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# First basin-scale assessment of PCB and DDT contamination in Mediterranean zooplankton: a baseline for the central–southern basin

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ABSTRACT

This study provides the first basin-scale assessment of polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) in zooplankton collected from the central–southern Mediterranean Sea. Zooplankton samples ($N = 26$) were collected along a ~ 1950 nautical mile transect (May–July 2025) and quantified for 24 PCB congeners and six DDT compounds using gas chromatography–mass spectrometry (GC–MS). QA/QC procedures were validated using certified reference material (recoveries 80–117%). PCBs were ubiquitously detected, with concentrations ranging from below detection limits to 96.5 ng $g^{-1}$ dry weight (dw) (mean 27.7 ± 20.2 ng $g^{-1}$ dw) and were dominated by penta- and hexachlorinated congeners. DDTs were detected less frequently (54.5% of samples), with lower concentrations (mean 3.30 ± 5.62 ng $g^{-1}$ dw), primarily as degradation products (DDE and DDD), indicating legacy inputs. Spatial patterns revealed higher PCB levels in coastal areas affected by industrial and urban pressures (e.g. Athens and eastern Sicily), while DDTs were more associated with regions influenced by historical agricultural use and riverine inputs (e.g., western Greece). Offshore sites showed low contamination levels, highlighting the influence of land-based sources. Comparative analyses revealed significantly lower PCB and DDT concentrations than those reported for the Tyrrhenian Sea (ANOVA, $p < 0.0001$; Kruskal-Wallis, $p = 0.0116$), supporting a longitudinal contamination gradient across the Mediterranean basin. This study provides a baseline for the central–southern Mediterranean and confirms zooplankton as an effective POP bioindicator. Future studies should extend monitoring and integrate chemical and eDNA approaches to improve basin-scale assessments of Mediterranean marine ecosystems.

Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT), have affected global ecosystems and human health. Their widespread impact arises from extensive historical use and production, combined with their remarkable environmental persistence, strong tendency to bioaccumulate, and significant toxic effects (Ross and Birnbaum, 2003). Although banned or heavily restricted for decades, these compounds continue to be detected worldwide, from heavily populated coastal regions to remote marine environments, due to their persistence, long-range transport, and continuous remobilization from environmental reservoirs. Following the ratification of the Stockholm Convention in 2004, regulatory measures were adopted worldwide to restrict their production and use (UNEP, 2019). The global ocean acts as a major receptor for POPs, making the assessment of spatiotemporal contamination trends in the marine environment essential for understanding their distribution, transport, and fate, as well as for evaluating the effectiveness of chemical management strategies.

The Mediterranean Sea is recognized as particularly vulnerable to POPs contamination: its semi-enclosed nature, together with high population density and intense industrial, touristic, and agricultural pressures, has resulted in significant pollutant accumulation in multiple areas (Berrojalbiz et al., 2011; Boldrocchi et al., 2023a; Villa et al., 2024). Despite this vulnerability, comprehensive basin-wide monitoring remains limited, with most available data derived from sporadic studies conducted in specific locations, leaving extensive portions of the Mediterranean Sea insufficiently investigated (Boldrocchi et al., 2023a). Previous research has documented PCB and DDT contamination throughout the Mediterranean Sea, including seawater and plankton at

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the basin scale (Berrojalbiz et al., 2011), sediments from the eastern Mediterranean and Aegean Sea (Kucuksezgin and Tolga Gonul, 2012; Pazi et al., 2011), and zooplankton from the Tyrrhenian and Adriatic Seas (Boldrocchi et al., 2023a; Villa et al., 2024). However, information from the central–southern Mediterranean remains fragmented, preventing a comprehensive basin-scale assessment. In the central-southern Mediterranean Sea, existing research has largely focused on legacy POPs such as PCBs and DDTs through localized investigations targeting sediments or higher-trophic-level biota. Several studies have employed fish and bivalves as bioindicators in highly impacted coastal areas, including İzmir Bay and Çandarlı Bay (Muzyed et al., 2017; Yurdakul and Kucuksezgin, 2020; Gonul et al., 2018; Gonul, 2024), while others have concentrated on sediment contamination in geographically restricted environments such as the İzmir Gulf and Keratsini Harbor (Galanopoulou et al., 2005; Pazi et al., 2011, 2012). Only a limited number of investigations have addressed broader spatial scales, either along the eastern Aegean coast (Kucuksezgin and Tolga Gonul, 2012) or at the basin scale (Parinos et al., 2026). Zooplankton is widely recognized as an effective bioindicator of marine water quality, playing a fundamental role in the trophic web and linking primary producers to higher trophic levels, thereby influencing contaminant transfer across food webs (Alfonso et al., 2023; Bettinetti et al., 2012; Piscia et al., 2023; Boldrocchi et al., 2020, 2022, 2023a, 2023b, 2025). While recent large-scale assessments of PCB and DDT contamination in zooplankton have been conducted in other Mediterranean sub-basins, such as the Tyrrhenian Sea (Boldrocchi et al., 2023a), the Adriatic Sea (Villa et al., 2024), and the northwestern Mediterranean Sea (Boldrocchi et al., 2026), studies in the central-southern Mediterranean remain scarce. Most available research in this area remains geographically fragmented and mostly limited to trace metals in coastal systems such as İzmir Bay and Edremit Bay (Kontas, 2008; Uluturhan et al., 2023). To date, only one study has investigated POPs in zooplankton over a broader portion of the central-southern Mediterranean Sea, based on a limited dataset collected in 2006 and excluding DDTs (Berrojalbiz et al., 2011).

