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Polychlorinated organic pollutants (PCDD/Fs and DL-PCBs) in loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) turtles from Central-Southern Tyrrhenian Sea

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HIGHLIGHTS

- Turtles bioaccumulate levels of PCDD/Fs lower than DL-PCBs.
- Turtles living in contaminated areas accumulate high levels of PCBs in liver.
- PCB profiles found in turtles are similar to the patterns of commercial mixtures.
- Female turtles show lower concentrations of PCBs in their tissues than males.

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ABSTRACT

This study assesses for the first time the levels of PCDD/Fs and DL-PCBs in sea turtles coming from Tyrrhenian Sea. The concentrations measured in liver of the 24 specimens analysed were 6.90 *vs* 5.65 pg g^{-1} wet weight (ww) for PCDD/Fs and 10.95 *vs* 0.79 ng g^{-1} ww for DL-PCBs in *Caretta caretta* and *Chelonia mydas*, respectively. The DL-PCB levels resulted very higher in *Caretta caretta* than *Chelonia mydas* probably due to the different eating habits between the two species investigated. Furthermore, the highest levels of DL-PCBs were determined in livers of the adult *Caretta caretta* turtles of male sex. Positive correlations were found out between PCB-81 and the body mass (BM) of turtles ($r^2 = 0.6561$; $p = 0.001$) and between PCB-81 and the curved carapace length (CCL) ($r^2 = 0.6250$; $p = 0.006$) suggesting that the body burden of contaminants is related to the body size. The mean TEQ values, as a matter of risk assessment for turtles, were 3.64 *vs* 1.62 pg TEQ g^{-1} ww for PCDD/Fs and 8.72 *vs* 2.16 pg TEQ g^{-1} ww for DL-PCBs in *Caretta caretta* and *Chelonia mydas*, respectively. The results reported in this study increase the data available about the consequences of the Mediterranean Sea contamination by organochlorine pollutants and highlight an evident PCDD/F and PCB bioaccumulation in sea turtle tissues that threatens the survival of these marine organisms.

1. Introduction

Anthropic marine pollution, caused by the spill of urban and industrial wastes, harmful chemicals and debris such as plastics, glass, paper, metals and rubber into the oceans and seas, is one of the main threat to marine organisms (Kühn et al., 2016; Guzzetti et al., 2018; Kögel et al., 2020) including all species of sea turtles (Yaghmour et al., 2011; Schuyler et al., 2014; IUCN, 2019).

The Mediterranean Sea is considered a marine area subject to a risk of chemical contamination (García-Fernàndez et al., 2009), affected by environmental pollution more than oceans, due to the feature of being a semi-enclosed sea with a wide costal development (Prada et al., 2019). In particular, in this area the anthropic pressure occurs mainly through urban and industrial activities, coastal tourism, maritime traffic and fishing (Coll et al., 2012; Karadirek et al., 2019). Moreover, the Mediterranean Sea is characterized by high concentrations of marine litter and retains between 6% and 8% of the particles introduced into a global ocean

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circulation model of floating marine debris, as reported by Lebreton et al. (2012).

The Mediterranean Sea pollution is a concern especially for the marine organisms that are more responsive to environmental contaminants. In fact, according to recent studies, the contamination of this marine area may potentially threat the survival of several living species that populate it, including fish (van der Oost et al., 2003; Storelli et al., 2011; Maisano et al., 2016; van der Hal et al., 2020), seabirds (Roscales et al., 2011), marine mammals (Pinzone et al., 2015; Genov et al., 2019) and all species of sea turtles (Corsolini et al., 2000; Lazar et al., 2011; Storelli and Zizzo, 2014). Seven turtle species inhabit seas and oceans and among them the loggerhead sea turtle (*Caretta caretta*, Linnaeus, 1758) is the most common turtle in the Mediterranean Sea. The loggerhead turtle is a long-lived reptile (up to 70–80 years) belonging to the order *Testudines*, and represents the most abundant species of the family *Cheloniidae* (Margaritoulis et al., 2003; Casale and Margaritoulis, 2010). The *Caretta caretta* is a migratory species capable to move long distances between the western and eastern Mediterranean basins following a seasonal pattern, probably driven by the temperature and food availability (Bentivegna, 2002). Turtles prefer deep and warm waters near the coasts, where they spend most of their lifetime on feeding of gastropods, crustaceans, echinoderms, molluscs, fish and jellyfish. The loggerhead turtles is classified as endangered species in the IUCN red list (IUCN, 2019), whose survival is threatened mainly by marine pollution and marine litter but also by accidental capture during trawling fishing, man-made alteration of their coastal habitats, pathogens such as fibropapilloma virus and climate change (Hamann et al., 2010; Casini et al., 2018). Among environmental contaminants, persistent organic pollutants (POPs), including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), represent a problem of great concern, as a direct cause of many harmful effects (Bergeron et al., 1994; Danzo, 1997). Although these chemicals are slightly hydrosoluble, they are found in aquatic environments adsorbed to the particulate matter, that remains suspended in the water and gradually accumulate in sediments. As a result, PCDD/Fs and PCBs bioaccumulate in benthic organisms and in marine animals through the ingestion of contaminated prey (Storelli et al., 2011). Sea turtles bioaccumulate polychlorinated organic pollutants as PCDD/Fs and PCBs throughout their life; the intake occurs mainly by diet, but even from marine sediment and sea water, depending on biological factors such as age and sex of the animal (Cammilleri et al., 2017). Actually, another important exposure pathway to environmental contaminants is the ingestion of marine litter, especially plastic debris, that the turtles mistake for food (Campani et al., 2013; Pham et al., 2017; Caron et al., 2018; Clukey et al., 2018). In fact, many authors reported that plastics, in particular microplastics (MP, between 0.3 and 5 mm) are capable to adsorb on their surfaces environmental pollutants, including PCBs (Yeo et al., 2020). The accidental ingestion of plastic debris by marine organisms causes, during digestion processes, the release of the contaminants adsorbed which are accumulated into tissues of the animal (Mato et al., 2001; Pascall et al., 2005; Rios et al., 2007; Bouhroum et al., 2019; van der Hal et al., 2020).

