Monitoring of dioxin-like, estrogenic and anti-androgenic activities in sediments of the Bizerta lagoon (Tunisia) by means of in vitro cell-based bioassays: contribution of low concentrations of polynuclear aromatic hydrocarbons (PAHs).


To cite this version:

HAL Id: ineris-00336985
https://hal-ineris.archives-ouvertes.fr/ineris-00336985
Submitted on 5 Nov 2008

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Monitoring of dioxin-like, estrogenic and anti-androgenic activities in sediments of the Bizerta lagoon (Tunisia) by means of in vitro cell-based bioassays: contribution of low concentrations of polynuclear aromatic hydrocarbons (PAHs)

I. Louiz \textsuperscript{a,b,c,*}, S. Kinani \textsuperscript{c,d}, M-E. Gouze \textsuperscript{c}, M. Ben-Attia \textsuperscript{b}, D. Menif \textsuperscript{a}, S. Bouchonnet \textsuperscript{d}, J.M. Porcher \textsuperscript{c}, O.K. Ben-Hassine \textsuperscript{a,*}, and S. Aït-Aïssa \textsuperscript{c,*}

\textsuperscript{a} Unité de recherche de Biologie, Écologie et Parasitologie des Organismes Aquatiques, Faculté des sciences de Tunis, 2092 El Manar, Tunisie

\textsuperscript{b} Unité d’Ecotoxicométrie & Chronobiométrie, Laboratoire de Biosurveillance de l’Environnement, Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisie

\textsuperscript{c} Unité d’Evaluation des Risques Ecotoxicologiques, INERIS, B.P. 2, F60550 Verneuil-en-Halatte, France

\textsuperscript{d} Département de Chimie des Mécanismes Réactionnels, Ecole Polytechnique, F91128 Palaiseau, France

* Corresponding authors:

selim.ait-aissa@ineris.fr (Phone +33 3 44 55 65 11; Fax. +33 3 44 55 67 67);
louizibtissem@yahoo.fr ; kalthoum.benhassine@gmail.com
Abstract

We used an array of in vitro cell-based bioassays to assess dioxin-like, estrogenic and (anti-)androgenic activities in organic extracts of sediments from the Bizerta lagoon, one of the largest Tunisian lagoons subjected to various anthropogenic and industrial pressures. The sediments were sampled both in winter and summer 2006 in 6 stations differently impacted and in one reference station located in the seawards entrance of Ghar el Mehl lagoon. Chemical analyses of the 16 priority PAHs showed that the sediments were low to moderately contaminated (2-537 ng/g dry weight). By using the estrogen- (MELN) and androgen-responsive (MDA-kb2) reporter cell lines, significant estrogenic and anti-androgenic activities were detected only in the Menzel Bourguiba (MB) site, the most contaminated site, both in winter and summer. By using 7-ethoxyresorufin-O-deethylase (EROD) induction in the fish PLHC-1 cell line after both 4 and 24 h of cell exposure, dioxin-like activities were detected in all analysed samples. Dioxin-like activities were higher after 4 h exposure, and varied according to the sites and the sampling season. While highly significant correlation was observed between bioassay- and chemical analyses-derived toxic equivalents (TEQs), PAHs accounted for only a small part (up to 4%) of the detected biological activities, suggesting that other readily metabolised EROD inducing compounds were present. This study argues for the use of short time exposure to assess biological TEQs in low contaminated samples and provides new induction equivalent factors (IEF_{4h}) for 16 PAHs in the PLHC-1 cell line. Finally, our results stress the need to further characterise the nature of organic chemical contamination as well as its long-term impacts on aquatic wildlife in the Bizerta lagoon.

Keywords: Bizerta lagoon sediments, in vitro cell bioassays, estrogenicity, anti-androgenicity, dioxin-like activity, PAH contamination, benzo[a]pyrene- and dioxin-equivalents.
1. Introduction

Bizerta lagoon, the second largest lagoon in Tunisia, is located in an economically very important area in northern Tunisia (Fig.1). This lagoon is submitted to many anthropic pressures including urbanisation, industrial activities (cement works, metallurgical industry, boatyard, tyre production factories...), as well as naval and commercial shipping harbours. Lagoon shores have also been used as open-air waste-dumping sites. The direct and indirect discharges of urban and industrial wastes and runoff lead to the chemical contamination of the lagoon by various toxic compounds such as organo-chlorinated pesticides (Cheikh et al., 2002), halogenated aromatics compounds like polychlorobiphenyls (PCBs) (Derouiche et al., 2004), polycyclic aromatic hydrocarbons (PAHs) (Trabelsi and Driss, 2005), heavy metals (Yoshida et al., 2002) and organotins (Mzoughi et al., 2005). The presence of such potentially toxic compounds in this aquatic ecosystem has led scientists to investigate biological impacts on aquatic organisms. Recently, some biological alterations have been reported, like imposex incidence in the muricid gastropod *Hexaplex trunculus* (Lahbib et al., 2007), inhibition of acetylcholinesterase activity in clams and mussels sampled (Dellali et al., 2001) or the oxidative damage to DNA in clam gills (Jebali et al., 2007). Fish are also possibly affected by lagoon pollution. Indeed, substantial vertebral deformities were recently reported in three Gobiidae species, sedentary and benthic fish sampled in different sites of the Bizerta lagoon (Louiz et al., 2007). Interestingly, those morphological alterations in adults were found to be correlated with the degree of sediment contamination by PAHs, suggesting that in situ long-term chemical exposure at these sites could be responsible for integrated biological effects related to essential physiological functions, like development or reproduction (Louiz et al., 2007). However, these data remain scarce and much information is still needed in order to characterise the toxic potency and the identity of biologically active chemicals that are present in this lagoon.

In this context, the present study was undertaken in order to provide a first evaluation of the presence of bioactive organic contaminants, namely dioxin-like and endocrine disrupting chemicals, using *in vitro* bioassays. It is now recognised that *in vitro* mechanism-based cellular bioassays serve as valuable bio-analytical tools for the detection and quantification of biologically active chemicals in environmental mixtures such as river sediment, surface waters or aqueous effluents (Eggen and Segner, 2003).
The principle of such assays relies on a common mode of action of chemicals, by which they initiate their toxicity, like their ability to bind to nuclear receptors or transcription factors and to subsequently activate or inhibit target genes. A very relevant group of contaminants are the so-called dioxin-like compounds, which include dioxins, furans, planar polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), etc. These chemicals bind to the aryl hydrocarbon receptor (AhR) and thereby activate the transcription of several genes, including those encoding for xenobiotic or hormone metabolising enzymes, such as CYP1A (Denison and Nagy, 2003). The AhR plays major roles in the mediation of developmental and reproductive toxicity of dioxin-like compounds (Hahn, 2002).

