Emerging endocrine disruptors in two edible fish from the Persian Gulf: Occurrence, congener profile, and human health risk assessment

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Footnote: This paper is dedicated to the memory of our wonderful colleague, Prof. Gustavo Damiano Mita (National Laboratory on Endocrine Disruptors, National Institute of Biostructures and Biosystems (INBB), Institute of Genetics and Biophysics of CNR, Via Pietro Castellino, 111, 80131, Naples, Italy) who gave the input and took active part in the work, but sadly passed away on 24th November 2019.

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Abstract (145 words)

The occurrence of endocrine disrupting chemicals (EDCs) has been determined in two widely consumed fish species from Persian Gulf *i.e.*, *Epinephelus coioides* and *Platycephalus indicus* by applying a validated analytical for the simultaneous detection of fourteen EDCs. The concentrations of all detected EDCs were greater in the liver than in the muscle (except for bisphenol A in *P. indicus*), suggesting a prolonged exposure of the fishes to these pollutants in the Persian Gulf. Specifically, the results showed that di(2-ethylhexyl) phthalate (DEHP) was the compound detected most frequently and at the highest concentration in both species. DEHP levels in ranged from 6.68 to 297.48 µg g-dw^{-1} and from 13.32 to 350.52 µg g-dw^{-1}, in muscle and in liver, respectively. A risk assessment study was conducted, and demonstrated that consuming two fish based- meals *per* week may result in a moderate risk especially for vulnerable population groups.

Keywords: Endocrine disruptors; Monte-Carlo simulation; fish; liver; muscle; Persian Gulf.

1- Introduction

Endocrine disrupting chemicals (EDCs) are pollutants able to interfere with the normal homeostasis of the endocrine system in animals and humans (ROPME, 2013; Scholz and Klüver, 2009). In fact, the occurrence of EDCs in fresh or seawaters has been associated with a potential risk of both aquatic organisms (Gong et al., 2016) and humans (Liu et al., 2011). Phenomena observed in animals are vitellogenin production in male organism and feminization with consequent threat to the conservation of biodiversity. Nowadays, it is well established that development of some diseases including prostate cancer (Di Lorenzo et al., 2018; Forte et al., 2016; Forte et al., 2019), breast cancer, endometrium cancer (Forte et al., 2016) and thyroid disfunction (Marotta et al., 2019) are related to the exposure to EDCs. The risks for humans come mainly thought the diet, specifically if this foresees great quantity of fish as often happens because of the nutritional value (Jia et al., 2017) EDCs can bioaccumulate and biomagnify in fish tissues, it is crucial for the human health to monitor and assess their content in this commodity.

EDCs reach the aquatic environments through different routes and have been found pervasive in fresh water, seawater, sediment, and biota (Ismail et al., 2018; Liu et al., 2017a; Omar et al., 2019). EDCs have been well-ascertained to be released to waterbodies from industrial discharges, runoff, landfill leachate, wastewater effluents, and wastewater treatment plants (Krishnapriya et al., 2017; Pahigian and Zuo, 2018; Park et al., 2018). The Persian Gulf is one of the most anthropogenically impacted waterbodies in the world (Cunningham et al., 2019; Mehdinia et al., 2015). Its high level of environmental contamination and human induced stress is plausible to effect in a more serious impact on the health of the Gulf's marine environment than open sea. This reverberates in as serious consequences related to food safety as well (Cunningham et al., 2019; Khatir et al., 2019). However, only few studies focused on the occurrence of phthalate and phenolic EDCs in the biota from the Persian Gulf so far

(Akhbarizadeh et al., 2020; Behfar et al., 2018; EbrahimSajjadi, 2017; Farasat et al., 2014; Jahromi et al., 2020; Yoon et al., 2019).

The Persian Gulf as a semi-enclosed system, is highly affected by anthropogenic contamination and fish caught from the Gulf were found to contain high level of pollutants due to its low depth and restricted circulation (Cunningham et al., 2019; ROPME, 2013). Indeed, the oil-related activities have driven economic, industrial, and urban development along the Gulf's coastal margins during the last century (Khatir et al., 2019). The human population settled in the Persian Gulf has grown from 46.5 to almost 150 million people during the last 50 years and it is expected to hit 200 million by 2030 (Khatir et al., 2019). This results in the Gulf's marine ecosystem facing even further anthropogenic pressure (Khatir et al., 2019). Domestic sewage, industrial discharge, effluent of desalination plants, landfills, fertilizers, plastic wastes, oil-related activities, and regional political conflicts (such as the 1980 Iran-Iraq War and 1991 Gulf War) are the main sources of EDCs in the Persian Gulf (Khatir et al., 2019; ROPME, 2013; Saeed et al., 2017; Smith et al., 2015). On the northern and western parts of the Gulf, EDCs are released to the Gulf though both direct discharge and river systems. The Shatt Al-Arab River system, is the largest freshwater discharge into the Persian Gulf and it drains the combined waters of the Tigris and Euphrates Rivers of Iraq and the Karun River of Iran. Moreover, other Iranian rivers *i.e.*, Helleh, Hendijan, and Mond, empty into the Gulf along its northwestern shoers of the Gulf (Cunningham et al., 2019). On the other hand, there is no riverine input along the southern part of the Gulf, and only direct discharge of runoff and effluent is reasonably expected (Figure 1). Recent studies confiremed the presence of EDCs in coastal waters of Kuwait (Saeed et al., 2017; Smith et al., 2015). Considering the importance and high consumption of seafood in these regions, monitoring EDCs in fish from Persian Gulf to evaluate the human health risk arising from their consumption emerged as a priority. However, to the best of our knowledge, the presence of phthalate and phenolic EDCs has been rarely investigated in muscle and liver of commercial fish from the Persian Gulf. Hence, this study aims at *i)* looking into the occurrence of some phthalates and phenols compounds in muscle and liver of two fish species (*Epinephelus coioides* and *Platycephalus indicus)* highly consumed on both Iranian and Arabian side of the Gulf and *ii*) estimating the health risk arising from the consumption of these fishes.