Although PCDDs, PCDFs and PCBs are considered among the most harmful environmental contaminants, the mortality rate resulting from their bioaccumulation in living organisms is difficult to assess. Several studies showed that chronic exposures of animals and humans to these chlorinated contaminants, even at low concentrations, alters the normal function of the immune, endocrine, developmental and reproductive systems (Safe, 1993; Keller et al., 2004, 2006; Schecter et al., 2006). Regarding turtles and in general all marine animals, the toxic effects that these contaminants may provoke on their health are still little known. In the literature, it has been described that the exposure of sea turtles to PCBs causes sub-lethal effects that alter the behaviour and conservation of these species, but are not the immediate cause of death (Holliday et al., 2009; Holliday and Holliday, 2012). Moreover, Ming-ch'eng Adams et al. (2016) reported that the harmful effects of PCBs in turtles include also decrease of the integrity of the skeletal structure development, alteration of embryonic development as well as sex reversal and ontogenetic development.

The present study introduces new information on bioaccumulation levels of organochlorine contaminants in two specimens of sea turtles from the Mediterranean Sea. For this purpose, the concentrations and the patterns of the most toxic PCDDs, PCDFs and PCBs, precisely the 12 dioxin-like PCBs (DL-PCBs), were determined in livers of loggerhead turtles and green sea turtles (*Chelonia mydas*, Linnaeus 1758), stranded along the coasts of the Campania region. Moreover, these marine organisms, due to their eating, migratory habits and longevity, tend to bioaccumulate high levels of organic and inorganic pollutants (Esposito et al., 2020) and therefore are considered a good bio-indicator of the sea contamination, providing important information about the environmental quality of their marine habitat (Keller et al., 2006).

2. Materials and methods

2.1. Sampling

During the years 2017–2018, 24 stranded sea turtles (22 *Caretta caretta* and 2 *Chelonia mydas*) were found dead in different coastal areas of the Campania region, Southern Italy (Fig. 1); locations details are reported in Table S1.

All the animals were found approximately 24 h after death and examined at laboratory of Istituto Zooprofilattico Sperimentale del Mezzogiorno, where they were subjected to necropsy, morphometric analysis and genetic confirmation of the species. The biometric parameters measured for each specimen are reported in Table S1. Life stage of turtles was determined by means the curved carapace length (CCL) value: individuals were classified as juvenile (CCL < 64 cm) and adult (CCL > 64 cm) according to Guerranti et al. (2014). Fresh organs and tissues were subjected to virological, microbiological and parasitological investigations; finally, liver samples were sent to the Department of Chemistry, where they were immediately frozen at −20 °C until analysis.

2.2. Reagents and standards

All solvents were of ultra-trace analysis grade. Toluene, *n*-hexane and sulphuric acid were purchased from Carlo Erba Reagents (Milan, Italy), dichloromethane from VWR International (Radnor, Pennsylvania, United States), diethyl ether and acetone from Honeywell International, Inc. (Morristown, New Jersey, United States), *n*-nonane from Alfa Aesar by Thermo Fisher Scientific (Haverhill, Massachusetts, United States). EXtrelut® was purchased from Merck KGaA (Darmstadt, Germania) and the PowerPrep consumables from Fluid Management System (FMS), Inc. (Lexington, Kentucky, United States). All ^{13}C labelled standard solutions were provided by Cambridge Isotope Laboratories (Tewksbury, Massachusetts, Stati Uniti).