This study provides the first basin-scale assessment of PCB and DDT contamination in zooplankton from the central-southern Mediterranean Sea, addressing a critical gap in current knowledge. By focusing on a biological indicator that is widely used worldwide but largely overlooked in this region, this work contributes essential baseline data for future monitoring efforts, trophic transfer assessments, and ecological risk evaluation in the Mediterranean marine environment. Moreover, the inclusion of scattered and remote stations strengthens the basin-scale perspective, allowing POPs monitoring to capture both localized coastal inputs and signals of long-range transport in offshore areas. This broad spatial coverage is particularly important for distinguishing local contamination hotspots from regional background conditions and for improving our understanding of pollutant distribution across interconnected Mediterranean sub-basins.

Field sampling has been carried out within the fourth campaign of the *Marine Adventure for Education and Research* (M.A.R.E.) initiative, carried out by Centro Velico Caprera (https://www.centrovelicocaprera.it/) and One Ocean Foundation (https://www.1ocean.org/), in the central-southern Mediterranean Sea. Zooplankton samples were collected on a daily basis, when possible, using a 200 µm mesh net, vertically at a depth of 20 m from the surface. The sampling campaign commenced in Taranto (Italy) on 17 May and followed a multi-basin transect across the central and eastern Mediterranean Sea (Fig. 1). From Taranto, the research catamaran navigated southward along the Ionian coastline before reaching Corfù Island. The route then extended through the Ionian archipelago, sequentially including Paxos, Lefkada, Kefalonia, and Zakynthos, while closely following the Peloponnese coastline toward the islands of Kithira and Antikithira (Fig. 1). Subsequently, the vessel entered the Aegean Sea, surveying the northern coastline of Crete before continuing eastward through the Dodecanese archipelago (Kasos, Karpathos, Rhodes, Kos, and Patmos). The cruise then proceeded westward across the Cyclades, reaching Athens and transiting the Corinth Gulf, before returning to the Ionian Sea via open waters and circumnavigating the eastern coast of Sicily in a clockwise direction (Fig. 1). The campaign concluded on 24 July 2025, providing extensive spatial coverage across the Mediterranean basin and encompassing a wide range of hydrographic and biogeochemical settings. Over a total of 68 days of navigation, the cruise covered approximately 1950 nautical miles and yielded 26 zooplankton samples collected along distinct Mediterranean seascapes (Table S1, Supplementary Materials).

Upon arrival at the laboratory at the University of Insubria (Como, Italy), zooplankton samples were freeze-dried for 72 h and weighed. Due to insufficient biomass at certain stations, eight low-biomass samples were pooled into four composite samples (E4 + E5, E6 + E7, E8 + E9 and E15 + E16) based on geographical proximity to meet the minimum requirement of 0.2 g dry weight (dw) for chemical analysis. The pooled samples were subsequently treated as single sampling units in all statistical analyses, and the final dataset comprised 26 samples. Analyses targeted six DDT isomers (p,p'-DDT, -DDE, -DDD; o,p'-DDT, -DDE, -DDD) and 24 PCB congeners (18, 28, 31, 52, 77, 95, 99, 101, 105, 110, 118, 123, 126, 128, 138, 146, 149, 153, 156, 169, 170, 180, 183, and 187). Concentrations are reported as ng $g^{-1}$ dw. Extraction and analytical procedures followed established protocols (e.g. Bettinetti et al., 2012). Briefly, ~0.2 g dw of each sample was Soxhlet-extracted for 2 h with 50


