structure, currents create eddies in the inlets of the strait, and cause elevated pollution in those inlets. Sampling stations 6, 8, 9 and 18 are good examples since they exhibit higher EROD 24-h values (15.86, 24.92, 19.77 and 26.07 pg g^{-1}, dry weight), respectively. Fig. 3 indicates the similarity between the currents and the accumulation of chemical compounds. It would appear that the counter current has the

FIGURE 2 - ISKI wastewater discharges along the Strait coastline (Squares with P show the discharge points) [33].

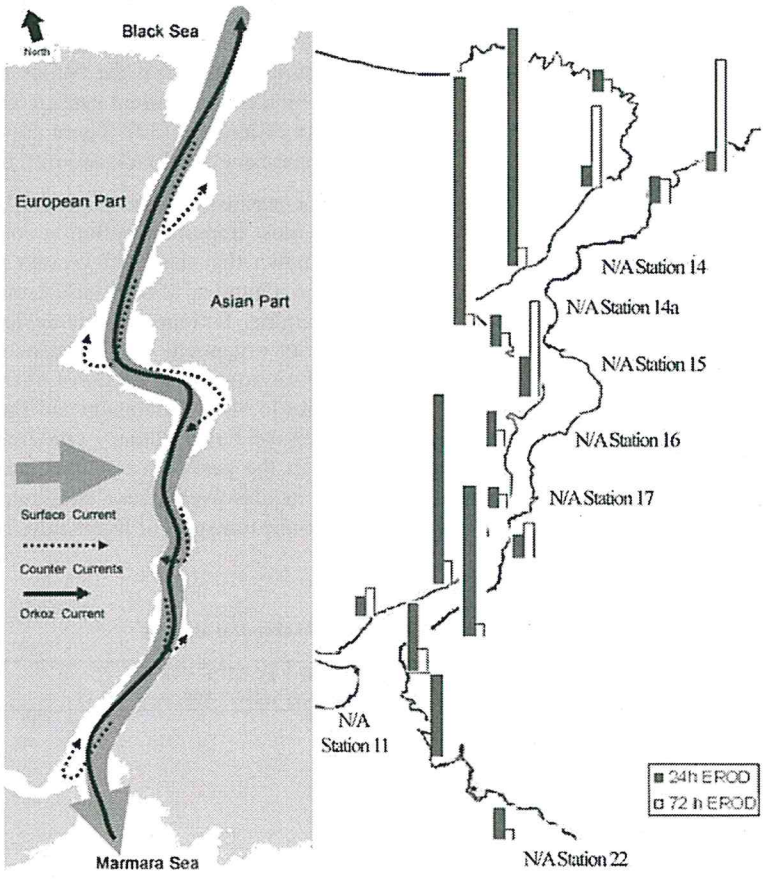

FIGURE 3 - Istanbul Strait water currents [36] and EROD (24-h/72-h) results.

most effect on the pollution in Istanbul Strait. EROD 24-h is comparable to PAH+PCDD/F+PCB analysis and EROD 72-h is comparable to PCDD/F+PCB analysis, because the PAHs are inactivated by the cells after 72-h.

The highest EROD 24-h values were found at the stations 8 (24.92 pg TCDD g^{-1}, dry weight) and 18 (26.07 pg TCDD g^{-1}, dry weight). Highest EROD 72-h values were determined at station 8 (11.84 pg TCDD g^{-1}, dry weight) and 18 (9.99 pg TCDD g^{-1}, dry weight). The EROD 72-h of station 23 is not presented, because it was lower than the limit of quantification of about 0.026 pg TCDD. Detailed EROD 24-h and 72-h results are given (Table 4).

TABLE 4 - EROD results (pg TCDD g^{-1}, dry weight).