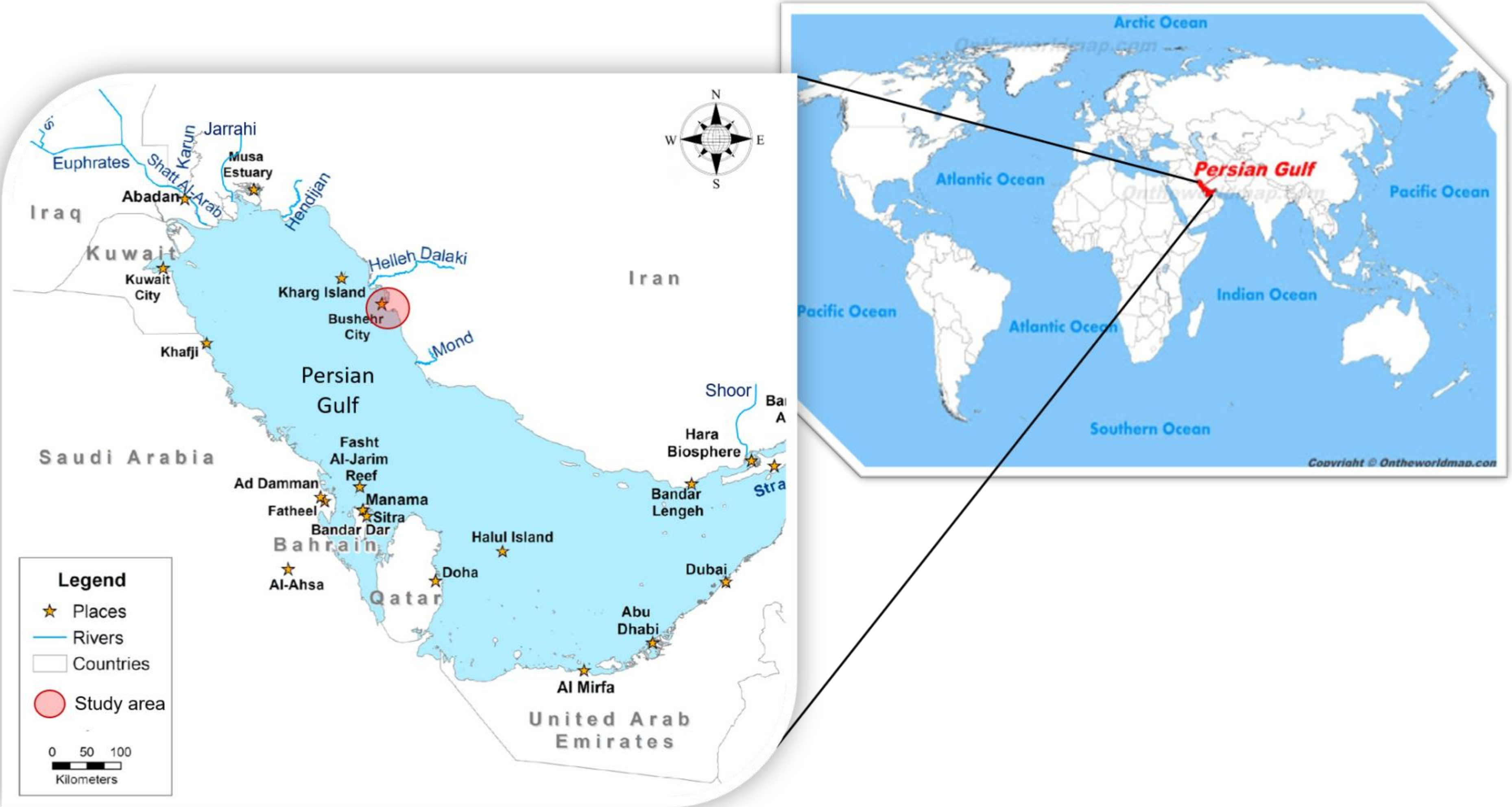

Figure 1: Sampling area in the Persian Gulf and the Gulf's riverine inputs (adapted from Cunningham et al., 2019 with minor revision).

2. Materials and Methods

2.1. Reagents and chemicals

Methanol (HPLC analytical grade) and formic acid (minimum purity ≥ 95%) were both purchased from Sigma Aldrich (Milan, Italy). Milli Q water was produced *in-house*, and its conductivity was 0.055 µS cm^{-1} at 25 °C (resistivity equals 18.2 MΩ cm). Analytical standards such as bisphenol F (BPF-minimum purity ≥ 99.0 %), bisphenol S (BPS-minimum purity ≥ 98%), BPA (minimum purity ≥ 99%), bisphenol A diglycidyl ether (BADGE-minimum purity ≥ 95%), carbamazepine (minimum purity ≥ 99%), 2-chlorophenol (2-CP-minimum purity ≥ 99%), 4-nonylphenol (4-NP-minimum purity ≥ 98%), 1,4-dichlorobenzen (DCB-minimum purity ≥ 99.0 %), 1,2,4,5-tetrachlorobenzene (TCB-minimum purity ≥ 98.0%), DEHP (minimum purity ≥ 98.0 %), Triclosan (TCS-minimum purity ≥ 97%) were purchased from Sigma-Aldrich (Dorset, United Kingdom). Other analytical standards such as bisphenol E (BPE-minimum purity > 98%), bisphenol B (BPB-minimum purity ≥ 99%), bisphenol AF (BPAF-minimum purity > 98%), bisphenol M (BPM-minimum purity > 98%) were purchased from TCI Europe (Zwijndrecht, Belgium) and used without further purification.

2.2. Sampling area and characteristics of fish

The Persian Gulf, with an average depth of 35 m and 240,000 km^2 area, is an important fishing hotspot in Iran and it hosts a wide variety of pelagic and benthic species. One of the important fishing ports of Iran is Bushehr (along the coast of South-Western Iran), where caught fish are exploited for trading both locally and internationally. In January 2018, 30 fishes were purchased from local fisheries at the Bushehr port (Figure 1). Two commercial species, namely *Epinephelus coioides* (n=15) and *Platycephalus indicus* (n=15) were selected for this research because of their being highly consumed and exported. Both species are carnivorous, being predators of demersal fish and benthic invertebrates (Table 1).

Samples were immediately put into ice boxes straight after purchase and transported to the laboratory, where they were first washed with tap water and then rinsed with distilled water. The morphometric measurements (total length and weight) of each fish were recorded before being dissection. Muscle tissue and liver of each species were dissected and homogenised, then freeze-dried (Alpha 2-4 LDplus freeze dryer, Martin Christ, Germany), and eventually ground. The such prepared samples were shipped to the laboratories of the Department of Pharmacy, University of Naples Federico II, Italy for HPLC analysis.

2.3. Biometric parameters

The gross assessment of fish health was assessed by calculating the condition factor (CF) and liver somatic index (LSI) using the following equations (Beg et al., 2015; Metón et al., 1999; Tenji et al., 2020):

$$CF = \frac{\text{Weight (g)}}{\text{Length (cm}^3\text{)}} \quad \text{Eq. (1)}$$

$$LSI = \frac{Liver\ weight\ (g)}{Body\ weight\ (g)} \times 100 \quad \text{Eq. (2)}$$

Table 1: The name, numbers, diet, living depth, size, lipid content, sex, condition factor, and liver somatic index of the analyzed fish from the Persian Gulf

Common name		**Orange-spotted grouper**	**Bartail flathead**
Scientific name		*Epinephelus coioides*	*Platycephalus indicus*
Family		Serranidae	Platycephalidae
Number of samples		15	15
Feeding behavior		Carnivorous	Carnivorous
Living depth in the studied area (m)		1 - 60	10 - 60
Length (cm)	Range	27.1 - 62.8	31 - 48.1
	Mean ± SD	37.64 ± 10.65	39.19 ± 5.21
Weight (g)	Range	229 - 3500	180 - 614
	Mean ± SD	810.47 ± 81.72	412 ± 141.21
Liver weight (g)	Range	3 - 45	1.9 - 12
	Mean ± SD	9.65 ± 3.98	6.91 ± 3.09
Age (yr)	Range	2 - 6	3 - 6
	Mean ± SD	3.1 ± 1.2	4.37 ± 0.76
Lipid (%)	Muscle	2.75	2.50
	Liver	23.45	22.64
Sex	Male (%)	44	39
	Female (%)	56	61
Condition factor (g $(cm^3)^{-1}$)	Range	0.9 - 1.41	0.55 - 0.78
	Mean ± SD	1.20 ± 0.11	0.65 ± 0.05
Liver somatic index (%)	Range	0.8 - 2.18	0.94 - 3.20
	Mean ± SD	1.31 ± 0.37	1.63 ± 0.56

