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Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon

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Abstract

We report a survey on the occurrence and distribution of natural (17β-estradiol, E2; estrone, E1) and synthetic (nonylphenol, NP; nonylphenol monoethoxylate carboxylate, NP1EC; bisphenol-A, BPA; benzophenone, BP; mestranol, MES; 17α-ethinylestradiol, EE2; diethylstilbestrol, DES) endocrine disrupting compounds (EDCs) in water, sediment and biota (Mediterranean mussel, *Mytilus galloprovincialis*) in the Venice lagoon, a highly urbanized coastal water ecosystem that receives both industrial and municipal wastewater effluents. The survey was preceded by the development of tailor made extraction and clean-up procedures for the simultaneous HPLC–ESI-MS determination of all examined EDCs in sediment and biota samples. Satisfactory extraction performances and method detection limits (MDLs) were obtained for almost all EDCs. Most of the selected compounds were found in water and sediment (concentration range: 2.8–211 ng/L, and 3.1–289 μg/kg, d.w., respectively), while only 17α-ethinylestradiol and nonylphenol were recorded in biota samples (conc. range: 7.2–240 ng/g, d.w.). 17β-estradiol and ethinylestradiol contributed mostly to the water estradiol equivalent concentration (EEQ) (1.1–191 ng/L, average: 25 ng/L), while synthetic EDCs (17α-ethinylestradiol, diethylstilbestrol) were mainly responsible of the sediment EEQ (1.1–191 μg/kg, average: 71 μg/kg, d. w.). Whenever diethylstilbestrol was not recorded in the sediment, water EEQs were similar to sediment EEQs. A remarkable increase of nonylphenol was observed in sediments over the last decade.

Keywords: Endocrine disrupting compounds; Lagoon waters; Lagoon sediment; Mediterranean mussel; Estradiol equivalents

1. Introduction

It is well-established that aquatic wildlife in marine and freshwater of the European Union is exposed to natural and synthetic endocrine disrupting chemicals (EDCs) which are able to interfere with the hormonal system, thus possibly causing adverse effects on the intact physiology of organisms (Desbrow et al., 1998; Spengler et al., 2001; Schlumpf et al., 2004). Both field and laboratory studies provided abundant evidence that exposure to these chemicals can lead to abnormal modulation or disruption of development and reproduction in aquatic wildlife, especially fish (Routledge and Sumpter, 1996; Harries et al., 1997; Jobling et al., 1998;Van den Belt et al., 2003). Abnormally elevated levels of plasma vitellogenin, a biomarker response for estrogens exposure, as well as a decrease in gonads development, reduced fecundity and/or fertility, are the most commonly observed effects. Literature reports show that concentrations of most effective estrogens as low as 0.1 ng/L in water samples can produce an increase of plasma vitellogenin in freshwater fish and that estrogenic compounds can act additively to cause intersex or other dysfunctions in aquatic wildlife (Seki et al., 2003; Gunderson et al., 2004; Brian et al., 2005). Very recently, estrogenic mixture has been moreover demonstrated to affect reproductive performance of fish even at individual non-effect concentration levels (Brian et al., 2007).

The EDCs most commonly found to be of concern for the aquatic wildlife are those originated by the discharge of either treated or untreated municipal and industrial wastewaters. The identified most potent EDCs contained in these effluents are the natural and synthetic steroid estrogens, such as 17β-estradiol (E2), estrone (E1), and 17α-ethinylestradiol (EE2), although less potent

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non-steroidal chemicals such as alkylphenols and bisphenol-A (BPA) are widely encountered at significant concentration levels.

Chemical analysis is essential for determining the identity and concentration of individual EDCs in the environment. As additive effects of the estrogenic activity of EDCs mixtures have been proved, the total estrogenic potential of environmental samples can be quantitatively evaluated in terms of EEQ (estradiol equivalent concentration), provided that individual concentrations of most active compounds are known (Körner et al., 1999; Brian et al., 2005). The EEQ is the sum of the concentrations of individual EDCs after normalization on E2 by means of estradiol equivalency factors (EEFs). The EEF is the quotient of $EC50_{E2}/EC50_{compound}$ and is conventionally set to 1 for E2. These factors cover a very wide range of values, from 2.7 for diethylstilbestrol (DES) to 1.1×10^{-7} for benzophenone (BP) (Körner et al., 1999; Gutendorf and Westendorf, 2001; Folmar et al., 2002).

A comprehensive evaluation of the potential impact of EDCs towards ecosystems should be based on reliable quantification of EDCs residual concentrations in water, sediment, as well as in organisms along the food-chain. The potential hazard posed by EDCs with relatively low polarity (e.g. EE2) was shown to depend on the removal from water through sorption and bioaccumulation (Lai et al., 2000; Bowman et al., 2002). Both in vitro and in vivo studies demonstrated that sediments can exhibit high estrogenic activity towards benthic organisms (Legler et al., 2002; Duft et al., 2003).