**Fig. 1.** Sampling route and zooplankton sampling stations across the central-southern Mediterranean Sea during the 2025 field campaign. This map was created by the authors using QGIS software (version 3.34.7).

mL of acetone:n-hexane (1:1, *v*/v). Lipids were removed by digestion with 7 mL concentrated $H_2SO_4$ (98%; Carlo Erba, Italy), and the hexane phase was purified using Florisil column cleanup (n-hexane:dichloromethane, 85:15). Extracts were concentrated to 0.5 mL under nitrogen and analyzed by gas chromatography–mass spectrometry (GC–MS; Thermo Fisher GC Trace 1600 coupled to a single-quadrupole ISQ 7610) equipped with a PTV injector and autosampler. Separation was achieved using a TG-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film), with detection in selected ion monitoring (SIM) mode.

Quality control and assurance procedures were rigorously applied. Quantification relied on certified multi-analyte PCB and DDT standards and eight isotopically labelled internal standards. Analytical performance was verified using certified reference material SRM 1946, yielding recoveries of 79.6–116.7% for PCBs and 84.8–102.4% for DDTs. Method limits of detection ranged from 0.1 to 0.2 ng $g^{-1}$ dw. For statistical analyses and the calculation of descriptive statistics (means and standard deviations), values below the limit of detection (<LOD) were replaced by LOD/2. Detection frequencies were calculated based on analyte occurrence, whereas only reliably quantified concentrations were used for quantitative comparisons and statistical analyses. Concentrations reported in the text and tables are indicated as <LOD where appropriate. Procedural blanks were processed with each analytical batch to assess potential contamination.

To support the interpretation of chemical data and provide qualitative information on planktonic community composition, environmental DNA (eDNA) analyses were performed. Cost-effective and non-invasive approaches for characterizing marine biodiversity are increasingly needed to support large-scale ecological assessments (Rezzolla et al., 2014), and eDNA metabarcoding has emerged as a powerful tool for detecting and monitoring marine biodiversity (Thomsen and Willerslev, 2015). Up to 10 water samples of 5 L each were collected using NatureMetrics eDNA filtration kits along the sampling route (Fig. S1, Supplementary Materials). Water was filtered through a 0.8 μm polyethersulfone membrane, and eDNA was preserved following the manufacturer's instructions (NatureMetrics, UK). All samples were taken from the water surface using a pre-cleaned pump, with operators wearing gloves to minimize contamination. The samples were then shipped to NatureMetrics (UK) for eDNA metabarcoding analysis.

Regarding statistical analysis, the significance threshold was set at $\alpha = 0.05$. Data normality was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated through Levene's test. To investigate differences in persistent organic pollutant concentrations in zooplankton from the central-southern Mediterranean (this study) compared with the Adriatic Sea ($N = 39$; Villa et al., 2024), the Tyrrhenian Sea ($N = 53$; Boldrocchi et al., 2023a) and the Northwestern Mediterranean ($N = 23$; Boldrocchi et al., 2026), either a one-way ANOVA or a Kruskal–Wallis test was applied depending on whether parametric assumptions were satisfied. Specifically, PCB data were square root transformed prior to analysis to meet assumptions of normality and homoscedasticity. Post hoc comparisons were then conducted using Tukey's test for parametric analyses or the Steel–Dwass procedure for non-parametric datasets, in order to identify significant differences among sub-basins.

The zooplanktonic community characterized through eDNA metabarcoding was dominated by copepods, particularly members of the genera *Clausocalanus* and *Centropages*. Additional taxa included appendicularians, pteropods, chaetognaths, gelatinous zooplankton, and protists. Overall, the results indicated a diverse planktonic assemblage typical of Mediterranean pelagic ecosystems. These data were generated to provide ecological context for the sampled zooplankton communities