Apart from dioxin-like compounds, a number of environmental chemicals can act as endocrine disrupters (EDs) by altering the normal functioning, synthesis and/or metabolism of endogenous hormones, and thereby affect growth, development and reproduction in wildlife and humans (Kavlock et al., 1996). One major molecular mechanism of action of EDs involves their ability to bind to steroid hormone receptors, i.e. estrogen (ER) or androgen (AR) receptors, and to subsequently modulate the expression of target genes responsible for hormonal cellular response. In the aquatic environment, a wide range of chemicals (e.g. natural and synthetic hormones, phytoestrogens, pesticides, alkylphenols, bisphenol A, phthalates, and so on) have been identified as estrogenic, anti-androgenic or androgenic compounds through their direct binding to the ER or AR (Kelce et al., 1995; Sohoni and Sumpter, 1998; Sonnenschein and Soto, 1998). In addition, environmental AhR ligands can also interact with the ER signalling pathway through a cross-talk between activated AhR and ER, leading to modulation of ER-regulated gene expression (Ohtake et al., 2003; Navas and Segner, 2001). Upon field exposure, EDs have been shown to adversely affect reproduction capabilities in aquatic wildlife (Tyler et al., 1998; Jobling et al., 2002).

The aim of this study was to assess the presence of compounds able to bind to the estrogen (ER), androgen (AR) or aryl hydrocarbon receptors (AhR) in organic extracts of Bizerta lagoon sediments, by using in vitro assays. The estrogenic and androgenic activities in the extracts were assessed by using established human reporter MELN (Balaguer et al., 1999) and MDA-kb2 (Wilson et al., 2002) cell lines that stably express the luciferase reporter gene under the control of the human ER or AR, respectively. The
AhR-mediated activities were detected in the hepatoma fish PLHC-1 cell line (Ryan and Hightower, 1994), by assessing CYP1A enzymatic activity. Because dioxin-like activities were found to be predominant in these sites, chemical analyses of target PAHs were performed in parallel in order to evaluate their contribution in measured biological activities.

2. Materials and methods

2.1. Chemicals

17β-estradiol (E2), 5α-dihydrotestosterone (DHT), as well as the 16 standard PAHs (listed in Table 2) were purchased from Sigma-Aldrich (St Quentin Fallavier, France). ICI 182,780 (ICI) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were obtained from Tocris Bioscience (Ellisville, USA) and Promochem (Molsheim, France), respectively. All standards were of 98.1-99.8% purity. For bioassay experiments, stock solutions (10 mM) were prepared in either dimethylsulfoxide (DMSO) or methanol and stored at -20°C. For chemical analyses of PAHs, stock solutions of each compound were prepared at a concentration of 1 mg.mL⁻¹ in toluene and stored at 4°C. Standard solutions were mixed to provide a solution of 10 µg.mL⁻¹ of each PAH. A 1000 µg.mL⁻¹ mixture solution of the following deuterated PAHs: [2H₁₀]phenanthrene (PA-d₁₀), [2H₁₀]acenaphthene (Acp-d₁₀), [2H₁₂]perylene (P-d₁₂) and [2H₁₂]chrysene (CHR-d₁₂) (St Quentin Fallavier, France), was used as the internal standard for quantification.

2.2. Studied sites and sample collection

- Study area

The sediment sampling sites were located in Bizerta and Ghar el Melh lagoons (Fig.1). The Bizerta lagoon is about 150 km² and is linked to the Mediterranean Sea and Lake Ichkeul by straight channels. The exchange of water with the Mediterranean Sea determine the salinity of the lagoon, which varies between 32.5‰ and 38.5‰. The water temperature ranged from 13°C to 32.5°C in 2006 in winter and summer, respectively. A relatively undisturbed site (Mahmoudi, 2003) located at the seawards entrance of Ghar el Melh lagoon (Fig. 1) was chosen as a reference station.

- Sediment sampling and extraction
Surface sediments were collected in January 2006 and July 2006 at six stations in Bizerta lagoon and one station in the Ghar el Melh lagoon. These stations were chosen as representative of the different anthropic pressures present in the study area (Table 1): CA, NJ, MB, MJ and ML were considered as impacted areas, while MR and GH were considered as low impacted or reference sites. The upper 10 cm-surface layer of sediment (about 500 g) were sampled, homogenised and stored in methanol-rinsed glass bottles at -20°C until processing. Before extraction, freeze dried sediments were sieved using a 2-mm sieve. Five grams of dry sediment were then extracted by a heptane:acetone (1:1) mixture using Accelerated Solvent Extraction (ASE 200; Dionex, France). ASE extraction was performed at 90°C, 103 Bars. Sediment extracts were completely evaporated under a gentle nitrogen stream and dissolved in 1 ml of methanol. The final methanol extracts were then subjected to chemical and bioassays analyses, as described below.

2.3. Chemical analyses

All PAH analyses were carried out on a Varian “CP3800” gas chromatograph system equipped with a “CP8400” autosampler and coupled to a Saturn “2000” ion trap mass spectrometer (Varian, Les Ulis, France). The chromatographic separation was performed on a 60 m “Factor Four VF-10-MS” capillary column (internal diameter: 0.25 mm, film thickness: 0.25 µm) from Varian. All experiments were performed by automatically injecting 1.5 µL of sample in the splitless mode at a rate of 50 µL.s⁻¹. Helium (purity: 99.999%) was used as carrier gas at a constant flow of 1.0 mL.min⁻¹. The injector temperature was set to 280 °C. The split valve opened after 1.5 min, with a split ratio of 35/100. The capillary column was increased from an initial temperature of 70°C, held for 0.5 min, increased at 10°C/min up to 280°C where it was held for 41.50 min. The total duration of GC analysis was 63.00 min. The manifold, ion trap source and transfer line temperatures were set to 120, 220 and 300°C, respectively. Ions were formed under electro-ionization at 70 eV with an emission current of 20 µA. The electron multiplier voltage was set to 1550 V. Spectra were recorded using the automatic gain controller (AGC) function with a target value of 20000. The mass spectrometer was operated in the "Selected Ion Storage" (SIS) mode. Molecular ions [M]⁺ as well as [M-H]⁺ and [M(¹³C)]⁺ ions were stored and monitored for each compound. The m/z ratios of the quantification/confirmation ions for all PAHs
were chosen according to the 96/23/CE directive published in the Journal of the European Communities and notified under the number C(2002)3044. Calibration curves were plotted using 10, 50, 100, 300, 500, 700, and 1000 ng.mL\(^{-1}\) standard solutions. Quantitative data were determined by internal standard calibration. The response was linear (\(r^2\) greater than 0.995) on the whole concentration range for each of the considered PAHs. Quantification limits (LOQ, estimated for a signal-to-noise ratio of 10) ranged from 0.02 to 1.20 ng.g\(^{-1}\) according to the PAH considered (Table 2).