While many works on freshwater and, more recently, river sediments (Bolz et al., 2001; Peck et al., 2004) were published, data sets on the occurrence and behaviour of EDCs in estuarine waters and sediments are still limited (Ferguson et al., 2001; Stuart et al., 2005; Houtman et al., 2006). In addition, the effects of transition environments on EDCs are still not fully understood (Bowman et al., 2002). A detailed review of available data has been recently reported (Langston et al., 2005).

The Venice lagoon (Italy) appears quite suitable for investigating the occurrence and effects of EDCs: it is a shallow coastal lagoon ecosystem connected to the sea by three inlets (Fig. 1). This ecosystem undergoes heavy anthropogenic pressure which increased greatly over the last century, following urban, industrial and agricultural development. The presence and distribution of natural and synthetic EDCs in the lagoon waters have been recently investigated for the first time (Pojana et al., 2004a,b).

In this study, we extended the previous investigation to sediment and biota with the aim of comparing the estrogenic potential of sediments and waters, and to obtain water/sediment partition coefficients and bioaccumulation factors for the selected EDCs. The latter included natural and synthetic steroids shown to be major estrogenic components of municipal wastewaters, as

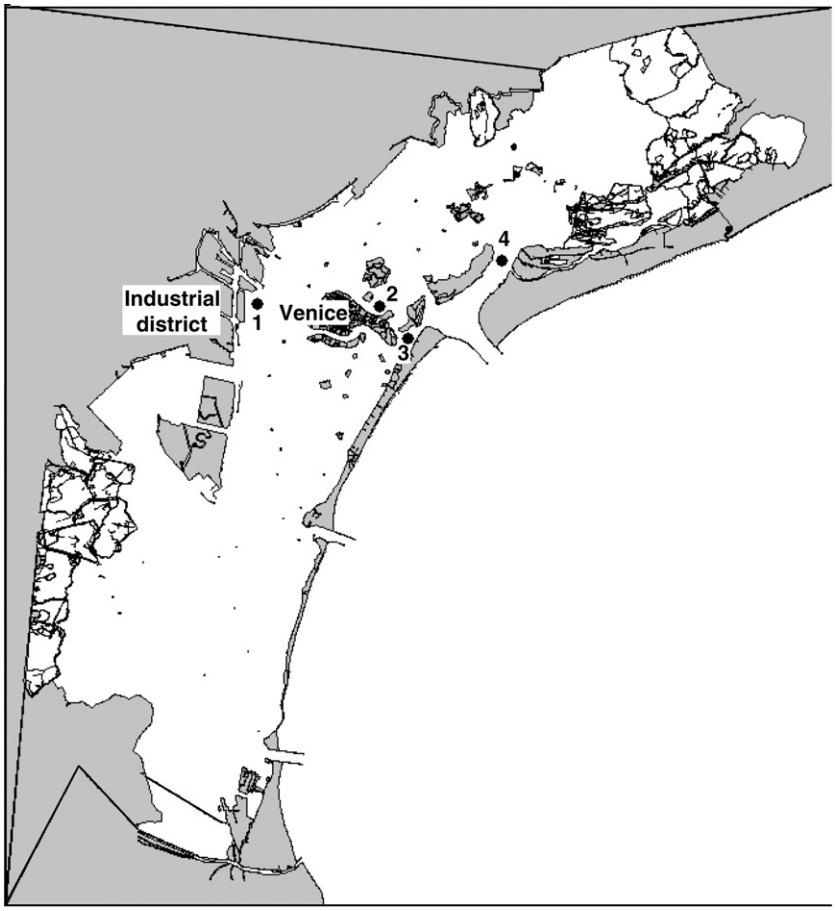

Fig. 1. Map of the Venice lagoon with location of the four sampling stations: 1. Isola della Tresse; 2. S. Nicolò; 3. Celestia; 4. S.Erasmo.

well as non-steroidal estrogens frequently detected in wastewaters affected by industrial inputs.

2. Materials and methods

2.1. Chemicals

Analytical standards of 17β-estradiol (E2), estrone (E1), estriol (E3), 17α-ethinylestradiol (EE2), mestranol (MES), diethylstilbestrol (DES), bisphenol-A (BPA), nonylphenol (NP), benzophenone (BP) (> 98% pure) were obtained from Fluka (Büchs, Switzerland). Nonylphenol monoethoxylate carboxylate (NP1EC, purity approx. 90%) was purchased from CIBA (Basel, Switzerland) and further purified by semi-preparative reversed-phase high performance liquid chromatography (HPLC) on a C-18 column. Isotope-labeled EDCs used as internal standards (tetradeuterated normal-4-nonylphenol, n-NP-d4, hexadeca-deuterated bisphenol-A, BPA-d16, tetradeuterated 17α-ethinylestradiol, EE2-d4 and trideuterated 17β-estradiol, E2-d3) were obtained from Chemical Research 2000 (Rome, Italy). Ammonium acetate ($AcNH_4$), HCl and NH_3 solutions (32%, v./v. in water, and 37%, v./v. in methanol, respectively), all > 99% pure, were from Fluka. All employed organic solvents were HPLC ultra-gradient grade from Romil (Dublin, Ireland). Water for chromatographic purposes was purified by a Milli-Q system (Millipore, Billerica, MS, USA). Standard stock solutions were prepared for all compounds, but MES and E1, at 1 μg/μL by dissolving solid standards in methanol. MES and E1 were dissolved in methanol at 0.1 μg/μL. All working solutions (100, 10, 1 ng/μL) were weekly prepared by diluting stock solutions in 2 mL Teflon capped glass vials from Agilent (Avondale, PA, USA).