	24-h EROD	72-h EROD
Station 1	2.58	3.05
Station 2	2.36	1.54
Station 3	3.74	1.86
Station 4	3.52	2.64
Station 5	3.49	1.45
Station 6	15.86	1.21
Station 7	2.46	1.26
Station 8	24.92	11.84
Station 9	19.77	8.63
Station 10	8.57	2.48
Station 12	2.25	2.53
Station 13	2.98	2.05
Station 18	26.07	9.99
Station 19	2.22	1.43
Station 20	2.32	1.49
Station 21	7.07	3.73
Station 23	4.24	-*

* lower than LOQ

TABLE 5 - PAH-TEF values for EROD response after 24-h [37].

PAH	TEF* VALUE (unitless)
Benzo(a)pyrene	$3.0x10^{-4}$
Benzo(a)anthracene	$2.7x10^{-5}$
Benzo(b)fluoranthene	$3.8x10^{-4}$
Benzo(k)fluoranthene	$2.9x10^{-4}$
Dibenz(a,h)anthracene	$7.8x10^{-5}$
Indeno(1,2,3-cd)pyrene	$8.6x10^{-5}$

*TEF – toxic equivalency factor

Potential EROD induction of PAH values was calculated using TEF values based on the individual PAH component's relative toxicity to 2,3,7,8-tetrachlorodibenzo-p-dioxin. TEF values are presented in Table 5. The PAH-TEQs were calculated as the sum of each individual PAH concentration multiplied by the corresponding TEF value [37, 38].

The biological and chemical results are shown for comparison (Table 6). The chemical analysis (PAH+ PCDD/F+ PCB) results from stations 6 and 8 show larger differences compared to the results for the EROD 24-h. Antagonism could be one possible reason for this result.

Stations 6 and 8, which have a connection with a creek, are more contaminated than the other sites. Station 6 is situated in a semi enclosed waterbody and has been affected by the industrial contamination carried by the İstinye River. The structure of the cove creates also reverse currents/eddies which may allow the pollutants to stay and accumulate. The station is heavily used by leisure boats and, most importantly, the area has been used as a shipyard for a long period of time (70 years). It is well-known that a shipyard is a workplace contaminated with spilled petroleum, paints, solvents, hydraulic liquids, etc. in relation to shipbuilding and repair activities The shipyard stopped its activities in 1991 [6]. Operation of the shipyard probably caused the release of several pollutants to accumulate in sediments. Station 8 has the second-highest total PAH concentration after station 6. The hydraulic properties of that station generate reverse currents/ eddies, and it is under the pressure of high population. This site is used as a marina for leisure boats which probably cause pollution by uncontrolled discharges (Fig. 4). The temperature differences between Bebek (station 8) cove and the main strait current show that the waters in the cove are not affected by the main current system, and these two waters do not mix efficiently [39]. There are also waste discharges from pharmaceutical factories into the tributaries.

The surface currents from the Black Sea are also one of the most important pollution sources of Istanbul. It is well-known that the northwestern part of the Black Sea receives a number of land-based sources of pollution from the Danube, Dnieper and Dniester river, an important source of organochlorine substances, as it flows through several regions with intensive agriculture and industrial activities [5, 41]. Maldonado and Bayona [42] showed that the Danube River estuary contains 4.3– 57.4 pg L^{-1} of PCBs in the particulate matter, and 77.7–102 pg L^{-1} of PCBs in dissolved phase showing the relevance of the river to the transport of PCBs into the Black Sea waters.