### 2.4. In vitro cellular bioassays

#### Estrogenic and (anti-)androgenic activities in reporter gene assays

The estrogenic and (anti-)androgenic activities of the extracts were monitored by using the MELN and MDA-kb2 cell lines, respectively. The MELN cell line was obtained from Patrick Balaguer (INSERM, Montpellier, France). These cells were obtained by stable transfection of MCF-7 cells by an ERE-\(\beta\)Glob-Luc-SVNeo plasmid and selected with 1 mg.ml\(^{-1}\) G418 (Balaguer et al., 1999). They were routinely cultured in phenol red containing Dulbecco’s Modified Eagles’s Medium (DMEM), supplemented with 5% foetal calf serum (FCS), 1% non essential amino acids and penicillin/streptomycin (50 U/ml each) in a 5% CO\(_2\) humidified atmosphere at 37°C. For experiments, MELN cells were left to incubate for two days in phenol red free DMEM supplemented with 3% dextran- charcoal coated FCS (DCC medium) before seeding in white opaque 96-wells culture plates. After another 24 h period, the cells were exposed for 16 hours in triplicates to vehicle (solvent control, 0.5% v/v), estradiol (E2; positive control), blank extraction procedure or serial dilutions of sediment organic extracts. The solvent concentration in the culture medium was always 0.5% v/v. At this concentration, it does not affect either cell viability or luciferase activity. After exposure, the medium was replaced by 50 \(\mu\)l of DCC-medium containing \(3.10^4\) M D-luciferin (Sigma) and the luminescence signal in living cells was read after 5 minutes with a microtiter plate luminometer (\(\mu\)Beta, Wallac). The MDA-kb2 cell line (ATCC, CRL-2713) was derived from MDA-MB-453 cells. They were stably transfected by a MMTV promoter-luciferase plasmid construct, which is under the control of endogenous androgen (AR) and glucocorticoid receptors (GR) (Wilson et al., 2002). These cells were routinely grown at 37°C under humidified air atmosphere in L15 Medium (Sigma) supplemented with 10% foetal calf serum (FCS), 1% (v/v) non essential amino acids and
penicillin/streptomycin (50 U/ml each). For sample analyses, cells were exposed to serial dilutions of extracts either in the absence (for agonist activity) or in the presence of 0.3 nM of DHT in the culture medium (for AR antagonist activity). Exposure to reference chemicals or sample extracts was carried out for 16 hours in 96-well white opaque plates (5 x 10^4 cells per well) in complete L15 medium, and luciferase activity was then monitored exactly as described with MELN cells. In both assays, results are expressed as percent of maximal luciferase activity induced by the positive controls, respectively E2 10 nM or DHT 1 nM in MELN or MDA-kb2.

- 7-Ethoxyresorufin-O-deethylase (EROD) activity assay in PLHC-1 cell line

The PLHC-1 cells (ATCC, CRL-2406) were routinely grown at 30°C in E-MEM culture media supplemented with 10% foetal calf serum and 1% antibiotics in a 5% CO₂ humidified atmosphere. For experiments, cells were seeded in 96-well plates at a rate of 5 x 10^4 cells per well. After 24 hours of incubation, cells were exposed to test chemicals or sample extracts for either 4 h or 24 h and then processed for EROD activity in intact cells, exactly as previously described (Laville et al., 2004). Results were expressed as percent of EROD activity induced by the positive control (TCDD 1 nM).

- Cell viability

Cellular viability after sample exposure of the different cell lines was evaluated by using the methyl-thiazol-tetrazolium (MTT) assay (Mosmann, 1983), as previously described (Laville et al., 2004).

2.5. Data analyses

In all assays, each sample was tested at various concentrations in at least two independent experiments, which always included both negative (solvent) controls and complete dose-response for reference chemical (TCDD, BaP, E2 or DHT). Experimental data were expressed as means ± standard deviation (SD). Statistical significance (p < 0.05) of treatment effects was tested by using Student t-test. Pearson’s linear regression was used for correlation analyses. The SPSS™ software version 10.1 was used for the statistical analysis.

Dose-response curves were modelled by using the Regtox 7.5 Microsoft Excel™ macro (Vindimian et al. 1983). This macro uses the Hill equation model and allows calculation of EC₅₀ (concentrations of test chemical or sediment extract that induce 50% of maximal response). Bioassay-derived TCDD- (TCDD-EQ), benzo[a]pyrene- (BaP-EQ)
or E2-equivalents (E2-EQ) were determined by dividing the EC$_{50}$ of the reference
chemical (expressed in ng/L) by that of the sample (expressed as equivalent gram of dry
sediment per litre). Instrumentally derived toxic-equivalents (TEQs) for PAHs were
determined according to the following equation: TEQ$_{\text{cal}}$ = Σ([PAH$_i$]-IEF$_i$), where,
[PAH$_i$] is the measured concentration in the sample and IEF$_i$ is the induction equivalent
factor, for a given compound ($i$). IEFs were determined as the ratio of EC$_{50}$ as mass
concentration of reference compound (TCDD or BaP) to that of compound $i$. In the
present study, because no IEF data after short term exposure to PAHs were found in the
literature for the PLHC-1 cell line, IEFs for all 16 PAHs were determined in PLHC-1
cells after both 4 and 24 h of exposure to individual PAHs (see results section).