2.2. Sampling

Samples of water, sediment and organisms (Mediterranean mussel, *Mytilus galloprovincialis*) were collected in 4 stations located in the central Venice lagoon. The sampling stations were selected according to known major potential sources of EDCs (Fig. 1): Station 1, Isola delle Tresse, is affected by the treated industrial effluents from the nearby industrial district of Porto Marghera and by the final effluent of a municipal mechanical–biological sewage treatment plant (approx. 310,000 equivalent inhabitants); Station 2, S. Nicolò, and Station 3, Celestia, receive municipal untreated sewage from the Lido island and the historical centre of Venice, respectively; Station 4, S. Erasmo, located near the sea inlet, was assumed to be little affected by direct discharges and thus chosen as "environmental background" site of the EDC contamination in the Venice lagoon. Grab water samples were collected during ten subsequent sampling sessions from October 2001 to October 2002 as previously described (Pojana et al., 2004b), while sediments (0–20 cm layer) and mussels were sampled on two separate occasions (October 2001, July 2002). Sediment samples (approx. 2 kg each one) were collected at the selected sites with a core sampler (4 sub-samples per site). They were then added with 100 ppm of $HgCl_2$, freeze-dried, homogenized in a glass mortar and stored in a freezer at −30 °C before analysis. Collected mussels (30–50 individuals per site, 4.2–5.6 cm long) were left for 24 h in an aerated tank with filtered sea water to allow their stabilization. Then they were sacrificed, and their soft tissues were collected, added with 100 ppm of $HgCl_2$, freeze-dried, homogenized in a glass mortar and then stored in a freezer at −30 °C before analysis. Typical physical–chemical parameters, such as dissolved O_2, pH, temperature and salinity for waters, and Eh values for sediments, were also recorded, and are reported in the R&D section. The organic carbon content of composite sediment samples was determined with a NA1500 NCS elemental analyzer by Fisons (Valencia, CA, USA).

The reported theoretical K_{ow}s and BCFs for the examined EDCs were calculated by using the EPIWIN® (US-EPA) software (http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm).

2.3. Extraction, chromatographic separation and detection

The determination of selected EDCs in water samples was performed as previously described (Pojana et al., 2004a). Briefly, 1000 mL water aliquots were extracted by SPE on a C-18 column, and the final extracts were analyzed by High Performance Liquid Chromatography coupled with a Mass Spectrometry detector via an ElectroSpray Interface (HPLC–ESI-MS). Tailored extraction and purification procedures were developed for the determination of the selected EDCs from sediments and biota samples, and hereafter described, while the chromatographic separation, detection and quantification conditions applied to sediment and biota extracts were the same as previously reported for water extracts (Pojana et al., 2004a). Twenty grams of freeze-dried sediment sample were sonicated, after addition of recovery standards (100 ng of n-NP-d4, BPA-d16, EE2-d4 and E2-d3 dissolved in methanol, 10 ng/μL each) to the dried samples, at 20 °C in a Branson (Danbury, CT, USA) sonication bath with a methanol:acetone mixture (80:20, v:v) for 2 h (50 mL, two times). The two 50 mL aliquots from each extraction step were then combined, filtered through a GFF filter (47 mm, 0.7 μm pore size) from Whatman (Maidstone, UK) and concentrated under gentle nitrogen flow down to 500 μL in an automated evaporator Zymark Turbovap II (Darlington, MA, USA) set at 20 °C. The concentrated extracts were then diluted to 1000 μL with Milli-Q water. Final extracts were then stored into 2 mL Teflon capped screw cap glass vials from Agilent at 2 °C before their analysis (100 μL injected volume) as previously reported (Pojana et al., 2004a). Five grams of freeze-dried mussel soft tissue, after addition of deuterated recovery standards (100 ng of n-NP-d4, BPA-d16, EE2-d4 and E2-d3, 10 ng/μL each in methanol), were sonicated with a *n*-hexane/acetone mixture (70:30, v:v) for 2 h (20 mL, two times). The two 20 mL aliquots from each extraction step were then combined, filtered through a glass fiber filter (47 mm, 0.7 μm pore size) from Whatman (Maidstone, UK) and concentrated under gentle nitrogen flow down to 500 μL in the automated evaporator set at 20 °C. The concentrated extracts from mussels samples were further purified on a clean-up glass column (25 cm long, 1 cm i.d.) filled with 2 g of Florisil (60–100 mesh, activated at 150 °C for 16 h). After conditioning the column with 50 mL of *n*-hexane, the extract from sonication was added at the top of the column, and elution was carried out by two solvent fractions: 50 mL of *n*-hexane (discarded fraction), followed by 50 mL *n*-hexane/isopropanol 9:1 (v:v), which was collected and then concentrated down to 500 μL in the automated evaporator. The concentrated extract was then diluted to 1000 μL with Milli-Q water. Final extracts were stored into 2 mL Teflon capped screw cap glass vials from Agilent at 2 °C prior to their analysis (100 μL injected volume) by HPLC–ESI-MS under conditions previously reported (Pojana et al., 2004a).