2.3. Analytical methods

Liver samples were analysed for the determination of the 7 PCDDs, 10 PCDFs, 12 DL-PCBs listed in the Commission Regulation (EU) n. 2017/644. For the purpose, samples were carefully homogenized and 2.0 g of each one was weighed, spiked with a

Fig. 1. Map of the Campania region. Stranding sites of the sea turtles are shown as closed circles.

standard solution containing the ^{13}C-isotope labelled PCDD/Fs and PCBs corresponding to the same congeners to be measured and lipid fraction extracted with diethyl ether for 24 h. The extract was filtered, dried using a rotary evaporator and cleaned up by an acidified multi-layer column followed by a three-steps purification on an automatic Power Prep® system, equipped with silica, alumina and carbon columns. The procedure described is based on the US EPA method 1613 revision B and US EPA method 1668 revision C (Lambiase et al., 2017).

2.4. Instrumental analysis

Instrumental analysis was performed by a high resolution gas chromatograph coupled with a high resolution mass spectrometer (DFS Magnetic Sector GC-HRMS system, Thermo Fisher Scientific). The splitless mode was used for the samples injection and the temperature of the inlet and the transfer-line was fixed at 280 °C. The chromatographic separation of PCDDs, PCDFs and four DL-PCB non-ortho congeners (coplanar 77, 81, 126 and 169) was performed on a TR-1 (60 m, id 0.25 mm, 0.1 μm, Thermo Fisher Scientific) fused silica capillary column, coated with 5% phenyl, 94% methyl, 1% vinyl silicone; on the other hand, eight PCB mono-ortho congeners (105, 114, 118, 123, 156, 157, 167, 189) were separated on a HT 8 (60 m, id 0.25 mm, 0.25 μm, SGE Analytical Science) fused silica capillary column, coated with 8% phenylpolycarborane-siloxane. For both chromatographies, 99.9999% purity helium was used as carrier gas, at 1.2 mL min^{-1} constant flow rate. The high resolution mass spectrometer tuning was performed at a resolution of 10,000 (10%

peak valley) and the data acquisition was carried out using the multiple ion detection (MID) analysis monitoring two isotopic masses for each PCDD/F and PCB congener to be measured.

2.5. Data collection and statistical analysis

Quality assurance and quality control (QA/QC) of the analysis were verified through control samples including blanks, spikes and duplicates according to the US EPA methods 1613 and 1668. In addition, QC includes the participation in proficiency tests and inter-laboratory studies achieving z-scores always within the range ±2. PCDD and PCDF concentrations were expressed in pg g^{-1} wet weight (ww) as sum of 17 congeners and DL-PCB concentrations in ng g^{-1} as sum of 12 congeners, using the upper-bound approach. Since the toxic equivalency factors (TEFs) for reptiles have not yet been determined, the toxic equivalent (TEQ) concentrations were calculated using the TEFs for birds (Van den Berg et al., 1998) and expressed in pg g^{-1} for both PCDD/Fs and DL-PCBs. Student's *t*-test with 95% confidence interval was performed to compare the data groups. For statistical analysis, the congeners below the LOQ were considered equal to LOQ (Cammilleri et al., 2017). Correlation between bioaccumulation of PCBs and size of turtles (CCL and BM) was carried out using the Spearman rank correlation test.

3. Results and discussion

3.1. PCDD/F and DL-PCB concentrations in liver of sea turtles

PCDD, PCDF and DL-PCB levels determined in liver of the sea turtles analysed in this study are reported in Tables 1 and S2; their concentrations followed the order DL-PCBs ≫ PCDD/Fs. The PCDD/F levels found out in the two species investigated resulted very low. In particular, the mean concentrations determined were 3.41 pg g^{-1} ww for PCDDs and 3.50 pg g^{-1} ww for PCDFs in *Caretta caretta* turtles and 2.39 pg g^{-1} ww for PCDDs and 3.26 pg g^{-1} ww for PCDFs in *Chelonia mydas* turtles. Statistical analysis performed using Student's *t*-test, showed no significant differences ($p > 0.05$) between the levels of PCDDs and PCDFs and neither between the concentrations of these pollutants determined in the two species analysed. Among the 17 PCDD/Fs, only 18% of congeners resulted above the LOQs and the most detected were 2,3,7,8-TCDF, 1,2,3,4,6,7,8-HpCDF and OCDD detected in 75%, 71% and 38% of samples, respectively. Their mean contribution to the total PCDD/Fs were 8.1% (mean: 0.51 pg g^{-1} ww), 9.1% (mean: 0.55 pg g^{-1} ww) and 16.3% (mean: 1.07 pg g^{-1} ww), respectively. The congener 2,3,7,8-TCDD of great toxicological interest was found out only in 3 turtles at very low concentration (mean: 0.26 pg g^{-1} ww).