2.4. Fish Samples Treatment

Due to the huge variety of known EDCs, we decided to focus our attention on fourteen model pollutants with a well-ascertained endocrine disrupting activity and assumed as markers of environmental pollution. We applied an extraction method from both matrices performed according a work reported in the literature (Cheng et al. 2018), slightly adjusted to fit our purpose. The EDCs under our investigation belong to various chemical classes: one phthalate (DEHP); two chlorobenzenes, (DCB and TCB); three phenol derivatives (2-CP, 4-NP, and TCS); and some bisphenols such as the parent compound BPA and is seven analogues (BPS, BPF, BPAF, BADGE, BPE, BPB and BPM). Sample treatment was performed as follows: 500.0 mg of either muscle or liver of both fish species were collected and weighted. Each tissue was added of 5.0 mL 0.01 M KCl solution and underwent homogenization. The resulted suspensions were added to a 9.0 mL of a 1:8 methanol: 50/50 (v/v) *n*-hexane/ethyl acetate solution. The samples underwent ultrasonication (Soniprep 150, MSE, London, UK) to destroy the cellular matrices, vortexed to allow further dispersion and centrifuged at 3,500 rpm for 15 minutes. The supernatant was collected and gently dried under nitrogen flow. The dry residue was re-dispersed in a suitable volume of acetonitrile (max 2.0 mL), filtered through a 0.20 µm nylon membrane (Merck Millipore, Darmstadt, Germany), properly diluted to fit within the range of the calibration curves and analyzed by high performance liquid chromatography (HPLC), coupled with both Ultraviolet (UV) and fluorescence detection (FD). Plastic equipment was treated to avoid any possible background contamination by keeping the plastic labware in contact with a 50/50 *n*-hexane : tetrahydrofuran solution for 30 minutes (Olivieri et al., 2012).

2.5. Chromatographic measurements and quality control

To establish the calibration curves for all the EDCs under examination, standard solutions were prepared in acetonitrile at a 2.0 mg mL^{-1} concentration of for all but DEHP (10.00 mg/mL), 4-NP (0.50 mg mL^{-1}), BPB and BPM (both 0.25 mg mL^{-1}) and TCB (1.00 mg mL^{-1}). Matrix-matched calibration curves were obtained by plotting peak areas against concentrations of the analytes. To evaluate the matrix effect, three samples of each tissue (liver and muscle) from *Gadus morhua*, previously verified as analytically free of the considered EDCs, were used as a blank, spiked at low and high concentration values of the EDCs under investigation and subject to the analytical procedure. The *Gadus morhua* fish was purchased from a fishery established in Naples (Italy) and used for method validation. The matrix effect was calculated according to Equation 3.

$$matrix\ effect = \left(\frac{\text{Signal of EDC in spiked fish sample}}{\text{Signal of EDC in standard solution}}\right) * 100 \qquad \text{Eq. (3)}$$

In Eq.(3), a value equal to 100 % indicates a null matrix effect, a value higher than 100 %, a signal enhancement. Limit of detection (LOD) and limit of quantitation (LOQ) were assumed as the analyte concentrations producing an analytical signal three (LOD) and ten (LOQ) times the ratio between the standard deviation of the intercept and the slope of the signal *vs* concentration calibration line. The results of validation parameters are shown in Table 2.

For the quantitative determination of the 14 EDCs we applied a validated separation chromatographic method already published which allows simultaneous determination of our target EDCs by means of HPLC/UV/FD (Russo et al., 2019). Each sample was injected three times to test the instrument repeatability. Carbamazepine (retention time 16.50 ± 0.30 min) was selected as internal standard to verify recovery to keep track of possible flaws during the sample preparation and analysis procedure, *e.g.* injection of some volumes of air instead of samples. Experimental values are at least the average value of three independent measurements. The %

standard deviation never exceeded 10.60%. For each retention value the 95% confidence interval associated never exceeded 0.04.

Table 2: Validation parameters for the quantitative analysis of the detected EDCs in muscle and liver of the fishes *E. coioides* and *P. indicus.*

Analyte	**Recovery (%)**				**Matrix effect (%)**	**LOD ($\mu g g^{-1}$)**	**LOQ ($\mu g g^{-1}$)**
	Fortification level						
	Muscle		Liver				
BPA	High[a]	Low[b]	High[a]	Low[b]	107.9	$5.61 * 10^{-3}$	$15.71 * 10^{-3}$
BPAF	95.7	77.5	92.7	75.4	91.8	$1.54 * 10^{-3}$	$3.45 * 10^{-3}$
DEHP	101.2	84.6	100.1	83.8	94.4	2.88	7.58
BADGE	97.8	94.9	97.5	92.9	101.0	$1.02 * 10^{-3}$	$3.12 * 10^{-3}$
TCB	98.3	96.4	96.3	94.5	105.8	7.29	26.44
TCS	95.8	95.1	94.0	93.1	97.4	0.29	0.95
4-NP	87.8	81.2	85.8	80.7	100.8	$4.22 * 10^{-3}$	$12.38 * 10^{-3}$

* Validation has been performed on *Gadus morhua*, previously verified as analytically free of the considered EDCs.

a = $50 * 10^{-3}$ $\mu g g^{-1}$ for BPA, BPAF, BADGE and 4-NP, 10 $\mu g g^{-1}$ for DEHP, TCB and TCS

b = $200 * 10^{-3}$ $\mu g g^{-1}$ for BPA, BPAF, BADGE and 4-NP, 20 $\mu g g^{-1}$ for DEHP, TCB and TCS.

2.6. Moisture and Lipid content

In order to measure the moisture content of each fish species, 10.00 g of homogenized muscle samples were placed in a drying oven at 105 °C for 5.0 h, then weighted and returned to the oven for more 1.0 h. The drying and weighing process was repeated until a constant weight was achieved (Gagnon et al., 2016; Pratoomyot et al., 2008). The moisture content of samples was measured to enable comparisons between EDCs level in fish from the Persian Gulf and the results of previous studies from different parts of the world.

Lipid content of homogenized muscle and liver samples were extracted using a modified method from the literature (Bligh and Dyer, 1959). First, 20.0 g muscle and 1.0 g liver of each fish sample was transferred to a 500 mL and 50 mL separator funnel, respectively. Then dichloromethane, ethanol, and distilled water was added to each sample. After complete stratification of the phases, the dichloromethane layer, which contained lipids, was transferred to a boiling flask, the solvent was evaporated by rotary evaporator and the residual fat was weighted using a Mettler Toledo balance with 5 decimal accuracies.

2.7. Data Analysis

All statistical analysis was done using Microsoft Excel 2016 and XLSTAT software (version 2016). The normality and homogeneity of variables were checked by Shapiro-Wilk test (significance level was considered at P value ≤ 0.05). Considering normality tests results, Spearman correlation tests were performed to determine the relationship among EDCs in fish organs.

2.8. Risk Assessment

The dietary exposure to the considered EDCs was calculated using the mean concentration of BPA, BPAF, BADGE, and DEHP in fish flesh. In all calculated risk factors (based on USEPA guidelines), the ingestion and absorbed dose were considered as equal, and cooking has been assumed to have no effect on pollutants concentration (Copat et al., 2013; USEPA, 2000).