3. Results

3.1. Responses of in vitro assays to reference ER, AR and AhR ligands

The sensitivity of the three cellular assays in our assay conditions was first evaluated by
using reference chemicals (Fig. 2). In our experiments, the EC$_{50}$ of E2 in MELN cells
and DHT in MDA-kb2 cells were 0.03±0.01 and 0.145±0.065 nM (mean ± SD of three
independent experiments), respectively (Fig 2a). In PLHC-1 cells, patterns of EROD
induction by BaP and TCDD varied according to the exposure duration (Fig 2b). BaP
was more potent after 4 h (EC$_{50}$ 4.1±3.3 nM) than after 24 h (EC$_{50}$ 380±140 nM),
whereas TCDD was almost equipotent at both duration of exposure (EC$_{50}$ 0.011±0.01 nM
at 4 h, 0.09±0.001 nM at 24 h). Maximal EROD activities induced by TCDD 1 nM were
about 30 and 160 pmol resorufin/mg protein/min after 4 and 24 h of exposure, respectively. Overall, the response induced by reference ligands, were in line with the
reported sensitivity of these cell lines, which validates their use in the present study.

3.2. Estrogenic and anti-androgenic activities in sediments extracts

The effects of sediment extracts on ER, AR and cytotoxicity assays were limited. At the
highest tested concentration (i.e. 0.5 % v/v of extract), none of the extracts exerted
significant alteration of cellular viability as assessed by the MTT assay in the three cell
lines (data not shown). Among all samples, significant estrogenic and anti-androgenic
activities were detected only in the MB site, both in summer and winter samples (Fig.
3). In MELN cells, these activities were observed at the same sample concentration for
the two seasons and corresponded to 0.22 and 0.29 ng E2-Eq/g in winter and summer,
respectively. All other samples were below the detection limit (i.e. 0.011 ng E2-Eq/g).

Co-exposure with the pure antiestrogen ICI 182,780 led to a complete inhibition of the estrogenic activity of MB extracts (result not shown), thus indicating that the detected activity involved an ER-dependent mechanism in MELN cells. Interestingly, MB sediment extracts also exerted anti-androgenic activity in MDA-kb2 cells, although these activities were weak (20 % decrease of luciferase induced by DHT 0.1 nM). In addition, no androgenic or anti-estrogenic activities were detected in any of the analysed samples (data not shown).

3.3. In vitro dioxin-like activities in sediment extracts

The induction of EROD in PLHC-1 cells by sediment extracts was assessed after both 4 and 24 hours of exposure in order to distinguish between HAP-like compounds that are rapidly biotransformed in the cells and dioxin-like chemicals that are persistent in the cells after 24 h. As shown in figure 4, all extracts were able to induce EROD activity that depended on concentration and exposure duration, and with varying potencies between the sites studied.

First, the time-course study showed that exposure duration affected both the magnitude of EROD induction and EC$_{50}$ values. Indeed, higher activities were observed after 4 hours of cell exposure (Fig. 4a,b) than after 24 hours (Fig. 4c,d). This pattern of EROD responses suggests that the chemicals responsible for activation are rapidly metabolised into the cells, which supports the hypothesis for a major role of PAH-like chemicals in the observed EROD induction. Second, sample EC$_{50}$s varied by up to two orders of magnitude between the different sites (Fig 4a, b). The extracts from MB site, which is subjected to human industrial and urban activity nearby (Table 1), were the most active samples with equal potency in winter and summer. On the contrary, the reference site GH, was about 15 and 360 times less active than MB in winter and summer, respectively. Regarding seasonal variation, it is noteworthy that, although similar site ranking was observed between summer and winter, higher activities were observed in winter in all stations, except for MB site, which was still highly potent in summer.

3.4. Concentrations of PAHs in sediment extracts

On the basis of the results provided by the in vitro PLHC-1 assay at various exposure duration, we suspected a significant contribution of PAHs to the biological responses. To test this hypothesis, we performed analyses of the 16 US EPA priority PAHs in the
organic extracts. Overall, the results given in Table 2 showed that sediments presented low to moderate PAH contamination. The concentrations of analysed PAHs ranged from 1 ng/g in the GH reference site up to 537 ng/g in MB site, identified as the most responsive site by in vitro assays. As observed with the EROD induction bioassays, some seasonal variations were also observed, the total PAH content being substantially higher in winter than in summer especially in sites CA, ML and MJ, but with the exception of the MB site which had higher PAH content in summer.

3.5. Determination and comparison of biological and chemical-derived TEQs in sediment extracts

In order to allow a quantitative comparison between chemical and biological data, we applied the "toxic equivalent" (TEQ) approach to our data set. To derive chemical analyses-based TEQ, we first determined EROD induction equivalent factors (IEF) of individual PAHs at both times of exposure in our assay conditions. To this end, we exposed PLHC-1 cells to various concentrations of individual PAHs (0.1 nM to 10 µM) for 4 h and 24 h and determined their EC₅₀. The results, as per Table 3, showed that, on the basis of EC₅₀ values, all tested PAHs were more active after 4 h than after 24 h of exposure. Such differences were also noticed when comparing IEFs relative to TCDD at both times of exposure (Table 3). For active PAHs, the EC50s were found 5-fold (Pyrene) up to 100-fold (Benz[a]Anthracene) more sensitive at 4 h as compared to 24 h. Furthermore, fluorene and acenaphtylene were found to induce EROD activity after 4 h, while they were inactive after 24 h within the range of tested concentrations, as previously described in this cell line (Fent and Bätscher, 2000). Fluoranthene, phenanthrene, benzo[ghi]perylenne, acenaphtene, and anthracene showed no significant response at both exposure durations.

The IEFs derived from these experiments were then used to derive toxic-equivalent values from chemical analyses in sediments extracts. BaP-EQs and TCDD-EQs were calculated by using IEF_{BaP 4h} and IEF_{TCDD 24h} given in Table 3, respectively. The results presented in Table 4 showed very good concordance between the ranking of sites given by biological- and instrumental-derived BEQs and TEQs. Furthermore, the regression analyses (Fig. 5) showed highly significant positive correlation between BEQchem and BEQbiol (R²=0.95, p<0.001) and, to a fewer extent, between TEQchem and TEQbiol (R²=0.90, p<0.01). However, when comparing toxic-equivalent values in
Table 4, the BEQchem and TEQchem values explained only a small part (0.6 to 3.7%) of the biological activities detected by the bioassay, suggesting that other EROD inducing compounds than the 16 analysed PAHs are present in the extracts.