2.4. Calculation of EEQ

The estradiol equivalent concentrations (EEQs) for each investigated chemical were calculated by using estradiol equivalent factor (EEF), defined as the quotient of $EC50_{E2}/EC50_{compound}$. EEF values previously reported in the literature were averaged and applied (Pojana et al., 2004b). Each individual average EEF value was multiplied by the measured molar concentration of the corresponding EDC to obtain a EEQ value for each analyzed chemical. The resulting sample EEQ was obtained by adding the individual EEQs.

3. Results and discussion

The pool of chemicals analyzed in this work included natural (estrone, E1; 17β-estradiol, E2; estriol, E3) and synthetic (17α-ethinylestradiol, EE2; mestranol, MES) steroids as well as non-steroidal compounds (diethylstilbestrol, DES; bisphenol-A, BPA; benzophenone, BP; nonylphenol, NP) likely to occur in sewage effluents and surface waters, and considered as the most potent endocrine active substances. The accurate simultaneous determination of these EDCs in marine sediment and biota was first undertaken. The resulting method was then applied to the environmental investigation that was carried out in four stations during six sampling sessions over a period of one year.

3.1. Analysis of selected EDCs in sediment and biota

Extraction and clean-up procedures were optimized in order to achieve satisfactory recoveries for all examined compounds. The extraction efficiencies were validated by triplicate analyses of samples spiked at two concentration levels, 100 and 20 ng/g (d.w.) of each

Table 1
Extraction efficiencies for examined EDCs from sediment (20 and 100 μg/kg, d.w., spiking level) and mussel (20 and 100 ng/g, d.w., spiking level) samples

Compound (acronym)	CAS number	Mean recovery (n=3) from sediment, % (RSD)		Mean recovery (n=3) from mussel, % (RSD)	
Spiking level		20 μg/kg	100 μg/kg	20 μg/kg	100 μg/kg
Estriol (E3)	50-27-1	75 (8)	79 (5)	56 (11)	62 (7)
17β-estradiol (E2)	50-28-2	71 (13)	77 (15)	52 (8)	55 (5)
Estrone (E1)	53-16-7	78 (6)	84 (3)	44 (15)	46 (14)
17α-ethinylestradiol (EE2)	57-63-6	65 (11)	70 (8)	48 (16)	52 (14)
Mestranol (MES)	72-33-3	77 (13)	82 (10)	51 (17)	55 (13)
Diethylstilbestrol (DES)	56-53-1	85 (9)	88 (6)	72 (10)	77 (6)
Bisphenol-A (BPA)	80-05-7	75 (12)	78 (8)	75 (8)	80 (5)
Benzophenone (BP)	11-61-9	59 (12)	63 (10)	70 (9)	74 (5)
Nonylphenol (NP)	25154-54-3	84 (6)	86 (5)	63 (14)	66 (11)
Nonylphenol monoethoxylate carboxylate (NP1EC)	n.a.	61 (15)	67 (14)	40 (16)	46 (11)

n.a.: not available.

compound, respectively. The obtained recoveries of selected EDCs in sediment and mussel tissues are presented in Table 1.

Two sonication extraction steps with methanol–acetone (80:20, vol:vol), each extraction lasting 2 h, followed by extract filtration, concentration, and solution reconstruction, permitted mean recoveries from sediment that ranged from 63% for BP, to 88% for DES, with relative standard deviations of 5–15%. The performance of the extraction procedure showed some individual recoveries lower than those previously reported, which was counterbalanced by the number (10) of analytes simultaneously extracted and separated. (Kuster et al., 2004).

The mussel tissues underwent the same extraction procedure (i.e. two steps of 2 h sonication), with hexane–acetone (70:30, vol:vol) as extractant. To avoid strong signal decrease on ion generation observed in the ESI interface caused by co-extracted interferences, an additional Florisil clean-up step was added to the procedure. Mean recoveries (Table 1) at both 20 and 100 ng/g spiking level were between 45 and 80%, except for NP1EC (35%), with relative standard deviations of 5 to 19%.

The proposed extraction procedures, followed by HPLC–ESI-MS determination, allowed to reach Method Detection Limits (MDL, s/n = 3) ranging from 0.1 to 2.6 ng/L for water samples, 0.2 to 5.0 μg/kg (d.w.) for sediment samples, and from 0.3 to 5.0 ng/g (d.w.) for biota samples, respectively (Tables 2–4). These MDLs are comparable with those recently reported (Liu et al., 2004).