The Average Daily Intake (ADI-μg $kg\text{-}day^{-1}$), target hazard quotient (THQ) and hazard index (HI) were calculated to express the risk of non-carcinogen effects. The ADI, THQ, and HI were calculated using the equations 4, 5 and 6 (Fattore et al., 2015; Liao and Kannan, 2013):

$$ADI = \frac{\mathrm{IR} \times CF \times C}{BW} \quad \text{Eq.(4)}$$

$$CF = 1 - \frac{[wet\ weight - dry\ weight]}{wet\ weight} \quad \text{Eq.(5)}$$

$$HI = \sum_1^n THQ = \sum_1^n \frac{ADI}{RfD_o} \quad \text{Eq.(6)}$$

where IR is the fish consumption rate (g $person^{-1}$ day^{-1}), CF is the conversion factor to convert the dry weight values to wet weight (species specific), C is the mean concentration of each EDC compound (μg $g\text{-}dw^{-1}$), RfD_0 is the oral reference dose (μg $kg\text{-}day^{-1}$), and BW is body weight (70 kg for adults and 16 kg for children) (Gu et al., 2016; USEPA, 2000).

THQ and HI values greater than 1 indicate that potential human health effects exist and related remedial action should be taken (Diao et al., 2017; Gu et al., 2016).

The total estrogenic activity in a sample is expressed as estradiol equivalent quotient (EEQ-ng $g^{-1})_t$, defined according to the equation 7:

$$EEQ_t = \sum EEQ_i = \sum C_i \times EEF_i \quad \text{Eq.(7)}$$

in Eq.(7) C_i is the concentration of each EDC (μg g^{-1}) in muscle samples and EEF_i is the estradiol equivalency factor defined as the median effective concentration (EC_{50}) of compound *i* relative to EC_{50} of 17β-estradiol (E2): $EEF_i = EC50_{E2}/EC50_i$ (Liu et al., 2017a; Liu et al., 2017b; Zhou et al., 2018). EEF_i of EDCs were taken from the literature (Diao et al., 2017; Leeuwen et al., 2019; Papapostolou, 2016).

Monte-Carlo simulation was performed by Quantum Xl, a Microsoft Excel add-in, developed by Sigmazone to evaluate the health risks of fish consumers and also calculate the uncertainty distributions. 10,000 interactions were randomly conducted for each simulation. HI and ADI_{EEQt} were estimated for both age groups (adults and children).

3. Results and discussion

3.1. Characteristics of marine organisms

The results of biometric measurements, the number of sampled organisms, their feeding behaviour, age, lipid content, sex, condition factor, and liver somatic index are listed in Table 1. Since the results of the normality test revealed that fish length and weight for all species were not normal *($p>0.05$)*, Spearman correlation analysis was carried out to investigate this relationship. Based on the results, a significant positive correlation existed between length and weight in both analysed fish species ($p< 0.01$, r=0.99 for *E. coioides*, r=0.98 for *P. indicus*, r=0.95).

E. coioides fish samples were between 2 to 6 years old (mean 3.10±1.24 years) and *P. indicus* fish samples were between 3 to 6 years old (mean 4.37±0.76). No significant differences were shown between CF and HIS in male and female fish and both indexes presented similar distribution in both sexes. The mean level of CF for *E. coioides* samples was 1.2 while this value for *P. indicus* samples was 0.65 (Table 1).

The mean lipid content of muscle and liver of *E. coioides* were slightly higher than that of *P. indicus*. Indeed, the lipid content of marine organisms is influenced by a variety of factors including species, swimming activity, diet, size, age, and reproductive stage (Ejike et al., 2015; Lundebye et al., 2017; Murillo et al., 2014).

3.2. EDCs concentration in fish

The range, mean concentration, and standard deviation of analysed EDCs in muscle and liver of sampled marine fish are summarized in Table 3. Only eight out of the fourteen endocrine disruptors were detected. Four EDCs *i.e.*, DEHP, BPA, BPAF and BADGE showed a frequency detection above 74%. The detection frequency ranking for these was DEHP >

BADGE > BPAF > BPA, and DEHP was found to be the EDC detected at the highest concentration levels (Diao et al., 2017).

Table 3: Descriptive statistics of the detected EDCs in muscle and liver of *E. coioides* and *P. indicus* from the Persian Gulf.

Species	**Tissue**	**EDCs**	**Polluted samples**	**Detection Frequency (%)**	**Range (μg g^{-1} dw)**	**Mean (μg g^{-1} dw)**	**Std-E**	**Coefficient of variation (%)**
E. coioides	**Muscle**	DEHP	15	100	6.68-246.08	69.25	57.51	83.05
		BPA	15	100	0.02-28.29	3.84	7.77	202.51
		BPAF	12	80	BDL -12.4	2.75	4.57	166.36
		BADGE	14	94	BDL -13.04	6.99	3.34	47.73
		4-NP	7	47	BDL-18.80	5.57	6.89	123.53
	Liver	DEHP	15	100	15.6-350.52	137.38	97.17	70.71
		BPA	14	94	BDL -47.16	8.45	14.95	176.85
		BPAF	12	80	BDL -23.32	3.51	6.86	195.52
		BADGE	15	100	6.72-23.36	13.78	4.17	30.24
		4-NP	8	53	BDL-13.72	2.99	4.46	149.11
		TCB	2	13	BDL-91.64	55.15	36.50	66.18
P. indicus	**Muscle**	DEHP	15	100	9.36-297.48	89.78	72.93	81.23
		BPA	14	94	BDL -12.48	1.96	3.17	162.03
		BPAF	15	100	0.03-8.12	0.70	1.99	284.87
		BADGE	15	100	0.56-16-16	6.47	4.37	67.52
		4-NP	8	53	BDL-22.00	3.02	7.18	237.54
		TCS	1	6	BDL-1.32	-	-	-
	Liver	DEHP	15	100	13.32-328.72	145.54	111.94	76.91
		BPA	11	74	BDL -1.93	0.51	0.61	119.28
		BPAF	13	87	BDL -26.64	3.12	7.05	226.21
		BADGE	14	94	BDL -28.59	12.44	6.47	52.03
		4-NP	5	33	BDL-20.06	4.48	8.07	180.10
		BPB	1	6	BDL-5.04	-	-	-

*BDL=Below Detection Limit

Figure 2 shows the average concentrations of the five most detected EDCs in the both muscle and liver of the two fishes under investigation. For both species and in both tissues, the levels of DEHP were significantly higher than other EDCs ($p<0.05$). These results suggest that the level of EDCs in biota might reflect their concentration in the surrounding environment (Diao et al., 2017). As expected, liver had concentrations of all the detected EDCs higher than muscle (except for BPA and 4-NP in *P. indicus,* and 4-NP in *E. coioides*) in both species. These results are consistent with data previously reported in the literature (Liu et al., 2011; Zhou et al., 2019). Indeed, the concentration and the exposition time to chemicals are mostly reflected in the level of these compounds in the liver (Staniszewska et al., 2014), as they first pass through the liver before reaching the muscle (Mita et al., 2011; Staniszewska et al., 2014). In fact, a liver/muscle level ratio greater than 1 indicates a chronic exposure to EDCs (especially thought the diet), while a muscle/liver ratio concentration higher than 1 indicates a short-term exposure to pollutants (Errico et al., 2017; Staniszewska et al., 2014; Zhou et al., 2019). Hence, liver can be assumed a good indicator of marine contamination. In fact, previous studies reiterated the importance of liver in storage, redistribution, detoxification, and transformation of pollutants depending on sex and age of the organisms (Staniszewska et al., 2014; Zhou et al., 2019). In the present study, the mean liver/muscle concentration ratio for DEHP in *E. coioides* and *P. indicus* was 2 and 1.6, respectively, suggesting a prolonged exposure of the fishes to these pollutants in the Persian Gulf. On the other hand, the mean liver/muscle level of BPA and 4-NP in *P. indicus* and 4-NP in *E. coioides* were lower than 1 implying a short-term exposure to BPA and 4-NP. Hence, the recent discharge and leachates of BPA and 4-NP from different sources including plastics, domestic and wastewater effluents, and landfills (Wei et al., 2011) to the Persian Gulf did impact this marine ecosystem.