4. Discussion

By means of an array of *in vitro* bioassays, this study examined for the first time the possible occurrence of ER-, AR- and AhR-mediated activities in sediments of the Bizerta lagoon. The main outcomes were a low contamination of this lagoon by estrogen-like or antiandrogen-like compounds while dioxin-like compounds were detected in all analysed samples. PAHs, but also other unidentified chemicals, contributed significantly to the dioxin-like activities detected by the bioassays.

Overall, the dioxin-like activities and chemical analyses showed large variations between the different sites, which could be related to the industrial and urban activities in the site areas. The site of Menzel Bourguiba (MB) provided the most active samples, followed to a lesser extent by the channel (CA) and Menzel Abderrahmen (ML). These sites are located in areas with intensive urban and industrial activities (Table 1) characterised by metallurgical industry and ship building (MB), solid waste landfills (MB, CA, ML), cement factories and commercial harbours (CA). Oppositely, the GH and MR sites, located in non-urbanised and non-industrial zones, were not exposed to any identified direct sources of pollution. Both sites had the lowest dioxin-like and total PAH content in our study. In addition, the site ranking given by our data correlates with previous studies that compared sediment contamination by PAHs (Trabelsi and Driss, 2005) or PCBs (Derouiche et al., 2001) in these sites among others of the Bizerta lagoon.

With regard to total PAH concentrations, Bizerta lagoon sediments can be considered as low to moderately polluted as compared to other marine ecosystems. In industrialized coastal areas, much higher PAH concentrations, *i.e.* up to several µg/g sediment, can be found, although the concentration ranges usually vary between neighbour sites depending on the presence or not of a local source of pollution. For instance, reported PAH concentrations in marine sediments ranged from 626 to 3766 ng/g in the Mersey Estuary, U.K. (Vane et al., 2007), 1 to 20,440 ng/g in French Mediterranean coasts (Baumard et al., 1998), or 7 to 640 ng/g in the Black Sea (Readman et al., 2002). In the
Bizerta lagoon, Trabelsi and Driss (2005) recorded slightly greater PAH concentrations (83-450 ng/g) in sediments sampled in 2001 than in our study. Interestingly, in their study, the MB site was found to be the most contaminated one with a total PAH content of 450 ng/g (Trabelsi et Driss, 2005), which is close to our data. Globally, the levels of concentrations of PAHs can be considered as relatively low considering the industrial activities and urbanization that have been developed in the vicinity of the Bizerta lagoon.

Seasonal variations were also observed when comparing winter and summer surveys, as shown by both *in vitro* dioxin-like activities and PAH concentrations that were lower in summer. In the same lagoon, Mzoughi et al. (2002) also reported similar seasonal differences with higher PAH concentration in winter samples than in summer. Such variations probably reflect a higher abiotic and biological degradation of organic chemicals in the hot season. In addition, several PAH degrading bacterial strains were recently isolated from Bizerta lagoon sediments (Ben Said et al., 2007), which demonstrated the high PAH biodegradation capabilities of sediment microflora in this lagoon.

In the PLHC-1 assay, higher AhR-mediated activities observed after 4 h of exposure than after 24 h (Fig.4) led us to question whether or not rapidly metabolised active compounds, like PAHs, were responsible for EROD induction. Indeed, the B[a]P-EQs and TCDD-EQs derived from the PLHC-1 bioassay were positively correlated to toxic-equivalents derived from PAH analyses (Table 4, Fig. 5). Similar significant correlation has been reported (Michallet-Ferrier et al., 2004, Vondracek et al., 2001) between bioassay and instrumentally derived TEQ in river sediments that have been highly contaminated by PAHs. Our study shows that such a relationship is also demonstrated for samples with low levels of contamination by the use of different duration of exposure (e.g. 4 and 24 h). However, comparison of the amounts of toxic-equivalents given by both methodologies (Table 4) indicated that only a small part of the B[a]P-EQs (up to 3%) was accounted for by the 16 PAHs measured in the samples.

It is however noted that some caution should be observed when estimating bioassay-derived TEQ values in complex mixtures because of non-parallel dose-response curves or variation in maximal levels of EROD induction, a common feature with environmental samples (Villeneuve et al., 2000). In our study, varying maximal EROD
induction levels were observed between samples (Fig. 4), and the TEQ values were derived from median activities (defined as EC$_{50}$) of the samples, as given by non linear regression of the data. This calculation mode was chosen in order to process all sample data in the same way and to allow inter-site comparison. In addition, in order to estimate a possible bias due to uncertainty in calculation, we determined TEQs on the basis of EC$_{20}$ ratios and found that EC$_{20}$-based TEQs were lower, but only slightly (by less than two fold), than EC$_{50}$-based TEQs (data not shown). Hence, although bioassay-derived TEQs may be considered as semi-quantitative data, we assume that uncertainty in their determination does not explain the differences by up to two orders of magnitude when compared to the TEQs derived from the chemical analyses in the present study.

Therefore, if assuming an additive model, the instrumentally derived TEQ values based only on the 16 priority PAHs clearly underestimated the real contamination of the samples by AhR activating compounds. The attempts to attribute the EROD inducing potency of organic sediment extracts to only 16 identified priority PAHs has often failed (Khim et al., 1999; Fent and Bätscher, 2000; Hollert et al., 2002; Brack et al., 2003; Qiao et al., 2006). The dioxin-like responses observed in our study were thus likely to have been caused by other ubiquitous biogenic and anthropogenic PAH-like AhR ligands that were not taken into account by the chemical analyses. For instance, various substituted PAHs such as oxidized and sulphured PAHs have been identified as AhR active chemicals in aquatic sediments by using bioassay-directed fractionation and toxic identification (TIE) approaches (Brack and Schirmer, 2003). It cannot be excluded that such compounds may have contributed to the AhR activities detected in our samples. However, to our knowledge, the presence or not of substituted PAHs in Bizerta lagoon sediments is not known.