TABLE 6 - Comparison EROD assay to chemical analysis

Station No.	PCDD/F + PCB (TEQ WHO 1998, pg g^{-1}, dry weight)	EROD 72-h (pg g^{-1}, dry)	PAH + PCDD/F + PCB (TEQ WHO 1998, pg g^{-1}, dry)	EROD 24-h (pg g^{-1}, dry weight)
2	0.01	1.54	1.02	2.36
6	27.4	12.1	313	15.9
8	1.8	11.8	125	24.9
10	0.4	2.48	21.7	8.6
13	0.21	2.05	0.96	2.98
18	1.8	10	25.1	26.1
20	0.04	1.49	1.43	2.32
23	0.2	*	15	4.2

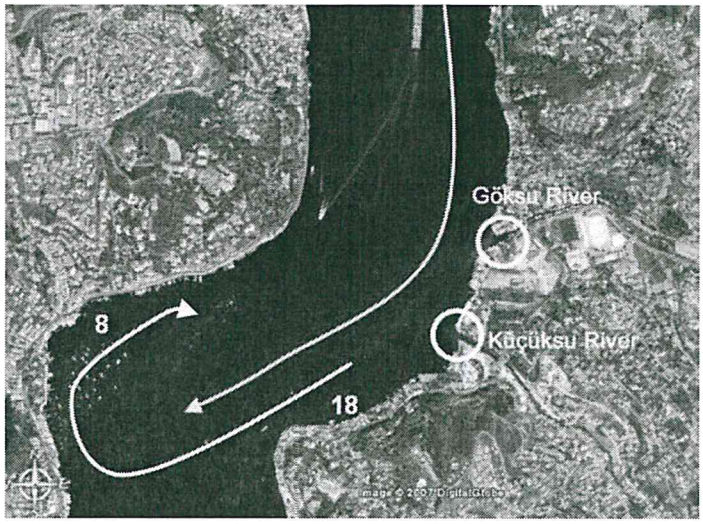

FIGURE 4 - Position of stations 8 and 18 [38].

Those values reported for the Black Sea are higher compared to the reported data from the other regions, such as PCB values for the particulate and dissolved phase were found in the Mediterranean Sea to be 28–63 pg L^{-1} and 1.7–16.6 pg L^{-1}, respectively [43]. Besides previous results of the chemical analysis, there are no EROD results from Istanbul Strait and Black Sea sediments to compare and to conclude about the presence of additional unknown Ah-receptor modulators.

The chemical analysis results indicate a high level of contamination. However, the EROD results, throughout, are not similar to the chemical analysis as depicted for the two stations 6 and 8. The measured organic chemicals and/ or unknown chemicals, such as pharmaceuticals, could cause modulating and, in this case, antagonistic effects. In most other cases, the difference between biological values and chemical analysis is substantial, and rises the question which unknown compounds could be responsible.

CONCLUSIONS

Sediments are environmental compartments, reflecting recent and past pollution. PAH, PCB, PCDD/F, and EROD-activity were detected in all samples, except EROD-values at 72-h from station 23, taken from an island in the Marmara Sea. The results in the Istanbul Strait are comparable to other areas around the world. In general, the highest levels of contaminants were found in sediments collected from urbanized sites including the bayous and river mouths within the strait, and from locations where the heavy ship traffic/ port activities occur. This study suggests that wastewater treatment plant (WWTP) discharges into the Istanbul Strait, the heavy ship traffic and the currents are important factors of pollution in the sediment, as evidenced by high concentrations of toxic organic chemicals, such as PAHs. Biological TEQ for sediment samples were measured using Micro-EROD assay complementary to the chemical analysis of PCB, PCDD/F, and PAH substances. It is expected, that the results of EROD 24-h are comparable to PAH+PCDD/F+PCB analysis, and EROD 72 h partly explains the PCDD/F+PCB analysis. The chemical analyses show lower values than Micro EROD data, or are in agreement for the majority of stations. PAH+PCDD/F+PCB results from stations 6 and 8 show interesting differences compared to EROD 24-h results. Antagonistic modulators, such as PCB 126 [2], could partly explain the differences, but most of the chemicals potentially responsible remain unknown. In general, the heavy ship traffic/port activities and the discharges of Istanbul city cause an accumulation through the Istanbul Strait water currents. Considering uncontrolled discharges from the rivers, WWTPs and heavy ship traffic, monitoring of the pollution should be helpful to examine the changes in Istanbul Strait.