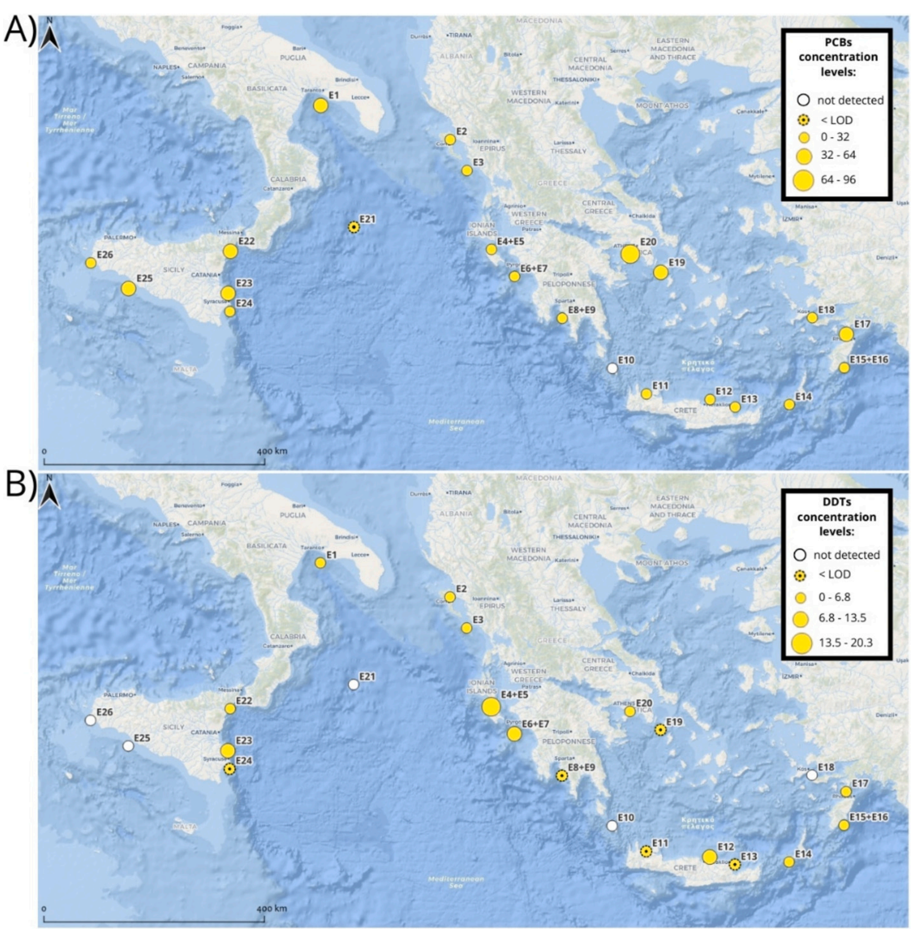


**Fig. 2.** Sampling stations ($N = 26$) in the central-southern Mediterranean Sea showing A) PCBs and B) DDTs concentration levels in zooplankton.

and establish a biodiversity baseline for future integration with contaminant monitoring activities. With regard to contamination analyses, PCBs were ubiquitously detected in Mediterranean zooplankton, with a mean concentration of 27.7 ± 20.2 ng $g^{-1}$ dw, spanning more than one order of magnitude (from <LOD to 96.5 ng $g^{-1}$ dw), and highlighting substantial spatial variability among sampling sites (Fig. 2). Most of the 24 PCB congeners were detected across the dataset (Table S2, Supplementary Materials). However, not all detected congeners could be reliably quantified. PCB congeners 28 + 31 were detected in 18% of samples but could not be reliably quantified and were therefore excluded from quantitative analyses. In addition, PCB 99, PCB 183, and PCB 187 remained <LOD in all samples.

Penta- and hexachlorinated biphenyls were the most frequently detected groups, occurring in 90.9% and 68.2% of samples, respectively, followed by tri-CBs (18.2%), tetra-CBs (13.6%), and hepta-CBs (9.1%). Consistently, these same groups also dominated the concentration profile. Hexa-CBs contributed most to the total PCB burden (19.1 ± 13.1 ng $g^{-1}$ dw), followed by tetra-CBs (15.1 ± 17.6 ng $g^{-1}$ dw) and penta-CBs (13.0 ± 9.30 ng $g^{-1}$ dw), whereas lower levels were observed for hepta-CBs (5.70 ± 7.04 ng $g^{-1}$ dw) and tri-CBs (1.44 ± 1.98 ng $g^{-1}$ dw) (Fig. S2). The predominance of penta-CBs and hexaCBs in both detection frequency and concentration is consistent with patterns observed across seawater, plankton, air and sediments (Berrojalbiz et al., 2011; Lammel et al., 2015; Mandalakis et al., 2014). In these environmental compartments, mid-chlorinated congeners emerge as the most abundant because they combine high persistence with strong hydrophobicity, which enhances bioaccumulation and reduces volatilization losses. As a consequence, penta- and hexachlorinated PCBs remain stored in seawater and sediments, continue to recirculate through air–water and particle-mediated processes, and undergo negligible degradation over decadal timescales.