As already pointed out by other authors (Jones et al., 2000, Vondracek et al., 2001, Machala et al., 2001), this study further argues for the use of different times of exposure in order to characterise the sample contamination by non-persistent AhR ligands and stresses the need to assess IEFs at the observed duration of exposure. The screening of individual PAHs confirmed that, on a concentration basis, a short exposure time led to a significantly higher AhR-mediated activity (e.g. by up to two orders of magnitude for BaA or BbF), thereby enhancing the sensitivity of the bioassay for the detection of active PAHs at low concentrations. The decrease of AhR-mediated response with
increased time of exposure to PAHs has been described in other AhR expressing cell lines from other species like HepG2 (Jones et al., 2000), H4IIE DR-CALUX (Machala et al., 2001) or RTL-W1 cells (Bols et al., 1999). In PLHC-1 cells, the potency of individual PAHs to induce EROD activity after 24 or 72 h has been previously described (Fent and Bätscher, 2000; Villeneuve et al., 2002) and our results using a 24 h exposure were in line with those previous studies (Fent and Bätscher, 2000). However, to our knowledge, such systematic screening of the EROD induction potencies of the 16 priority PAHs after short-term exposure is reported here for the first time in these cells. Since EROD potencies of individual PAHs were differently affected by cell metabolism, the use of IEFs determined at a specific time (e.g. IEF₄₄h) should be recommended for TEQ calculation of samples contaminated by weakly persistent AhR agonists.

Lagoon sediment extracts were also screened for their ability to interfere with estrogen and androgen receptor–mediated gene expression. The activities were limited since, among the different sites, a significant estrogenic activity (0.22-0.29 ng E2-EQ/g dw) was detected only at the MB station in the two surveys. Nonetheless, these activities were of the same order of magnitude as those reported by the use of the ER-CALUX assay in contaminated sediment from Zierikzee harbour (0.46 ng E2-EQ/g dw) (Houtman et al, 2006) and fromm Aa station (0.22 ng E2-EQ/g dw) in Dutch rivers (Houtman et al., 2007). Interestingly, the MB site was the one most contaminated by PAHs and it is not unlikely that those compounds, among others, may have contributed to the observed estrogenticity. Indeed, hydroxylated PAH metabolites produced by phase I biotransformation, can bind to the ER and induce its transactivation in MCF-7 cells (Charles et al., 2000, Fertuck et al., 2001). They may also indirectly activate the ER-responsive genes through a positive cross-talk between ER and liganded AhR (Ohtake et al., 2003). In MELN cells, we have recently shown that many of the PAHs studied here are able to induce luciferase activity and are thus susceptible to be detected in environmental mixtures using this cell line (Aït-Aïssa et al., unpublished). However, both activities are not necessarily due to the same activating chemicals, as demonstrated by Pillon et al (2005) who used an ER-affinity column that allowed the separation of ER and AhR activities from the same sediment samples. Thus, other anthropogenic chemicals that act as xeno-estrogens are likely to be present, like organo-chlorinated
pesticides and break down products (DDD, DDE) or PCBs and their hydroxylated metabolites, which have been shown to be present in sediments at the Menzel Bourguiba site (Derouiche et al., 2004; Cheikh et al., 2002). Besides xenoestrogenic compounds, natural estrogenic compounds are an important source of estrogenic activity in the aquatic environment (Sumpter al, 2005; Peck et al, 2004). The MB site receives wastes from large urban and industrial settlements. Thus, the estrogenic activities were to be expected due to both natural and synthetic hormones released from municipal wastes.

Interestingly, anti-androgenic activity was also detected in MB extracts, at similar sample concentrations as those necessary to induce estrogenic activity. Because several environmental xeno-estrogens are known to possess anti-androgenic activity as well (Sohoni and Sumpter, 1998), it can be expected that active endocrine disrupters, other than typical AhR ligands detected in the PLHC-1 cells, are present at this station. However, further studies using bioassay-directed chemical analyses will be necessary to identify the nature of the bio active chemicals in the Bizerta lagoon.

In summary, the occurrence of dioxin-like, estrogenic and anti-androgenic activities by using *in vitro* assays is thus reported for the first time in Bizerta lagoon sediments. A first attempt to identify the nature of active chemicals showed that the 16 priority PAHs significantly contributed to the measured dioxin-like activities, although a large part of the EROD-inducing potencies were due to non identified PAHs or other metabolisable compounds, possibly toxicologically important. The bioanalytic approach proved its strength in the assessment of low concentrations of PAH incomplex environmental mixtures. Furthermore, this work suggests the relevance of the implementation of different exposure duration in order to differentiate between AhR-mediated activities exerted by readily metabolised and persistent dioxin-like compounds. Finally, additional studies using biochemical biomarkers (EROD, oxidative stress) in Gobiidea fish sampled in these sites are underway in order to evaluate the environmental chemical exposure of and possible impacts on benthic aquatic vertebrates in Bizerta lagoon.

**Acknowledgements**
We wish to thank Olivier Diago (INERIS) for ASE extraction of the sediments, Matthew Loder for editorial assistance, so as an anonymous reviewer who helped to improve the manuscript. This work was supported by a doctoral fellowship from the University of Tunis El Manar (to I.L.) and by the French Ministry of Ecology and Sustainable Development (Prog189) (to S.A.A and J.-M.P). S.K. was supported by a doctoral fellowship from INERIS and ANRT.