Table 2
Concentrations of analyzed EDCs in lagoon water samples

Sampling period	Station	Average concentration (ng/L)						
Compound		E2	E1	EE2	BPA	BP	NP	NP1EC
Oct, 2001	1	<1.0	<1.2	<0.8	3.4	<2.6	4.0	5.0
	2	7.2	<1.2	8.4	<1.0	15	5.8	3.3
	3	8.0	5.2	<0.8	4.7	16	4.3	2.8
	4	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]
Nov, 2001	1	3.0	<1.2	8.0	30	12	39	15
	2	8.0	<1.2	4.6	4.9	20	69	42
	3	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]	_[a]
	4	<1.0	<1.2	34	<1.0	<2.6	7.1	<0.1
Feb, 2002	1	3.1	<1.2	<0.8	7.6	36	31	82
	2	175	4.6	10	6.4	37	23	71
	3	15	<1.2	7.1	<1.0	16	27	16
	4	36	10	<0.8	9.7	<2.6	16	10
May, 2002	1	<1.0	3.2	<0.8	7.9	6.5	<0.5	<0.1
	2	<1.0	<1.2	<0.8	5.3	<2.6	<0.5	<0.1
	3	<1.0	6.7	<0.8	8.8	<2.6	<0.5	<0.1
	4	<1.0	<1.2	<0.8	6.6	<2.6	<0.5	<0.1
July, 2002	1	<1.0	<1.2	10	3.5	<2.6	<0.5	3.3
	2	<1.0	<1.2	<0.8	<1.0	<2.6	<0.5	10
	3	<1.0	<1.2	<0.8	<1.0	5.0	<0.5	2.3
	4	<1.0	<1.2	<0.8	3.5	5.5	<0.5	<0.1
Oct, 2002	1	15	<1.2	28	18	113	211	<0.1
	2	14	<1.2	25	145	128	103	<0.1
	3	14	<1.2	23	15	136	146	<0.1
	4	9.8	<1.2	18	16	87	211	<0.1

Abbreviations: E2, 17β-estradiol; E1, estrone; EE2, 17α-ethinylestradiol; BPA, bisphenol-A; BP, benzophenone; nonylphenol, NP; NP1EC, nonylphenol monoethoxylate carboxylate.
[a] Sample not collected.

Table 3
Concentrations of detected EDCs in lagoon sediments

Sampling period	Station	Average concentration (μg/kg, dry weight)				
Compound		EE2	DES	BPA	BP	NP
July, 2001	1	<2.0	55	118	33	192
	2	12	<2.0	2.1	30	57
	3	41	<2.0	60	110	47
	4	<2.0	11	25	19	97
July, 2002	1	<2.0	63	99	15	91
	2	<2.0	<2.0	<2.0	36	101
	3	<2.0	34	32	14	73
	4	35	<2.0	21	58	53

Abbreviations: E2, 17β-estradiol; E1, estrone; EE2, 17α-ethinylestradiol; BPA, bisphenol-A; BP, benzophenone; nonylphenol, NP; NP1EC, nonylphenol monoethoxylate carboxylate.

3.2. Composition and spatial distribution of EDCs in lagoon water samples

Concentrations of recorded EDCs in the lagoon water samples are presented in Table 2. Except for E3, MES and DES (MDLs: 1.0, 2.0 and 0.6 ng/L, respectively), all other steroidal and non-steroidal EDCs were detected in all stations. In some more detail, at station 1, located near the large industrial district of Porto Marghera, the concentrations

Table 4
Concentrations of detected EDCs in mussels

Sampling period	Station	Average concentration (ng/g, dry weight)	
Compound		EE2	NP
July, 2001	1	<3	170
	2	17	115
	3	7.2	240
	4	7.2	206
July, 2002	1	<3	131
	2	38	211
	3	11	171
	4	20	170

See Table 1 for abbreviations.

of non-steroidal EDCs (e.g. NP and BPA) were generally higher than those of steroidal estrogens (e.g. EE2), as result of the proximity to both industrial treated effluents and effluents of a mechanical–biological sewage treatment plant (STP) receiving municipal and industrial wastewaters. The vicinity of the STP is likely to explain the significant concentrations of E2 and EE2 during the winter period (3.0–15 ng/L and 8.0–28 ng/L, respectively). At stations 2 and 3, expected to be affected mainly by municipal wastewaters, non-steroidal EDCs were in general more abundant than steroidal EDCs, except for the peak concentration (175 ng/L) of E2 at station 2 in the February sampling session. The overall results showed that stations 1–3 were affected by EDCs of both municipal and industrial origin (Pojana et al., 2004b). Unexpectedly high contamination levels of steroidal and non-steroidal EDCs were found at station 4, selected as "environmental background" site: the mean concentrations of E2, EE2, NP and BPA were 9.5 ng/L, 11 ng/L, 47 ng/L, 7.5 ng/L, respectively. The redistribution of contaminants inside the central lagoon by tidal hydrodynamics is likely to explain this finding and confirms previous reported results (Pojana et al., 2004b). The seasonal sampling permitted to identify possible influence of seasonality on concentrations. Approx. 40% of concentrations in the summer period were < MDL, achieving 69% during the May/July sessions. Temperature was the only recorded parameter that changed significantly in the sampling sessions, exhibiting a minimum value of 9.3 °C in February and a maximum value of 29.2 °C in July, respectively. The only other parameter showing a significant change was dissolved O_2, ranging from 13 mg/L in November to 29 mg/L in February. All other recorded parameters remained fairly constant throughout the sampling period: pH (7.6–8.2) and salinity (26–34 g/L, as NaCl) were as expected over the entire sampling campaign and as previously found (Pojana et al., 2004b).