In the literature there are still few studies concerning the PCDD/F levels in sea turtles, therefore, it is difficult to have a clear overview on contamination of these reptiles. Moreover, a comparison between the concentrations measured in this study and those determined in turtles coming from other marine areas is difficult to realize, also because the analytical methods (extraction, purification and instrumental techniques employed and number of congeners measured) and the approaches used for the expression of the results (wet weight or lipid weight) are often different. Nevertheless, the concentrations measured in the present study were compared to the levels of PCDD/Fs found out in the turtles from the Adriatic Sea, stranded along the Apulian coast, as reported by Storelli and Zizzo (2014). The range of concentrations found out in these turtles (0.20 pg g^{-1} ww - 24 pg g^{-1} ww) was slightly different compared to that measured in this study, while the mean concentration (7 pg g^{-1} ww) was very similar. According to this study, the predominant congeners that they determined were 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 2,3,7,8-TCDF and 1,2,3,4,7,8,9-HpCDF. Contrariwise, significant levels of PCBs were determined in all sea turtles as reported in Tables 1 and S2. The mean concentrations determined into the liver samples were 10.95 ng g^{-1} ww in *Caretta Caretta* turtles and 0.79 ng g^{-1} ww in *Chelonia mydas* turtles. Although the PCB levels found out in the loggerhead turtles resulted higher than those found in the green turtles, it was not possible to perform statistical tests because the number of *Chelonia mydas* specimens found was very small, thus not comparable to *Caretta caretta*. Anyway, the difference in DL-PCB concentration was probably due to the different eating habits between the two species. In fact, the adult *Chelonia mydas* turtles are herbivores and feed mainly on macroalgae and phanerogams (da Silva et al., 2016); this behaviour leads them to a minor accumulation of lipophilic contaminants than the *Caretta caretta* turtles, which are omnivorous animals. Moreover, the highest DL-PCB concentrations were determined in livers of adult *Caretta caretta* turtles of male sex as shown graphically by the mean values reported in the box plots (Fig. 2). This result is attributable to the capability of bioaccumulation of these POPs. In fact, turtles, as well as other animals, accumulate environmental contaminants during their entire life, consequently the higher DL-PCB concentrations in adults than juveniles were likely due to a longer exposition period to these

Table 1
PCDD, PCDF (pg g^{-1} ww), DL-PCB (ng g^{-1} ww) and WHO-TEQ (pg g^{-1} ww) concentrations in liver of stranded turtles from the Campania coasts.

	Caretta caretta turtles (n = 22)	
Congener	Mean ± SD	Congeners above the LOQs (%)
PCDDs		
2,3,7,8-TCDD	0.09 ± 0.08	13.6
1,2,3,7,8-PeCDD	1.99 ± 1.68	0
1,2,3,4,7,8-HxCDD	0.04 ± 0.02	0
1,2,3,6,7,8-HxCDD	0.04 ± 0.02	0
1,2,3,7,8,9-HxCDD	0.04 ± 0.02	0
1,2,3,4,6,7,8-HpCDD	0.14 ± 0.13	13.6
OCDD	1.07 ± 1.21	36.4
PCDFs		
2,3,7,8-TCDF	0.53 ± 0.45	72.7
1,2,3,7,8-PeCDF	0.49 ± 1.17	13.6
2,3,4,7,8-PeCDF	0.91 ± 1.30	27.3
1,2,3,4,7,8-HxCDF	0.15 ± 0.17	13.6
1,2,3,6,7,8-HxCDF	0.11 ± 0.08	9.1
1,2,3,7,8,9-HxCDF	0.07 ± 0.04	0
2,3,4,6,7,8-HxCDF	0.16 ± 0.23	18.2
1,2,3,4,6,7,8-HpCDF	0.49 ± 0.42	68.2
1,2,3,4,7,8,9-HpCDF	0.07 ± 0.07	9.1
OCDF	0.51 ± 0.48	9.1
PCDD/Fs[a]	6.90 ± 3.60	
PCDD/Fs[c]	3.63 ± 2.51	
DL-PCBs		
PCB 77	0.04 ± 0.03	100
PCB 81	0.01 ± 0.01	90.9
PCB 105	1.89 ± 3.14	100
PCB 114	0.23 ± 0.39	100
PCB 118	5.88 ± 9.87	100
PCB 123	0.10 ± 0.21	81.8
PCB 126	0.06 ± 0.07	100
PCB 156	1.38 ± 2.74	100
PCB 157	0.29 ± 0.54	100
PCB 167	0.90 ± 1.50	100
PCB 169	0.01 ± 0.01	95.5
PCB 189	0.17 ± 0.31	100
PCBs[b]	10.95 ± 18.40	
PCBs[c]	8.72 ± 8.98	