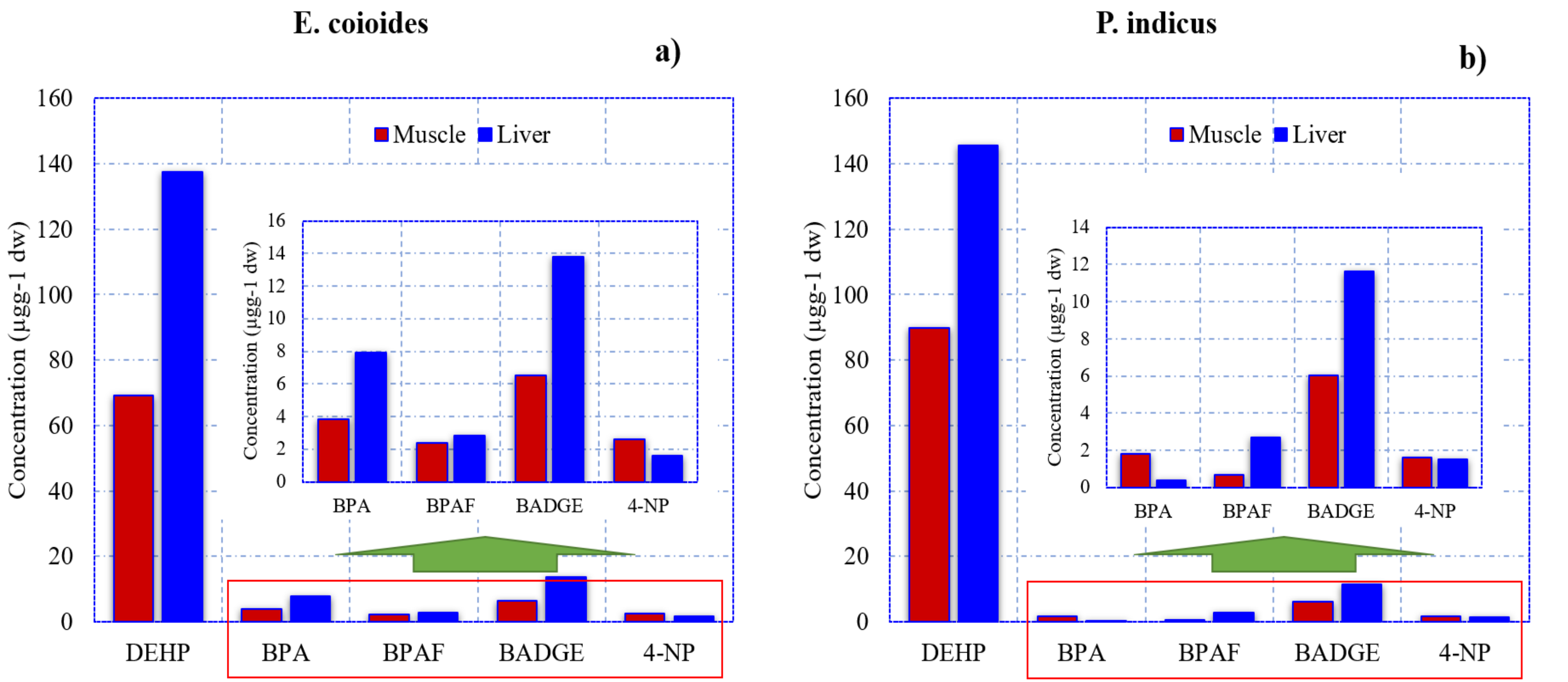

Figure 2: Histograms reporting the mean concentrations of the five predominant EDCs in a) *E.coioides* and b) *P.indicus*.

As shown in Figure 3, the mean concentration of all predominant EDCs (except DEHP) in both muscles and livers were higher in *E. coioides* than *P. indicus*. There were significant variations in the EDCs content among the studied fish ($p < 0.05$). The occurrence of diverse EDCs levels in these different fish species is reasonable as it reflects their peculiarities in ecological and nutritional requirements, feeding behaviour, living environment, growth rate, and metabolism (Jia et al., 2017; Liu et al., 2011; Zhou et al., 2019). Furthermore, since the lipid content of muscle and liver as well as trophic level of *E. Coioides* is higher than *P. indicus* (Table 1), a hypothesis that may support these results is the bioaccumulation of lipophilic EDCs in adipose tissues and their plausible biomagnification (Akhbarizadeh et al., 2020; Gu et al., 2016; Staniszewska et al., 2014; Wei et al., 2011) in the marine food web of the Persian Gulf.

Both studied fish species are demersal, and they are not large migrators, hence, the presence of EDCs in their organs (muscles and livers) might be attributed to the environmental pollution of the Persian Gulf. Indeed, sediments can act as sinks of many EDCs in aquatic organisms (Gu et al., 2016). Deposition of various pollutants on marine sediment may lead to their transportation to benthic organisms or to those that fed on benthic organisms (Diao et al., 2017; Gu et al., 2016; Wei et al., 2011). The main sources of EDCs in the Gulf might be petrochemical plants, plastics pollutants, untreated industrial and domestic effluents, regional political conflicts (such as the 1980 Iran-Iraq War and 1991 Gulf War), and atmospheric deposition (Khalililaghab et al., 2017; Khatir et al., 2019; Saeed et al., 2017; Smith et al., 2015). The presence of EDCs in the coastline and marine areas of Kuwait was investigated by Smith and co-workers (Smith et al., 2015) and the measured concentration were low (ng L^{-1}). However, the effects of EDCs on fish can be detected even where concentrations were below 1 ng L^{-1} E2 equivalent (Henneberg et al., 2014; Smith et al., 2015). Moreover, microplastics (MPs) are also known as an important source of bisphenols (especially BPA) and phthalates in the marine environment and organisms (Auta et al., 2017; Hermabessiere et al., 2017; Luo et al., 2017; Smith, 2018). Recently, some studies reported the occurrence of bisphenols and MPs in the Persian gulf's organisms (Abbasi et al., 2018; Akhbarizadeh et al., 2018; Akhbarizadeh et al., 2019; Akhbarizadeh et al., 2020; Naji et al., 2018). Apart from these, there is no further published research dealing with the concentration levels and possible sources of EDCs in the Northern edge of the Persian Gulf.

As shown in Table 4, the mean levels of BPA in *E. coioides* and *P. indicus* from Persian Gulf are much lower than results obtained from similar species in Hong Kong (Wong et al., 2017) and much higher than those obtained in fish from Pearl River delta, China (Wei et al., 2011). *P. indicus* from East China showed comparable levels of BPA with those of Persian Gulf. Moreover, the concentration of 4-NP in *P. indicus* from East China was higher than that measured in fish from the Persian Gulf (Gu et al., 2016; Niu et al., 2015). The reported values of DEHP in *E. coioides* from Hong Kong were much lower than the values measured in the flesh of the fish from the Persian Gulf (Cheng et al., 2018). To the best of our knowledge, there are no other reports indicating the presence of EDCs in muscles and livers of *E. coioides* and *P. indicus*. These differences may be attributed to the terrestrial sources of contaminants, environmental fate of the contaminants in different media such as marine water and fresh water, organisms' habitats, organisms' age, dietary sources, and the feeding of the organisms (Liu et al., 2017a; Zhao et al., 2019; Zhou et al., 2019).