Results from the sampling of stations 1 and 3 are in good agreement with those obtained recently at sampling stations located approx. 500 m from the location of stations 1 and 3 in this investigation. In this survey, however, average concentration levels of the same selected EDCs dissolved in water were lower or in the typical concentration range previously reported for fresh and marine waters (Baronti et al., 2000; Pojana et al., 2004b; Céspedes et al., 2005; Langston et al., 2005). Diethylstilbestrol (DES), analyzed for the first time in the Venice lagoon, was not detected in all water samples.

3.3. Composition and spatial distribution of EDCs in lagoon sediments

The Venice lagoon sediment was analyzed to determine distribution and partitioning of examined EDCs in this compartment. To the best of our knowledge, these are the first data about contamination from EDCs in the Venice lagoon sediments. The average concentrations of the detected EDCs from the four sampling stations are reported in Table 3. While concentrations of synthetic non-steroidal EDCs showed a relevant increase from 2001 to 2002 in collected sediments, synthetic steroidal EDCs showed to decrease. The most contaminated sediment was found at station 1. Generally, NP was found at the highest concentration scores (47÷192, average 89 μg/kg) as expected by its hydrophobic properties (K_{ow}: 5.76), which caused a preferential accumulation in both sediments and aquatic organisms. The relevant contamination recorded at station 1 could indicate a continuous dumping during the sampling period. However, the reported concentrations were generally similar or lower than those previously recorded in other environments (Ferguson et al., 2001; Ferrara et al., 2001). In contrast with NP, NP1EC (K_{ow}: 2.2), was not detected in any sediment sample. Bisphenol-A (K_{ow}: 3.64) was instead found up to a 118 μg/kg concentration level, approx. ten times higher than previously reported for other coastal sediments (1.4–13 μg/kg) (Kawahata et al., 2004). Diethylstilbestrol (K_{ow}: 5.64), which was not detected in water samples, was detected at stations 1, 3 and 4 in the 11÷63 μg/kg range, much higher than other reported environmental concentrations (0.3–2.0 μg/kg) (Lopez de Alda et al., 2002). Benzophenone (K_{ow}: 3.15), showed instead concentrations (up to 110 μg/kg) lower than the typical environmental levels reported elsewhere (100÷800 μg/kg) (Burkhardt et al., 2005). Ethinylestradiol (K_{ow}: 4.12) was the only steroidal EDC detected at relevant concentrations (up to 41 μg/kg) in the collected sediment samples, similarly to previously reported for river sediments (Langston et al., 2005). The relatively high concentrations of synthetic EDCs, such as EE2 and NP, are in good agreement with previous laboratory experiments with marine sediments which showed no or little degradation under anaerobic conditions, which are the typical Venice sediment conditions: the monitored average Eh values at stations 1 and 2 were −69 and −28 mV, respectively, while at stations 3 and 4 Eh values of 44 and 28 mV were found, respectively (Ying and Kookana, 2003). The fast degradation rate of natural estrogens (E2, E1) in the water column (half lives: 2.8÷3.0 days), together with their potential interaction with colloidal particles (Bowman et al., 2002), can explain their almost total absence in the sediment samples. On the basis of the measured concentrations in waters and sediments and of the measured organic carbon content (9.7%, 6.5%, 1.6% and 1.8% at stations 1, 2, 3 and 4, respectively), rough experimental partitioning coefficients could have been estimated for NP, BPA and BP. Carbon-normalized sediment–water partition coefficients were calculated for each station according to Eq. (1):

$$K'_{OC} = \left(C_s/C_{aq}\right)/f_{OC} \quad (1)$$

where C_s is the average concentration in the analyzed sediment sample (as μg/kg), C_{aq} is the determined mean concentration in water (as ng/L), and f_{OC} is the mass fraction of organic carbon in the collected sediment sample. Despite the limited number of samples, experimental LogK'_{OC} values were 3.7–5.0 for BP (mean: 4.5), 3.3–5.5 (mean: 4.7) for BPA, and 4.5–5.0 (mean: 4.8) for NP; the values calculated for NP are in good agreement with those reported for other estuarine sediments (Isobe et al., 2001), but lower than in estuarine suspended particulate matter (Jonkers et al., 2003).