[a] Sum of the analytical concentrations of the 17 PCDD/F congeners.
[b] Sum of the analytical concentrations of the 12 DL-PCB congeners.
[c] Sum of the WHO-TEQ concentrations calculated using the TEFs.

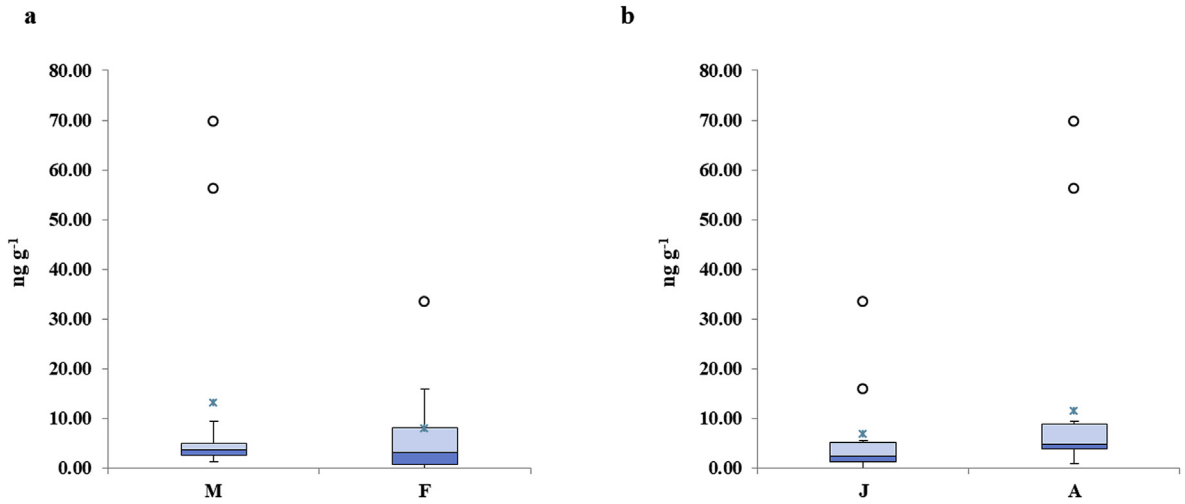

Fig. 2. Box plot of the DL-PCB concentrations (ng g^{-1} ww) determined in liver of *Caretta caretta* turtles sorted by a) sex and b) age of specimens. The graphs show the first and third quartiles, the medians and the means. The lowest and highest concentrations, excluding outliers, are represented by the lower and the upper whiskers, respectively (° = outliers; * = mean values; M = male; F = female; J = juvenile; A = adult).

substances. Furthermore, the DL-PCB concentrations in male specimens resulted higher than in females. Many authors describe that females show lower concentrations of environmental contaminants in their tissues than males because they may transfer part of their contaminant burden to the progeny (McKenzie et al., 1997; García-Besné et al., 2015; Ming-ch'eng Adams et al., 2016). In fact, Guirlet et al. (2010) in a study on the maternal transfer of chlorinated contaminants in the leatherback turtles, found out that all contaminants detected in blood of females were also detectable in their eggs.

Studies on ecological and health risk assessment report that PCBs may also affect the morphology of the turtles (Salice et al., 2014; Ming-ch'eng Adams et al., 2016). Holliday et al. (2009), studying the impact of PCBs on the growth of diamondback terrapins, observed that all individuals exposed to PCB 126 grew significantly smaller in size (length and mass) than uncontaminated turtles. In this regard, the concentrations of total DL-PCBs and those of the single congeners determined in the current study should be correlated to the BM and CCL of the turtles, which are morphometric parameters related to the age of the animal. Slight positive correlations, statistically significant, were obtained between CCL and PCB 81 ($r^2 = 0.6250$; $p = 0.006$) and BM and PCB 81 ($r^2 = 0.6561$; $p = 0.001$). Therefore, the statistical tests showed that the highest concentrations of PCB 81 were in turtles with higher CCL and BM values. Since the CCL is used to determine the age of the animals, these results confirmed that the highest PCB concentrations were detectable in adults because to their longer exposition period. Similar correlations were found out also by other authors (Kelly et al., 2008; Holliday et al., 2009; Lazar et al., 2011; Salice et al., 2014). Kelly et al. (2008) explained the positive relationships between blood PCB levels and size of female and male *Chelydra serpentina* turtles supposing that larger specimens feed on preys with higher PCB concentrations than smaller specimens. Lazar et al. (2011) also hypothesised that the correlations between PCBs and size were due to feeding behaviour of turtles; in their study in fact, oceanic stage loggerheads with CCL< 40 cm, which feed mainly on planktonic organisms, showed lower PCB levels than neretic stage loggerheads with CCL> 40 cm, which instead feed mainly on benthic organisms.