ACKNOWLEDGEMENTS

This research has been supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) and International Bureau of the Federal Ministry of Education and Research, Germany through a Joint Research Project (Project no: 106Y302) entitled "Determination of polluted zones in the coastal areas of Istanbul Strait by using ecotoxicological biomonitoring techniques".

REFERENCES

[1] Li, W., Wu, W., Xu, Y., Zhang, Y., Schramm, K-W. and Kettrup, A. (1997). 7-Ethoxyresorufin-O-deethylase (EROD) as Bioindicator of 2,3,7,8-Tetrachlorinated Dibenzo-p-dioxin Toxicity to Exposed Gobiocypris Rarus. Acta Hydrobiologica Sinica 21, 216-222.

[2] Li, W., Wu, W.Z., Beck, B., Schramm, K-W. and Kettrup, A. (1999). A new enzyme immunoassay for PCDD/F TEQ screening in environmental samples: Comparison to micro-EROD assay and to chemical analysis. Chemosphere 38, 3313-3318.

[3] Fried, KW., Bazzi, R., Levy Lopez, W., Corsten, C., Schramm, K-W., Bell, D.R. and Rozman, K. (2007). Relationship between aryl hydrocarbon receptor-affinity and the induction of EROD activity by 2,3,7,8-tetrachlorinated phenothiazine and derivatives. Tox Appl Pharm 224, 147-155.

[4] Klotz, A.V., Stegeman, J.J. and Walsh, C. (1984). An alternative 7-ethoxyresorufin O-deethylase activity assay: a continuous visible spectrophotometric method for measurement of cytochrome P450 monooxygenase activity. Anal Biochem 140, 138-145.

[5] Okay, O.S., Karacık, B., Başak, S., Henkelmann, B., Bernhöft, S. and Schramm, K-W. (2009). PCB and PCDD/F in sediments and mussels of the Istanbul strait (Turkey). Chemosphere 76 (2), 159-166.

[6] Karacik, B. (2008). Determination of PAH pollution and some oceanographics characteristic through the Istanbul strait (Bosphorus), M.Sc. Thesis, Istanbul Technical University

[7] Can, S., Celik, F., Yilmaz, H., and Bak, O. A. (2007). Oil spill simulation: a case study in the strait of Istanbul. Fresen. Environ. Bull. 16 (11b), 1517-1522

[8] Balkıs, N. (2007). Variations of nutrients and chlorophyll-a in the coastal area of Baltalimani (Bosphorus-Turkey). Fresen. Environ. Bull. 16 (11b), 1429-1434.

[9] Hofmaier, A.M., Schwirzer, S.M.G., Wiebel, F.J., Schramm, K.W., Wegenke, M. and Kettrup, A. (1998). Bioassay zur Bestimmung von TCDD-Toxizitätsäquivalenten (TEQ) von Umweltproben und Reststoffen. UWSF- Z. Umweltchem. Ökotox., 11, 2-8.

[10] Schwirzer, S.M.G., Hofmaier, A.M., Kettrup, A., Nerdinger, P.E., Schramm, K-W., Thoma, H., Wegenke, M. and Wiebel, F.J. (1998). Establishment of a simple cleanup procedure and bioassay for determining 2, 3, 7, 8-Tetracholoro-p-dioxin toxicity equivalents of environmental samples. Ecotoxicol. Environ. Saf., 41, 77-82.

[11] Smith, P.K., Krohn, R.J., Hermanson, G.T., Malia, A.K. and Gartner, F.H. (1985). Provanzano, M. D. Measurement of protein using bicinchroninic acid. Anal Biochem 150, 76-85.

[12] Hanberg, A., Strahlberg, M., Georgellis, A., de Wit, C. and Ahlborg, U.G. (1991). Swedish Dioxin Survey: Envalution of the H4IIE Bioassay for Screening Environmental Samples for Dioxin-like Enzyme Induction. Pharmocol and Toxicol. 69, 442-449.