3.4. Composition and spatial distribution of EDCs in Mediterranean mussels

In addition to water and sediment samples, biota samples were also analyzed in order to investigate potential bioaccumulation of EDCs by lagoon organisms. The Mediterranean mussel (*M. galloprovincialis*) was selected as target, since it's a representative filtering organism of this estuarine ecosystem with a great ability in bioaccumulating persistent pollutants (Sabik et al., 2003; Gagné et al., 2004).

Only two of the examined EDCs were systematically found in mussel tissues: EE2 and NP. The average concentrations of EE2 and NP in the collected samples are reported in Table 4. The exhibited levels of NP (115÷240 ng/g, d.w.) were much lower than those previously reported in a more extended survey along the Adriatic Sea coast, with values in the 243÷265 ng/g, fresh weight, range, which correspond to 2025–2208 ng/g, dry weight, according on the average measured water content (Fulvio Ferrara, personal communication) (Ferrara et al., 2001). The steroidal synthetic estrogen EE2 was instead found in the 7.2÷38 μg/kg, d.w. concentration range. Diethylstilbestrol was also recorded in mussel samples from two stations (3, 4), during the 2002 sampling session, at concentrations of 9.6 and 13 ng/g (d.w.), respectively. The presence of DES in these samples could not have been explained, since the locations are far from any potential source,

Table 5
Total measured estrogenicity (EEQ) in the Venice lagoon waters and sediments at each sampling station, and relative mean contribution (%) of individual EDCs to the total EEQ

	Station — matrix							
	1 — water	1 — sediment	2 — water	2 — sediment	3 — water	3 — sediment	4 — water	4 — sediment
Average (range) EEQ	16 (1.1–57) ng/L	155 (145–165) µg/kg	20 (1.1–52) ng/L	12 (4–21) µg/kg	17 (1.2–49) ng/L	77 (64–90) µg/kg	21 (1.2–52) ng/L	43 (30–55) µg/kg
Compound	Average contribution (%) to estradiol equivalent concentration (EEQ)							
E2	44	<0.1	49	<0.1	51	<0.1	43	<0.1
E1	2	<0.1	1	<0.1	7	<0.1	2	<0.1
EE2	56	<0.1	49	2	42	41	56	56
DES	<0.1	100	<0.1	97	<0.1	59	<0.1	43

See Table 1 for definitions. Not reported EDCs exhibited <0.1% EEQ values in each analyzed sample. The outlying value recorded in February (175 ng/L) at Station 2 was discarded in the EEQ calculation.

except the sediment itself. Bisphenol-A was detected only in mussels from station 1 in the 2001 sampling campaign (11 ng/g, d.w.). The other examined EDCs were not detected in any mussel sample, i.e. concentrations were < MDLs: 5, 1.5, 1.5, 4, 3 and 0.2 ng/g, d.w., for E3, E1, E2, MES, BP and NP1EC, respectively. No comparison with other environments could be done since no data were available so far, to the best of our knowledge, for these EDCs. On the basis of these preliminary results (the first data about the occurrence of EDCs in the Venice lagoon biota), approximate experimental bioconcentration factors (BCFs) could be estimated for NP and EE2. In case of samples with EE2 concentrations < MDL, half of the experimental MDL was used for both water and mussel concentrations. The BCF values were calculated according to the following equation:

$$\mathrm{BCF} = C_m / C_{aq} \quad (2)$$

where C_m is the average concentration in the analyzed mussel tissues (as ng/g, f.w.) and C_{aq} is the average concentration in water (as ng/L) from the same station. The resulting BCFs were 1300 to 1500 (mean: 1400) and 3200 to 5800 (mean: 4400) L/g for EE2 and NP, respectively. The BCF of NP was also obtained by the EPIWIN® software leading to a value of 7200, which might suggest an underestimation of the calculated experimental BCF. However, a similar value of BCF (3400) was previously reported for NP in laboratory experiments (Ekelund et al., 1990). The BCF value for EE2 obtained by EPIWIN® (i.e. 134) was at least ten times lower than that calculated here from field experimental results. The experimental values found were at least ten times higher than those previously predicted for river organisms by mass-balance food-web models (Lai et al., 2002). No field literature data on EE2 BCFs were instead available, to the best of our knowledge, for a comparison.

3.5. Estimation of estrogenicity of waters and sediments

The recent demonstration from in vivo experiments that the mixture of examined EDCs shows a toxicological behaviour according to the principles of concentration addition in both freshwater and marine fish species, encouraged to estimate the estrogenic potential of lagoon water and sediment (Brian et al., 2005). The concentrations of individual EDCs were those presented in this work from field experimental results, while the EEFs were obtained from scrutin examination of most recent values reported in the literature (Pojana et al., 2004b). They were 5.8×10^{-2}, 1, 1.5, and 2.6 for E1, E2, EE2, and DES, respectively, the EDCs contributing significatively (> 0.1%) to the total EEQ. The