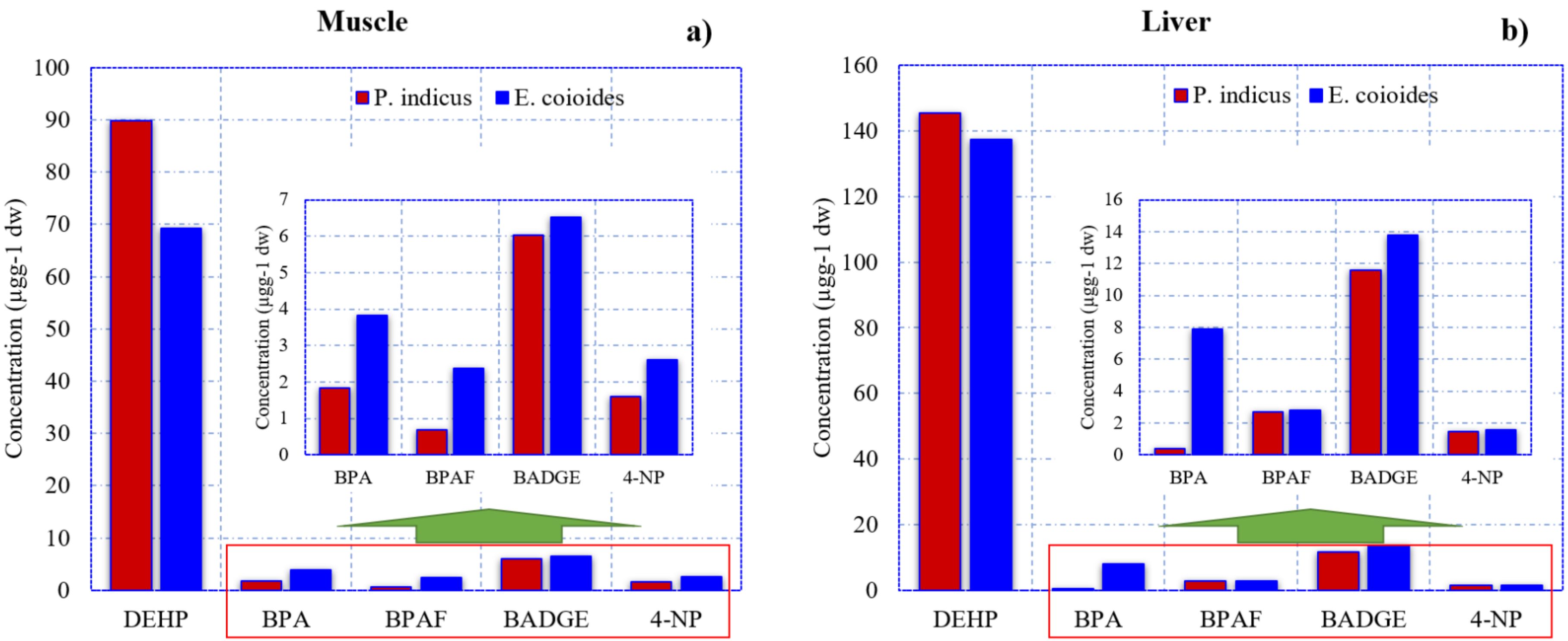

Figure 3: Comparison of the levels of EDCs in a) muscles and b) livers of *E.coioides* and *P.indicus*.

Table 4: EDCs concentration in analyzed *E. coioides* and *P. indicus* from different parts of the world (µgkg^{-1} ww).

Study area	**Species**	**Organ**	Bisphenol A (BPA) Mean + SD	4-NP Mean	DEHP Mean±SD	Reference
Hong Kong	*E. coioides*	muscle (wet weight)	8.97 ± 5.88	-	-	(Wong et al., 2017)
	P. indicus		6.20 ± 2.44	-	-	
East China Sea, China	*P. indicus*	muscle (wet weight)	2.58	158.35	-	(Gu et al., 2016)
Pearl river, China	*P. indicus*	muscle (wet weight)	0.6 ± 0.08	-	-	(Wei et al., 2011)
	E. coioides		0.7 ± 0.2	-	-	
China	*P. indicus*	muscle (lipid weight)	-	196.6	-	(Niu et al., 2015)
Hong Kong	*E. coioides*	muscle (dry weight)	-	-	0.20±0.06	(Cheng et al., 2018)

3.3. Relationships between EDCs in fish organs

The relationships between the mean concentration of each EDC in muscle and liver samples of the two studied fish species were tested using Spearman correlation analysis. The results demonstrated that the relationship between bisphenol analogues (BPAF, BPA, BPB, and BADGE) were significantly positive in muscles and livers of *E. coiodes* and in livers only of *P. indicus* (Table 5). Moreover, a significant positive relationship was found to exist between the concentrations of DEHP and BADGE and also of BPAF and 4-NP in muscles of *P. indicus* (r = 0.74 and 0.99, respectively, p < 0.05). In *E. coiodes*, the relationship between BADGE and DEHP in livers (r=0.68, p< 0.05) and 4-NP and BPAF in muscles (r=0.68, p< 0.05) were found to be significantly positive. However, no significant relationship was observed for the other investigated EDCs. The significant positive relationship between contaminants suggests similar sources and/or common environmental behaviour (co-occurrence) (Liao and Kannan, 2014; Liu et al., 2017c; Wong et al., 2017). Most of the bisphenol analogues share a strong structural similarity and alike physiochemical properties with BPA (Serra et al., 2019; Usman et al., 2019; Wang et al., 2019). Hence, their environmental behaviour and biological effects should reasonably be similar in the environment and organisms (Gramec Skledar and Peterlin

Masic, 2016). The significant positive relationship in muscles revealed a possible co-exposure risks to EDCs via fish consumption (Wong et al., 2017).

Table 5: Spearman correlation coefficients between level of EDCs in muscles and livers of *E.coioides* and *P.indicus* from the Persian Gulf.

	Muscles of *P.indicus*						**Livers of *P.indicus***						
Variables	DEHP	BPA	BPAF	BADGE	4-NP	TCS	Variables	DEHP	BPA	BPAF	BADGE	4-NP	BPB
DEHP	**1**	-0.263	-0.256	**0.741***	-0.269	-0.295	DEHP	**1**	-0.050	-0.293	0.225	0.128	-0.273
BPA		**1**	-0.102	-0.183	-0.129	0.091	BPA		**1**	-0.205	0.320	-0.140	**0.735***
BPAF			**1**	-0.351	**0.999***	-0.087	BPAF			**1**	-0.458	-0.112	-0.102
BADGE				**1**	-0.371	-0.043	BADGE				**1**	-0.442	0.246
4-NP					**1**	-0.079	4-NP					**1**	-0.078
TCS						**1**	BPB						**1**
	Muscles of *E. coioides*						**Livers of *E. coioides***						
Variables	DEHP	BPA	BPAF	BADGE	4-NP			DEHP	BPA	BPAF	BADGE	4-NP	TCB
DEHP	**1**	-0.005	-0.104	-0.180	-0.277		DEHP	**1**	-0.387	-0.206	**0.666***	-0.267	0.118
BPA		**1**	**0.793***	0.224	0.378		BPA		**1**	**0.530***	0.118	0.023	-0.172
BPAF			**1**	0.066	**0.634***		BPAF			**1**	0.216	0.229	-0.138
BADGE				**1**	-0.361		BADGE				**1**	-0.197	-0.189
4-NP					**1**		4-NP					**1**	-0.142
							TCB						**1**

*. Correlation is significant at the 0.05 level (2-tailed).