[13] Roots, O., Henkelmann, B. and Schramm, K-W. (2004). Concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in soil in the vicinity of a landfill. Chemosphere 57, 337-342.

[14] Thielen, D.R. and Olsen, G. (1988). Optimization of alumina selectivity for tetrachlorodibenzo-p-dioxins and the isomer-specific determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Anal Chem. 60, 1332-1336.

[15] Henkelmann, B., Schramm, K-W., Klimm, C. and Kettrup, A. (1996). Quality criteria for the isotope dilution method with HRGC/MS. Fresenius J Anal Chem., 354, 818-822.

[16] Martens, D., Balta-Brouma, K., Brotsack, R., Michalke, B., Schramel, P., Klimm, C., Henkelmann, B., Oxynos, K., Schramm, K-W., Diamadopoulos, E. and Kettrup, A. (1998). Chemical impact of uncontrolled solid waste combustion to the vicinity of the Kouroupitos ravine, Crete, Greece. Chemosphere., 36, 2855-2866.

[17] Karacik, B., Okay, O.S., Henkelmann, B., Bernhöft, S. and Schramm, K-W. (2009). Polycyclic aromatic hydrocarbons and effects on marine organisms in the Istanbul Strait. Environment International., 35 (3), 599-606.

[18] Wang, J., Bi, Y., Pfister, G., Henkelmann, B., Zhu, K. and Schramm, K.-W. (2009). Determination of PAH, PCB, and OCP in water from the Three Gorges Reservoir accumulated by semipermeable membrane devices (SPMD). Chemosphere., 75, 1119-1127

[19] Ramšak, A., Stopar, K., Sepčić, K., Berden-Zrimec, M., Bajt, O. and Malej, A. (2007). Reflection of hydrocarbon pollution on hepatic EROD activity in the black goby (Gobius niger). Environmental Toxicology and Pharmacology., 24, 304–310.

[20] Mouhri, A., Motelay-massei, A., Massei, N., Fournier, M. and Laignel, B. (2008). Polycyclic aromatic hydrocarbon transport processes on the scale of a flood event in the rural watershed of Le Bebec, France. Chemosphere., 73, 443–450.

[21] Vane, C.H., Harrison, I., Kim, A.W., Moss-Hayes, V., Vickers, B.P. and Horton, B.P. (2008). Status of organic pollutants in surface sediments of Barnegat Bay-Little Egg Harbor Estuary, New Jersey, USA. Marine Pollution Bulletin., 56, 1802–1814.

[22] Fang, M-Der., Chang, W-Kai-., Lee, C-Lin. and Liu, J.T. (2009). The use of polycyclic aromatic hydrocarbons as a particulate tracer in the water column of Gaoping (Kaoping) Submarine Canyon. Journal of Marine Systems., 76, 4 , 457-467.

[23] Readman, J.W., Fillmann, G., Tolosa, I., Bartocci, J., Villeneuve, J.-P., Catinni, C. and Me, L.D. (2002). Petroleum and PAH contamination of the Black Sea, Mar Pollut Bull., 44, 48–62.

[24] Tolun, L., Martens, D., Okay, O.S. and Schramm, K.-W. (2006). Polycyclic aromatic hydrocarbon contamination in coastal sediments of the Izmit Bay (Marmara Sea): case studies before and after the Izmit earthquake, Environ Int., 32, 758–765.

[25] Ünlü, S. and Alpar, B. (2006). Distribution and sources of hydrocarbons in surface sediments of Gemlik Bay (Marmara Sea, Turkey), Chemosphere., 64, 764–777.

[26] Gogou, A., Bouloubassi, I. and Stephanou, E.G. (2000). Marine organic geochemistry of the Eastern Mediterranean: 1. Aliphatic and polyaromatic hydrocarbons in Cretan Sea surficial sediments, Mar Chem., 68, 265–282.