Specific patterns of the 12 DL-PCB congeners, characteristic of the tissue and the species analysed, were observed. These patterns were penta-CB > hexa-CB > tetra-CB ≈ hepta-CB in *Caretta caretta* turtles and penta-CB > hexa-CB > tetra-CB > hepta-CB in *Chelonia mydas* turtles (Fig. 3). In both species, penta-CB and hexa-CB amounted to more than 96% of the total residue. Furthermore, similar profiles were observed also comparing juvenile specimens with adults and females with males. These congener distributions resulted very similar to the patterns in some of the most common PCB mixtures used in the past years, such as Aroclor 1254, which contained penta-CB (49.3%) > hexa-CB (27.8%) > tetra-CB (7.1%) > hepta-CB (3.9%) > octa-CB ≈ nona-CB (<0.05%) (IARC, 2016; Lavandier et al., 2019). The correspondence between the PCB profile of Aroclor 1254 and those found out in the liver of sea turtles analysed suggests that the contamination source had most likely an industrial origin.

Among DL-PCBs, the levels of the 8 mono-*ortho* congeners (105, 114, 118, 123, 156, 157, 167 and 189) resulted higher than the levels of the 4 non-*ortho* congeners (77, 81, 126 and 169) in both species of sea turtles analysed. The concentrations ranged between 0.03 ng g^{-1} ww and 69.29 ng g^{-1} ww for the mono-*ortho* PCBs and between 0.01 ng g^{-1} ww and 0.40 ng g^{-1} ww for non-*ortho* PCBs. The most abundant congeners determined in livers were mono-*ortho* PCBs 118, 105, 156 and 167; their mean contribution to the total DL-PCBs were 51.7% (mean: 5.43 ng g^{-1} ww) for PCB 118, 17.3% (mean: 1.75 ng g^{-1} ww) for PCB 105, 11.5% (mean: 1.27 ng g^{-1} ww) for PCB 156 and 9.0% (mean: 0.83 ng g^{-1} ww) for PCB 167. Hence, non-*ortho* PCBs represented only a small fraction of the total DL-PCBs which was less than 4.0% of the total residue. Among non-

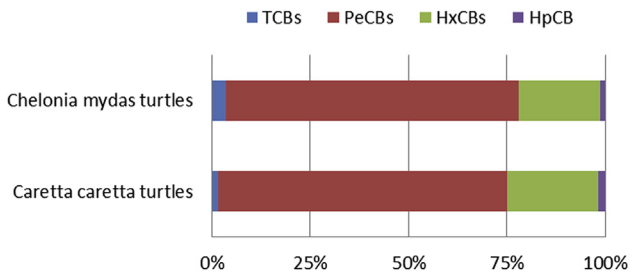

Fig. 3. Contributions of the single congeners to the sum of the 12 DL-PCBs in livers of *Caretta caretta* and *Chelonia mydas* turtles. (TCBs = Tetrachlorobyphenyls; PeCBs = Pentachlorobyphenyls; HxCBs = Hexachlorobyphenyls; HpCBs = Heptachlorobyphenyls).