3.4. Health risk assessment

Diet is an important pathway of human EDCs intake (Diao et al., 2017; Gu et al., 2016). Previous clinical observations and epidemiological analysis indicated that EDCs can negatively affect the nervous and reproductive systems, thus causing development of obesity and cancer (Diao et al., 2017; Kabir et al., 2015). Moreover, fetuses and infants exposed to EDCs in their early stage of life could develop dysfunction or even disease in later life (Diamanti-Kandarakis et al., 2009; Diao et al., 2017). Fish consumption often contributes to a significant proportion of the total intake of EDCs in human diets (Wei et al., 2011). Since both studied fish are traded species and valuable for fish exports, we assumed the USEPA recommended meal size (0.227 kg for adults) for the estimation of health risk (USEPA, 2000). Hence, the consumption rate for ADI calculations of EDC via fish was assumed 65 g and 33 g per day for adults and children, respectively. The calculated ADIs for EDCs in both age groups are presented in Table 6.

Based on the results, the ADI_{BPA}, ADI_{DEHP}, and ADI_{BADGE} values were below the tolerable daily intake (TDI) of 4, 50, and 150 µg $kg^{-1}day^{-1}$, respectively (Dietrich and Hengstler, 2016; FDA et al., 2001; Søeborg et al., 2007). However, it should be noted that the estimated daily dietary intake may be greatly underestimated since other food commodities, including non-canned and canned foodstuff such as meat, milk, dairy products and vegetables were not included in this estimate.

The calculated HI and THQ of BPA and DEHP in the studied fish for adults and children are showed in Table 6. Since RfD_0 value was not available for BADGE and BPAF, the THQ calculation for these EDCs was not applicable. The calculated THQ_{BPA} for both age classes were well below 1. While the THQ_{DEHP} and HI were higher than 1 for children consuming both studied species and adults eating *P. indicus* twice a week. All in all, these results suggest that routine consumption of the studied fish may pose a health threat to consumers. It should be noted that such an accounted for HI does not take into account the non-dietary routes to BPA and DEHP exposure, such as the dermal contact or uptake through airways, as well as other food sources (Diao et al., 2017; Wong et al., 2017). In addition, the possible synergistic effects arising by co-exposure to various pollutants have not been included in this estimate, albeit these being well established.

Table 6: The average daily intakes (ADIs), target hazard quotient (THQ), and hazard index (HI) of EDCs through the Persian Gulf's fish consumption in two age classes. THQ and HI >1 are reported as bold.

		ADI (μg kg^{-1} day^{-1})				**HQ**		**HI**
		BPA	**BPAF**	**BADGE**	**DEHP**	**BPA**	**DEHP**	
E. coioides	Adults	8.24E-01	5.90E-01	1.50	1.49E+01	1.65E-02	7.43E-01	7.60E-01
P. indicus		4.61E-01	1.65E-01	1.52	2.11E+01	9.22E-03	1.06	1.07
E. coioides	Children	1.83	1.31E	3.33	3.30E+01	3.66E-02	1.65	1.69
P. indicus		1.02	3.66E-01	3.38	4.69E+01	2.05E-02	2.35	2.37

The EEQ of the target EDCs and EEQ_t were calculated and reported in Table 7. The EEQ_t in the edible parts of the studied fish was 0.53 and 0.63 ng g-dw^{-1} for *E. coioides* and *P.indicus*, respectively. DEHP was the most relevant compound in terms of estrogenic activity in both *E. coioides* and *P. indicus.* On the other hand, BPAF was the second contributor of estrogenic activity in the studied fishes. According to Blair *et al.* (Blair et al., 2013) the risk is low when: 0.01 ng g^{-1} < EEQ_t < 0.1 ng g^{-1}, moderate if 0.1 ng g^{-1} < EEQ_t < 1 ng g^{-1}; high if EEQ_t >1 ng g^{-1}. Our data implies that consuming both *E. coioides* and *P.indicus* from Persian Gulf represents a moderate risk for their consumers. Hence, reducing the level of EDCs in marine water and sediments should be seen as mandatory to protect marine organisms and their consumers (Diao et al., 2017).

Table 7: Estrogenic activity of EDCs in fish from Persian Gulf.

	EEQ_i (ngg^{-1} ww)				EEQ_t (ngg^{-1} ww)
	BPA	BPAF	BADGE	DEHP	
EEF_i (reference)	1.50E-05 (Leeuwen et al., 2019)	1.50E-04 (Leeuwen et al., 2019)	1.50E-05 (Leeuwen et al., 2019)	2.50E-05 (Coffin et al., 2019)	
E. coioides	0.01	0.10	0.02	0.40	0.53
P. indicus	0.01	0.03	0.02	0.57	0.63
	Contribution (%)				
E. coioides	2.50	17.89	4.55	75.07	-
P. indicus	1.19	4.24	3.92	90.65	-

The average daily intake of EDCs in terms of EEQ (ADI_{EEQ}) for adults and children was estimated and it is shown in Figure 4. As expected, the ADI_{EEQ} values for children were higher than adults. The tolerable daily intake of E2 (ADI_{E2}) is 0.05 µg kg^{-1} day^{-1}, according to the joint FAO/WHO expert committee on food and additives (JECFA) (Liu et al., 2017b). As shown in Fig. 4, the calculated ADI_{EEQ} for both age groups were much lower than ADI_{E2}, indicating no estrogenic effects of the target EDCs on fish consumer's health.

The Monte-Carlo simulation was performed to handle the uncertainty of HI, EEQ_t, ADI_{BPA}, ADI_{DEHP}, and ADI_{BADGE}, and ADI_{EEQt} via corresponding probabilistic modeling through 10,000 iterations. The results are presented in Table 8. The HI distribution indicated the probability of people with an HI > 1 was more than 35% for adults and more than 60% for children. In addition, the probability of EEQ_t between 0.1-1 ng/g-ww (moderate risk) for both age groups were higher than 60%. Moreover, the 95th percent values for ADI_{BPA} and ADI_{DEHP} were above the acceptable range in most cases. Hence, high consumption of these two fish species may pose considerable health risk to vulnerable consumers. On the other hand, the probability for ADI_{BADGE} and ADI_{EEQt} greater than tolerable daily intake of BADGE (150 µg/kg-day) and E2 (50 ng/kg-day) were less than 1%. These results indicate no estrogenic effects of the target EDCs on fish consumers' health for the USEPA recommended meal sizes.