[27] Yılmaz, K., Yılmaz, A., Yemencioğlu, S., Sur, M., Salihoğlu, İ. and Karabulut Z. (1998). Polynuclear aromatic hydrocarbons (PAHs) in the Eastern Mediterranean Sea, Mar Pollut Bull., 36, 922–925.

[28] Caricchia, A.M., Chiavarini, S., Cremisini, C., Martini F. and Morabito R. (1993). PAHs, PCBs, and DDE in the Northern Adriatic Sea, Mar Pollut Bull., 26, 581–583.

[29] Bihari, N., Fafandel, M., Hamer, B., and Kralj-Bilen, B. (2006). PAHs content, toxicity and genotoxicity of coastal marine sediments from the Rovinj area, Northern Adriatic, Croatia, Sci Total Environ., 366, 602–611.

[30] Cardellicchio, N., Buccolieri, A., Giandomenico, S., Lopez, L., Pizzulli, F. and Spada, L. (2007). Organic pollutants (PAHs, PCBs) in sediments from the Mar Piccolo in Taranto (Ionian Sea, Southern Italy), Mar Pollut Bull., 55, 451–458.

[31] Baumard, P., Budzinski, H., Garrigues, P., Dizer, H. and Hansen, P.D. (1999). Polycyclic aromatic hydrocarbons in recent sediments and mussels (Mytilus edulis) from the Western Baltic Sea: occurrence, bioavailability and seasonal variations, Mar Environ Res., 47, 17–47.

[32] Artüz, I. (1992). Marine Pollution, Istanbul Technical University, Istanbul, Turkey.

[33] The Istanbul Water and Sewage Authority (ISKI) (1999). Masterplan report, Istanbul Municipality, Istanbul.

[34] Oguz, T., Ozsoy, E., Latif, M.A., Sur, H.I. and Unluata, U. (1990). Modeling of hydraulically controlled exchange flow in the Bosphorus Strait. Journal of Physical Oceanography., 20 (7), 945-965.

[35] Ozsoy, E., Di Iorio, D., Gregg, M.C. and Backhaus, J.O. (2001). Mixing in the Bosphorus Strait and the Black Sea continental shelf: observations and a model of the dense water outflow. Journal of Marine Systems., 3 (1-3), 99-135.

[36] Coşkun, H.G. (1992). Uydu verileri ile İstanbul Boğazı ve Haliç'de su kirliliğinin makro düzeyde belirlenmesi. PhD Thesis, ITU, Istanbul

[37] Schramm, K-W., Klimm, C., Hofmaier, A. and Kettrup, A. (2001). Comparison of dioxin-like-response in vitro and chemical analysis of emissions and materials. Chemosphere., 42, 551-557.

[38] EPA (2008). yosemite.epa.gov/R10/CLEANUP.NSF/.../$FILE/Appendix%20B%20-%202007%20Data%20management.pdf. Accessed 12 December 2008.

[39] TUDAV (2008). Türk Deniz Araştırmaları Vakfı, http://www.tudav.org. Accessed 24 December 2008.

[40] Google earth (2007). http://earth.google.com. Accessed 21 November 2007.

[41] Veningerova, M., Prachar, V., Uhnak, J. and Kovacicova, J. (1996). Levels of chlorinated pesticides in water of Danube River. Fresen. Environ. Bull. 5, 361–368.

[42] Maldonado, C. and Bayona, J.M. (2002). Organochlorine compounds in the Northwestern Black Sea water: distribution and water column process. Estuar. Coast. Shelf Sci.. 54, 527–540.

[43] Dachs, J., Bayona, J.M. and Albaiges, J. (1997). Spatial distribution, vertical profiles and budget of organochlorine compounds in Western Mediterranean seawater. Mar. Chem., 57, 313–324.

Received: March 15, 2010
Accepted: April 22, 2010

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FEB/ Vol 19/ No 7/ 2010 – pages 1245 - 1253