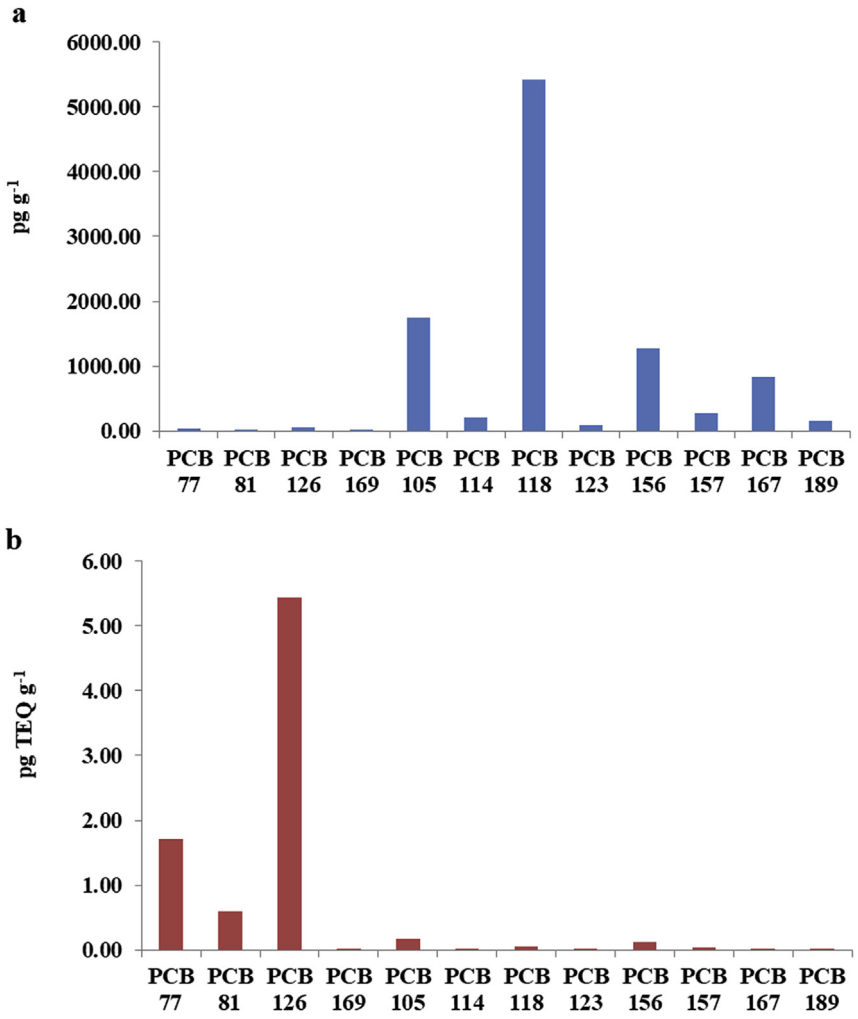

Fig. 4. Mean concentrations of DL-PCBs in livers of sea turtles reported as analytical concentrations expressed in pg g^{-1} ww (a) and analytical concentrations multiplied by TEFs expressed in pg TEQ g^{-1} ww (b).

ortho PCBs, the concentrations decreased in the order PCB 126 > PCB 77 > PCB 169 > PCB 81 in *Caretta caretta* turtles and PCB 77 > PCB 126 > PCB169 > PCB 81 in *Chelonia mydas*. Similar patterns were observed also in liver of the loggerhead turtles from north Adriatic Sea analysed by Corsolini et al. (2000) who found out a high prevalence of mono-*ortho* congeners although in concentrations lower than those determined in the current study. Storelli et al. (2007) instead measured similar levels of the PCBs 118, 105 and 156 in liver of the loggerhead turtles form Adriatic and Ionian Sea, while the mean concentration of non-*ortho* PCB 77 (0.11 ng g^{-1} ww) was about 3 times higher than the concentrations determined in this study and PCBs 126 and 169 resulted below the detection limit in all samples analysed. Also the concentrations of non-*ortho* PCBs found out in liver of green turtles coming from the Hawaiian Island showed a pattern similar to that observed in the green turtles stranded along the coasts of the Campania region (Miao et al., 2001). This characteristic bioaccumulation pattern of the 12 DL-PCBs in liver of the sea turtles, is a direct consequence of a higher presence in the marine environment of some congeners, but is also due to the ratio and amount of PCB metabolism which depends on the presence of vicinal proton position in the molecule (Grimm et al., 2015). The congeners with *ortho-metha* vicinal protons are substrates for CYP1A that is little expressed in turtles; hence, these PCBs are bioaccumulated in turtle tissues more than other congeners (Richardson et al., 2010; Grimm et al., 2015).

3.2. WHO-TEQ concentrations

For assessing the impact of PCDD/Fs and DL-PCBs on health of sea turtles and to evaluate the harmful effects that these toxic pollutants may provoke to them, the concentrations of each congeners were calculated also as 2,3,7,8-TCDD toxic equivalent (TEQ) concentrations. The mean TEQ concentrations found out in liver of the sea turtles analysed in this study were 2.09 pg TEQ g^{-1} ww for PCDDs and 1.55 pg TEQ g^{-1} ww for PCDFs in *Caretta caretta* turtles and 1.07 pg TEQ g^{-1} ww for PCDDs and 0.56 pg TEQ g^{-1} ww for PCDFs in *Chelonia mydas* turtles. The congeners that gave the highest contribution to the total PCDD/F TEQ values were 2,3,7,8-TCDF (19.4%) and 2,3,4,7,8-PeCDF (20.1%), while the contributions of 1,2,3,4,6,7,8-HpCDF and OCDD became negligible, 0.3% and <0.01% in terms of TEQ, respectively. These results were in accordance with the data reported by Storelli and Zizzo (2014), although the PCDD/Fs TEQ concentrations found out in the turtles coming from the Central-Southern Tyrrhenian Sea resulted slightly higher than those measured in the turtles from Adriatic and Ionian Sea. The mean DL-PCBs TEQ concentrations were 8.72 pg TEQ g^{-1} ww in *Caretta Caretta* turtles and 2.16 pg TEQ g^{-1} ww in *Chelonia mydas* turtles, resulting lower than those determined in the turtles from