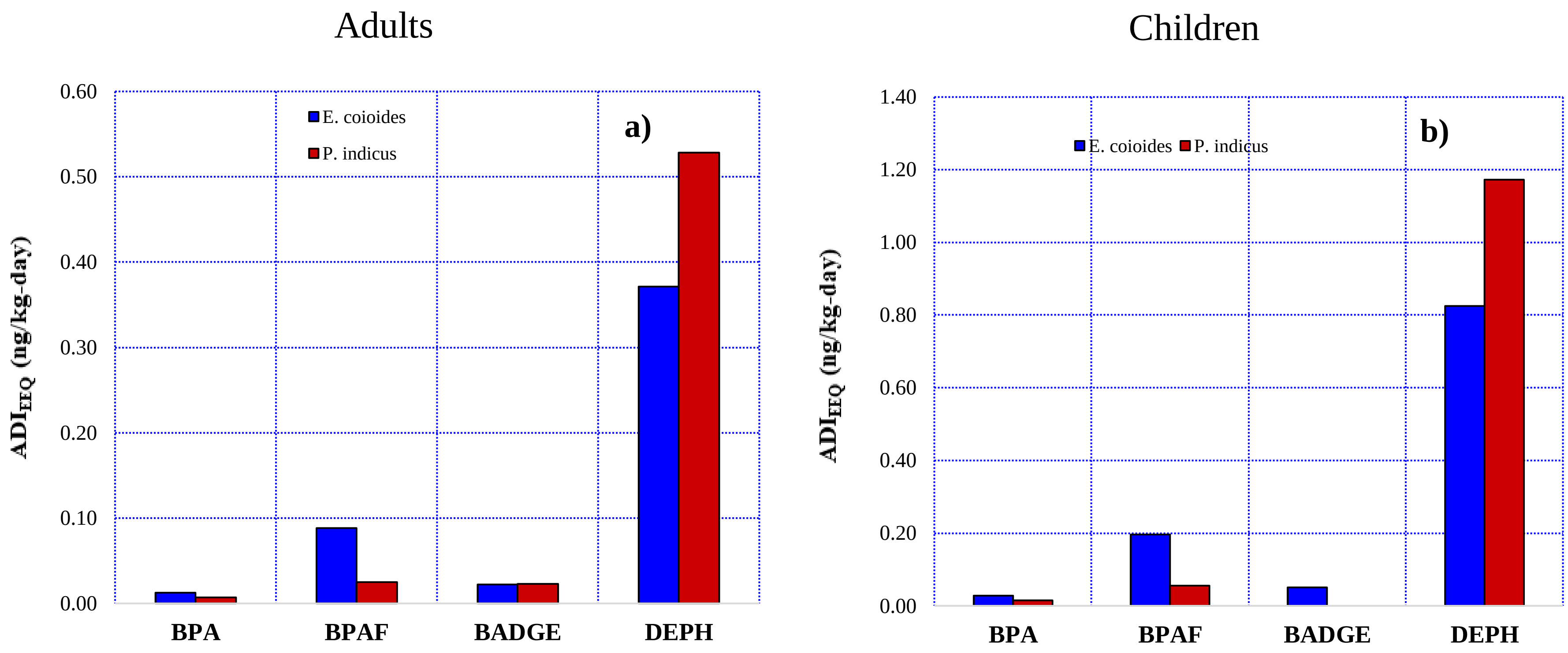

Figure 4: The average daily intake of EDCs in terms of EEQ (ADI_{EEQ}) for a: adults and b: children.

Table 8: Simulated EDCs exposures with various percentiles and model distributions by Monte Carlo simulation.

		Mean	SD	25th Percentile	50th Percentile	95th Percentile			
		EEQ$_t$ (ngg^{-1} ww)							
		Mean	SD	25th Percentile	50th Percentile	95th Percentile	Percentage of EEQ$_t$ (%)		
							0.01 -0.1	0.1 -1	>1
P. indicus		0.55	0.58	0.16	0.56	1.51	22	61	20
E. coioides		0.51	0.35	0.16	0.50	1.33	21	66	18
		HI							
		Mean	SD	25th Percentile	50th Percentile	95th Percentile	Percentage of HI> 1 (%)		
P. indicus	Adults	0.94	1.05	0.19	0.96	2.68	46		
E. coioides		0.74	0.88	0.12	0.76	2.24	32		
P. indicus	Children	2.16	2.43	0.55	2.18	6.15	68		
E. coioides		1.68	1.37	0.33	1.70	4.84	64		
		ADI$_{EEQ}$ (ngkg^{-1}day^{-1})							
		Mean	SD	25th Percentile	50th Percentile	95th Percentile	Percentage of ADI$_{EEQ}$> 50 (%)		
P. indicus	Adults	0.53	0.39	0.15	0.52	1.44	0		
E. coioides		0.48	0.46	0.16	0.48	1.24	0		
P. indicus	Children	1.17	0.86	0.35	1.15	3.13	0		
E. coioides		1.07	0.74	0.35	1.04	2.78	0		
		ADI$_{DEHP}$ (µgkg^{-1}day^{-1})							
		Mean	SD	25th Percentile	50th Percentile	95th Percentile	Percentage of ADI$_{DEHP}$ > 50 (%)		
P. indicus	Adults	20.31	22.25	5.76	19.85	55.17	8		
E. coioides		14.85	12.33	1.83	13.43	40.20	3		
P. indicus	Children	42.78	34.78	7.05	41.92	121.36	45		
E. coioides		30.64	37.92	6.12	32.16	91.21	31		
		ADI$_{BPA}$ (µgkg^{-1}day^{-1})							
		Mean	SD	25th Percentile	50th Percentile	95th Percentile	Percentage of ADI$_{BPA}$ > 4 (%)		
P. indicus	Adults	0.39	0.66	-0.27	0.36	2.01	0		
E. coioides		0.82	1.66	-0.69	0.78	4.74	9		
P. indicus	Children	0.87	1.47	-0.53	0.93	4.22	8		
E. coioides		1.83	3.7	-1.60	1.66	10.33	35		
		ADI$_{BADGE}$ (µgkg^{-1}day^{-1})							
		Mean	SD	25th Percentile	50th Percentile	95th Percentile	Percentage of ADI$_{BADGE}$ > 150 (%)		
P. indicus	Adults	1.3	0.97	0.30	1.22	3.42	0		
E. coioides		1.4	0.78	0.72	1.45	3.22	0		
P. indicus	Children	2.88	2.15	0.05	2.72	7.93	0		

4. Conclusions

To the best of our knowledge, this is the first research article reporting evidence of the occurrence of phthalate and phenolic EDCs in the seafood from the Persian Gulf. In the present work four bisphenols (BPA, BPB, BPAF and BADGE) one phthalate (DEHP), two phenol derivatives (4-NP, and TCS), and one chlorobenzene (TCB) compound were found in muscles and livers of two fish species from the Persian Gulf.

The average concentrations of EDCs determined in the muscle and in the liver of both species are many times greater than those normally found in seafood of animals that live in the sea different from that of the Persian Gulf, regardless of species investigated and from their being or not predators at the top of the food chain. This circumstance, even considering the phenomenon of biomagnification and bioaccumulation, indicates that the marine waters of the Persian Gulf are many times more polluted than those of other seas. This is justified by the high traffic of oil tankers and the anthropic activity located especially along the coasts. In addition, the concentrations found for DEHP are much greater than those of the other EDCs found in this research. Our main findings are that DEHP concentrations in the liver are greater than those found in the muscle of both fish species and that concentrations found in muscle and liver in *E.coioides* are greater than those of *P.indicus*.

With regards to risk assessment, it can be concluded that, following a diet conforming to the international guidelines (0.227 kg fish/meal) poses a moderate risk to the consumers.

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