5. References


**Table 1:** GPS location and general characteristics of the studied sites

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GPS coordinates</td>
<td>N 37 15 22</td>
<td>N 37 14 22</td>
<td>N 37 09 26</td>
<td>N 37 09 53</td>
<td>N 37 13 08</td>
<td>N 37 13 34</td>
<td>N 37 09 14</td>
</tr>
<tr>
<td></td>
<td>E 9 52 05</td>
<td>E 9 48 43</td>
<td>E 9 48 40</td>
<td>E 9 54 18</td>
<td>E 9 55 57</td>
<td>E 9 51 40</td>
<td>E 10 13 10</td>
</tr>
<tr>
<td>Pressures</td>
<td>Commercial port, ceramic industries, solid waste landfill</td>
<td>-</td>
<td>Urban, metallurgy activities, ship building, solid waste landfill</td>
<td>Agricultural run-off</td>
<td>Urban, industrial, aquaculture</td>
<td>Urban, solid waste landfill</td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 2: PAHs content (ng/g dry sediment) measured in organic extracts of sediments from the 7 studied sites sampled in summer and winter 2006.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphtalene</td>
<td>Nap</td>
<td>0.04</td>
<td>0.12</td>
<td>0.14</td>
<td>0.14</td>
<td>0.13</td>
<td>0.12</td>
<td>0.11</td>
<td>0.07</td>
<td>0.13</td>
<td>0.06</td>
<td>0.09</td>
<td>0.06</td>
<td>0.12</td>
<td>0.08</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>Acpy</td>
<td>0.02</td>
<td>0.90</td>
<td>2.58</td>
<td>0.47</td>
<td>0.93</td>
<td>0.77</td>
<td>2.27</td>
<td>1.12</td>
<td>2.99</td>
<td>1.02</td>
<td>0.91</td>
<td>1.38</td>
<td>1.18</td>
<td>0.78</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>Acp</td>
<td>0.42</td>
<td>0.76</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Fluorene</td>
<td>Flu</td>
<td>0.04</td>
<td>0.67</td>
<td>0.70</td>
<td>0.55</td>
<td>0.36</td>
<td>0.18</td>
<td>0.77</td>
<td>nd</td>
<td>0.46</td>
<td>nd</td>
<td>nd</td>
<td>0.24</td>
<td>0.20</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>Phe</td>
<td>0.04</td>
<td>22.21</td>
<td>1.13</td>
<td>8.45</td>
<td>5.48</td>
<td>nd</td>
<td>0.81</td>
<td>nd</td>
<td>44.05</td>
<td>0.14</td>
<td>0.44</td>
<td>nd</td>
<td>0.50</td>
<td>0.39</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Anthracene</td>
<td>Ant</td>
<td>0.10</td>
<td>5.03</td>
<td>0.13</td>
<td>3.37</td>
<td>1.40</td>
<td>0.34</td>
<td>0.20</td>
<td>nd</td>
<td>12.83</td>
<td>0.23</td>
<td>0.21</td>
<td>nd</td>
<td>0.10</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>Flt</td>
<td>0.06</td>
<td>49.04</td>
<td>1.71</td>
<td>10.72</td>
<td>11.10</td>
<td>1.04</td>
<td>1.26</td>
<td>0.82</td>
<td>170.33</td>
<td>0.99</td>
<td>5.84</td>
<td>1.55</td>
<td>0.36</td>
<td>0.53</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>Pyr</td>
<td>0.14</td>
<td>35.54</td>
<td>1.15</td>
<td>8.43</td>
<td>8.74</td>
<td>0.92</td>
<td>0.91</td>
<td>0.63</td>
<td>133.51</td>
<td>0.93</td>
<td>4.70</td>
<td>1.39</td>
<td>0.30</td>
<td>0.43</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>B[a]A</td>
<td>0.06</td>
<td>14.48</td>
<td>0.44</td>
<td>nd</td>
<td>1.68</td>
<td>0.43</td>
<td>nd</td>
<td>0.09</td>
<td>15.21</td>
<td>nd</td>
<td>0.56</td>
<td>0.23</td>
<td>0.11</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Chrysene</td>
<td>Chr</td>
<td>0.06</td>
<td>7.32</td>
<td>0.52</td>
<td>nd</td>
<td>1.06</td>
<td>0.35</td>
<td>nd</td>
<td>0.08</td>
<td>11.23</td>
<td>nd</td>
<td>0.42</td>
<td>nd</td>
<td>0.26</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>B[b]F</td>
<td>0.50</td>
<td>19.10</td>
<td>0.79</td>
<td>3.27</td>
<td>3.05</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>34.38</td>
<td>nd</td>
<td>2.40</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>B[k]F</td>
<td>0.50</td>
<td>8.10</td>
<td>0.77</td>
<td>2.06</td>
<td>3.13</td>
<td>0.74</td>
<td>nd</td>
<td>nd</td>
<td>8.72</td>
<td>nd</td>
<td>1.51</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>B[a]P</td>
<td>0.50</td>
<td>16.59</td>
<td>0.79</td>
<td>2.65</td>
<td>4.82</td>
<td>nd</td>
<td>nd</td>
<td>1.58</td>
<td>12.91</td>
<td>0.67</td>
<td>3.13</td>
<td>0.53</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>Ind</td>
<td>1.20</td>
<td>15.03</td>
<td>nd</td>
<td>4.68</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>54.86</td>
<td>nd</td>
<td>2.60</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Dibenz[a,h]anthracene</td>
<td>DBA</td>
<td>1.20</td>
<td>1.50</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Benzo[ghi]pyrene</td>
<td>B[ghi]P</td>
<td>1.20</td>
<td>12.66</td>
<td>nd</td>
<td>3.46</td>
<td>3.76</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>35.92</td>
<td>nd</td>
<td>2.13</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
</tbody>
</table>

ΣPAHs (ng/g dry wt.)

| Winter 2006 | 209.1 | 10.9  | 48.3  | 45.6  | 4.9   | 6.3   | 4.4   |
| Summer 2006  | 537.5 | 3.8   | 25.0  | 5.4   | 3.1   | 2.5   | 1.5   |