Adriatic and Ionian Sea (Storelli and Zizzo, 2014) and from the Baja California peninsula of Mexico (Richardson et al., 2010). The amount of each DL-PCB in relation to total TEQ concentration, which was calculated by multiplying the analytical concentration of each compound by its TEF, resulted very different if compared to the results calculated without TEFs (Fig. 4). In fact, the sum of the 4 non-*ortho* congeners accounted for the 96.4% of the total DL-PCBs TEQ value, while the sum of the 8 mono-*ortho* congeners represented only the 3.6%. Hence, considering TEQ concentrations, the turtle exposure to PCBs consisted almost totally of the non-*ortho* congeners, which are the PCBs that cause more concern due to their high toxicity. Studies for risk assessment report that birds, that are considered similar to reptiles and therefore to turtles for the three taxa, are more responsive to non-*ortho* PCBs than fish and mammals; in fact, for birds, the TEF values fixed for PCBs 77 and 81 are higher than those fixed for other organisms (Van den Berg et al., 1998). Moreover, it is important to consider that the observations on toxicity of non-*ortho* PCBs are related to the induction of hepatic CYP1A1 protein which has been demonstrated to be low expressed in turtles (Van den Berg et al., 1998; Richardson et al., 2010; Grimm et al., 2015). Moreover, birds have also been showed a high sensibility to mono *ortho* PCBs; hence, considering the concentrations found out in the current study, it was possible to hypothesize that this group of congeners might have caused harmful effects on the health of the turtles (Van den Berg et al., 1998).

In terms of TEQ, the pattern observed in livers of turtles was PCB 126 (62.1%) > PCB 77 (27.4%) > PCB 81 (6.8%) > PCB 105 (1.6%) > PCB 156 (1.0%) > PCB 118 (0.5%) > PCBs 157 and 114 (0.2%) followed by PCBs 169, 167, 189 and 123 (<0.1%). Similar PCB profiles were found out also in the turtles coming from other marine areas (Richardson et al., 2010; Storelli and Zizzo, 2014). Moreover, the statistical analysis of the results showed a significant correlation between some morphometric parameters (BM and CCL) and PCB 81 which is, together with PCB 126, the DL-PCB congener that exhibit the highest toxicity in relation to 2,3,7,8-TCDD.

4. Conclusion

This study assesses for the first time the PCDD, PCDF and DL-PCB contamination levels in liver of *Caretta caretta* and *Chelonia mydas* turtles from Central-Southern Tyrrhenian Sea. The results described an evident accumulation of these organochlorine contaminants in the turtles indicating their widespread presence in the investigated area. Although the levels of PCDD/Fs resulted lower than those of DL-PCBs, it is well known that these polychlorinated compounds may cause very toxic effects even at low concentrations. However, it remains very difficult to attribute the death of turtles to the PCDD/F and DL-PCB levels assessed in their liver because the harmful effects provoked by these contaminants could manifest slowly over time and, moreover, the TEFs for birds, used for health risk assessment, might not be completely suitable for marine reptiles. Nevertheless, considering that the negative long-term effects caused by these organochlorine contaminants on health of living organisms have been widely described, it is possible to assume that the levels of PCDD/Fs and DL-PCBs found in turtles potentially threatened their survival. Further surveys to monitor marine pollution and damages induced by it over time are needed in order to find new solutions to protect turtles and safeguard ecosystems.

Credit author statement

Conceptualization: Esposito; Lambiase; Fiorito. Methodology: Serpe. Software: Lambiase; Serpe. Validation: Esposito; Fiorito. Formal analysis: Lambiase; Iaccarino; Pilia. Investigation: Lambiase; Iaccarino; Pilia. Resources: Esposito; Iaccarino. Data curation: Lambiase; Iaccarino; Pilia. Writing – original draft: Lambiase. Writing – review and editing: Serpe. Visualization: Lambiase; Serpe. Supervision: Gallo. Project administration: Esposito. Funding acquisition: -.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.128226.

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