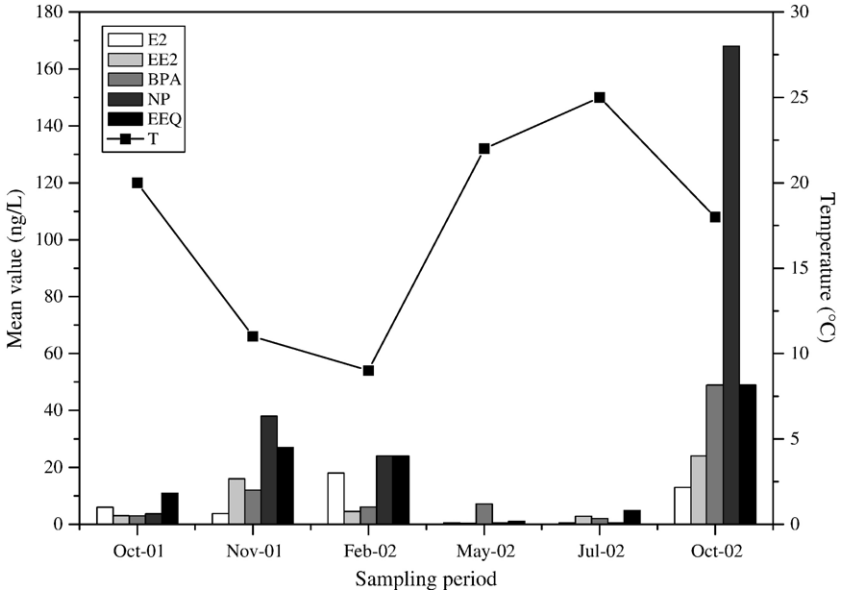

Fig. 2. Seasonal trend of mean EEQ values (ng/L) and temperature (T) in the Venice lagoon waters during the sampling period.

resulting EEQ values for both water and sediment are shown in Table 5. The water estrogenic potential ranged between 1.1 ng/L and 52 ng/L, and did not include the peak value (175 ng/L) of EE2 at station 2. A marked seasonability was shown by the EEQs as well as by the chemical concentrations. Fig. 2 shows the average concentrations of E1, E2, EE2, as well as of EEQs, against temperature (9 °C to 25 °C) over the six sampling campaigns: at temperatures below 20 °C EEQ values recorded that the sampled stations were between 3.7 and 57 ng/L (mean: 32 ng/L), while at temperatures above 20 °C EEQs were 3.4–20 ng/L (mean: 5 ng/L). This suggests that the time trend of EEQs is more marked than the concentration trend, i.e. effect concentrations decrease more markedly with temperature than chemical concentrations. In the sediment, EEQs ranged between 4 and 165 μg/kg d.w. (Table 5). Station 1 exhibited the highest sediment EEQs (145÷165 μg/kg), while station 2 was the least contaminated (4–21 μg/kg). The occurrence of DES increased greatly the sediment EEQ value at station 1, as well as at station 3, since its high EEF (approx. 2.6). The calculated EEQs in both water and sediment samples were higher than those previously reported in literature from bioassays carried out on sewage treatment plant effluents, surface waters and sediments (2.0–7.4 ng/L and 3.3×10^{-3}–11×10^{-3} μg/kg, respectively) (Oh et al., 2000).

A recent study on the biological effects of contaminated sediments on the freshwater mudsnail *Potamopyrgus antipodarum* reported a manifest increase in the number of sheltered embryos already at 1 μg/kg (d.w.) EEQ level (Duft et al., 2003). Taking into account that 81% of the analyzed sediment samples exhibited EEQ >1 μg/kg, the Venice lagoon sediment is strongly expected to be, expecially for the benthonic organisms, an additional source of exposure to EDCs.

An advantage of estimating EEQs from chemical analysis is to identify the compounds most contributing to the total estrogenic potential. The percentage contributions of analyzed compounds to the total EEQ of lagoon waters and sediments are also reported in Table 5. Estradiol, estrone, ethinylestradiol and diethylstilbestrol were the main contributors to the overall EEQ. Steroidal EDCs (E2, E1, EE2) showed to contribute mostly to the water EEQs, while EE2 and DES accounted mainly for the sediment EEQs levels. When detected, DES alone contributed up to a >99% of the overall sediment EEQ.

The water EEQs recorded in this work were lower than those found in a previous survey, but it is to be noticed that 84% of the previous samples were collected around the Venice historical centre, mostly in the inner city channels receiving untreated wastewaters, where higher concentrations of EDCs, expecially the steroidal ones (E2, EE2) were expected to be found (Pojana et al., 2004b). During this survey, only 59% of samples exhibited EEQ values >2.5 ng/L, in comparison with a >90% percentage of samples in the previous survey. This finding further confirms the Venice historical centre as major source of EDCs for the Venice lagoon.

Based on the exposure data reported in this investigation for water and sediment, on internal exposure on biota and on effect data from literature, effects such as increase of plasma vitellogenin, decrease in gonadal maturity, development of hermafroditic gonads and testis-ova, are likely to be observed in the aquatic organisms of the Venice lagoon. Very recent in vivo laboratory investigations confirmed these hypothesis: the investigated EDC mixture was found to significantly affect hemocites parameters of mussels at concentration comparable with those found in the Venice lagoon (Canesi et al., 2007).

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