*a: LOQ: Limit of quantification determined as described in Materials and Methods; n.d.: below quantification limit."
Table 3: Maximal EROD activities, EC50 values and induction equivalency factors (IEF) relative to B[a]P (IEF_{BaP}) and TCDD (IEF_{TCDD}) in PLHC-1 cells exposed for 4 and 24 h to individual PAHs^a.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>EC50 (M)</th>
<th>Maximal EROD (pmol/min/mg)</th>
<th>IEF_{BaP,4h}</th>
<th>IEF_{TCDD,4h}</th>
<th>IEF_{TCDD,24h}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
<td>24 h</td>
<td></td>
<td>ME</td>
<td>IEF</td>
</tr>
<tr>
<td>TCDD</td>
<td>1.07E-10</td>
<td>9.87E-11</td>
<td>29</td>
<td>156</td>
<td>30.19</td>
</tr>
<tr>
<td>DBA</td>
<td>1.02E-09</td>
<td>1.87E-08</td>
<td>32</td>
<td>192</td>
<td>3.66</td>
</tr>
<tr>
<td>B[k]F</td>
<td>1.40E-09</td>
<td>2.98E-08</td>
<td>29</td>
<td>143</td>
<td>2.94</td>
</tr>
<tr>
<td>B[a]P</td>
<td>4.12E-09</td>
<td>2.45E-07</td>
<td>19</td>
<td>51</td>
<td>1</td>
</tr>
<tr>
<td>Ind</td>
<td>4.47E-09</td>
<td>6.99E-08</td>
<td>35</td>
<td>68</td>
<td>8.43E-01</td>
</tr>
<tr>
<td>B[b]F</td>
<td>5.94E-09</td>
<td>2.72E-07</td>
<td>29</td>
<td>31</td>
<td>6.94E-01</td>
</tr>
<tr>
<td>Chr</td>
<td>1.56E-08</td>
<td>3.71E-07</td>
<td>40</td>
<td>177</td>
<td>2.92E-01</td>
</tr>
<tr>
<td>B[a]A</td>
<td>1.77E-08</td>
<td>1.42E-06</td>
<td>39</td>
<td>55</td>
<td>2.58E-01</td>
</tr>
<tr>
<td>Flu</td>
<td>4.76E-07</td>
<td>n.i.</td>
<td>16</td>
<td>n.i.</td>
<td>1.44E-02</td>
</tr>
<tr>
<td>Acpy</td>
<td>1.23E-06</td>
<td>n.i.</td>
<td>12</td>
<td>n.i.</td>
<td>5.56E-03</td>
</tr>
<tr>
<td>Pyr</td>
<td>1.44E-06</td>
<td>5.43E-06</td>
<td>11</td>
<td>10</td>
<td>3.58E-03</td>
</tr>
<tr>
<td>Flt</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Phe</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>B[ghi]P</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Acp</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Ant</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
<tr>
<td>Nap</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
<td>n.i.</td>
</tr>
</tbody>
</table>

^a: n.i.: no EROD induction detected within the 0.1 nM-10 µM concentration range
Table 4: B[a]P- and TCDD-equivalent values derived from chemical analyses (BEQchem, TEQchem) and *in vitro* PLHC-1 bioassay (BEQbiol, TEQbiol) in organic extracts of Bizerta and Ghar el Mehl lagoon sediments\(^a\).

<table>
<thead>
<tr>
<th>Samples</th>
<th>BEQs, 4h exposure</th>
<th>TEQs, 24h exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bioassay-derived</td>
<td>PAHs analyses-derived</td>
</tr>
<tr>
<td></td>
<td>BEQs (ng/g)</td>
<td>BEQs (ng/g)</td>
</tr>
<tr>
<td><strong>Winter 2006</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>6475</td>
<td>77.8</td>
</tr>
<tr>
<td>ML</td>
<td>1566</td>
<td>17.9</td>
</tr>
<tr>
<td>CA</td>
<td>1887</td>
<td>17.5</td>
</tr>
<tr>
<td>MJ</td>
<td>859</td>
<td>6.5</td>
</tr>
<tr>
<td>NJ</td>
<td>253</td>
<td>2.9</td>
</tr>
<tr>
<td>MR</td>
<td>17</td>
<td>0.1</td>
</tr>
<tr>
<td>GH</td>
<td>183</td>
<td>3.1</td>
</tr>
<tr>
<td><strong>Summer 2006</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td>5938</td>
<td>112.7</td>
</tr>
<tr>
<td>ML</td>
<td>751</td>
<td>11.7</td>
</tr>
<tr>
<td>CA</td>
<td>133</td>
<td>2.1</td>
</tr>
<tr>
<td>MJ</td>
<td>81</td>
<td>1.5</td>
</tr>
<tr>
<td>NJ</td>
<td>89</td>
<td>1.1</td>
</tr>
<tr>
<td>MR</td>
<td>17</td>
<td>0.6</td>
</tr>
<tr>
<td>GH</td>
<td>n.c.</td>
<td>0.1</td>
</tr>
</tbody>
</table>

\(a\): BEQ = B[a]P-equivalents; TEQ = TCDD-equivalents; n.c.: not calculated, slight EROD induction was detected but the low magnitude of the response did not allow EC\(_{50}\) determination; n.d.: not detected.
Figure captions

Figure 1: Map of study area and location of sampling sites in Bizerta and Ghar el Melh lagoons.

Figure 2: (a) Dose-response for luciferase activation by E2 in the MELN cell line and by DHT in the MDA-kb2 cell line; (b) dose-response for EROD induction by TCDD and BaP after 4 and 24 h in the PLHC-1 cell line. Values are means of triplicates ± S.D.

Figure 3: Estrogenic and anti-androgenic activities detected in MB site sampled in (a) summer and (b) winter 2006. Results are expressed as percentage of maximal luciferase activity induced by E2 10 nM or DHT 0.3 nM in MELN or MDA-kb2 cells, respectively. Values are means of triplicates ± S.D.

Figure 4: EROD induction in PLHC-1 cells exposed for either (A-B) 4 h or (C-D) 24 h to serial dilution of sediment extracts sampled (A-C) in summer and (B-D) in winter 2006. Results are expressed as percentage of maximal EROD activity induced by TCDD 1 nM. Values are means of triplicates ± S.D.

Figure 5: Correlation between chemical analyses- and bioassays-derived (a) BEQs and (b) TEQs determined in sediments extracts after 4 h and 24 h of exposure, respectively.
Figure 1- Map of study area and localisation of sampling sites in Bizerta and Ghar el Melh lagoons.
Figure 2: (a) dose-response for luciferase activation by E2 in the MELN cell line and by DHT in the MDA-kb2 cell line; (b) dose-response for EROD induction by TCDD and BaP after 4 and 24 h in the PLHC-1 cell line.
Figure 3: Estrogenic and anti-androgenic activities detected in MB site sampled in (a) summer and (b) winter 2006. Results are expressed as percentage of maximal luciferase activity induced by E2 10 nM or DHT 0.3 nM in MELN (ER responsive cells) or MDA-kb2 cells (AR responsive cells), respectively.
Figure 4: EROD induction in PLHC-1 cells exposed for either (A-B) 4 h or (C-D) 24 h to serial dilution of sediment extracts sampled (A-C) in summer and (B-D) in winter 2006. Results are expressed as percentage of maximal EROD activity induced by TCDD 1 nM. Error bars (sometimes obscured by markers) indicate the standard deviation of triplicate measurements.
Figure 5: Correlation between chemical analyses- and bioassays-derived (a) BEQs and (b) TEQs determined in sediments extracts after 4 h and 24 h of exposure, respectively.