Spatial patterns of PCB concentrations showed clear variability across the study area, with higher levels generally observed in coastal regions compared to offshore sites. Elevated PCB concentrations were detected in the area close-by Athens (E20) and Saronic Gulf (E19), with the highest value reaching up to 96.5 ng $g^{-1}$ dw. This pattern is consistent with the documented presence of intense industrial and urban pressures in one of the most densely populated metropolitan areas in the Mediterranean (Fig. 2A), as well as with previous studies identifying the Saronic Gulf as a major PCB contamination hotspot (Galanopoulou et al., 2005; Parinos et al., 2026; SoHelME, 2005). This latter area receives treated effluents from the Psittalia Wastewater Treatment Plant (WWTP), the second largest in Europe, and hosts a high density of industrial facilities, particularly within Elefsis Bay, a semi-enclosed and heavily impacted inlet that has long acted as a sink for POPs (Galanopoulou et al., 2005; Parinos et al., 2026; SoHelME, 2005).

Zooplankton samples collected near Rhodes Island showed concentrations of 29.5 ng $g^{-1}$ dw (E15 + E16), 35.1 ng $g^{-1}$ dw (E17) and 26 ng $g^{-1}$ dw, indicating an elevated PCB burden in the area. The PCB levels observed around Rhodes may be related to urban and harbor-related pressures in the area (Parinos et al., 2026). While our dataset cannot confirm specific sources, these factors may contribute to the observed spatial pattern.

Similarly, the elevated PCB concentrations observed along the eastern Sicilian coast are consistent with the documented presence of one of the largest petrochemical districts in Italy and Europe (Augusta–Priolo industrial complex), with values of 51.0, 43.8, and 37.5 ng $g^{-1}$ dw at stations E22, E23, and E25, respectively (Fig. 2A), although specific sources cannot be identified from the present dataset. Historical discharges from facilities such as the chlor-alkali plant operated by Montedison/Syndial have contributed to long-term PCB accumulation in the surrounding marine environment (Lumia et al., 2018; Romano et al., 2016; Benedetti et al., 2022).

High PCB concentrations (35.3 ng $g^{-1}$ dw) were also detected in the Gulf of Taranto (E1), compatible with the presence of major industrial activities, including the ILVA S.p.A. steel plant, historically recognized as a significant source of environmental contamination, and consistent with documented legacy pollution in local biota (Annicchiarico et al., 2011; Giannico et al., 2020; Villa et al., 2024). In contrast, offshore samples (e.g., E10 and E21) were generally characterized by concentrations close to or below detection limits, reinforcing the dominant influence of land-based sources on PCB distribution (Fig. 2).

Overall, these findings emphasize the strong spatial heterogeneity of PCB contamination in the central–southern Mediterranean and the influence of localized anthropogenic inputs.

DDTs were quantifiable in 54.5% of the zooplankton samples, while concentrations in the remaining samples were either below the LOD or not detected. Overall, total DDT concentrations exhibited a mean value of 3.30 ± 5.62 ng $g^{-1}$ dw, ranging from <LOD to 20.3 ng $g^{-1}$ dw, indicating considerable variability across sampling sites. Among DDT-related compounds, o,p'-DDE was the most frequently detected analyte, occurring in 59.1% of samples, followed by o,p'-DDT (36.4%), p,p'-DDE (31.8%), and o,p'-DDD (18.2%). In contrast, the p,p'-DDT isomer was not detected in any sample, whereas p,p'-DDD was detected in 13.6% of samples but only at concentrations below the LOD. DDE was also reported as the predominant DDT metabolite in mussels from the Aegean Sea according to the State of the Hellenic Marine Environment report, where DDE consistently represented 41.7–92.3% of total DDTs (SoHelME, 2005). In terms of concentrations, o,p'-DDD displayed the highest mean level (6.46 ± 6.34 ng $g^{-1}$ dw), followed by p,p'-DDE (4.01 ± 6.83 ng $g^{-1}$ dw), o,p'-DDT (1.97 ± 2.05 ng $g^{-1}$ dw), and o,p'-DDE (0.21 ± 0.42 ng $g^{-1}$ dw) (Fig. S3).

Regarding the spatial distribution of DDTs, the highest concentrations were observed in zooplankton collected along the western Ionian coast of Greece (Fig. 2B), particularly near Zakynthos Island (E4 + E5; 20.4 ng $g^{-1}$ dw) and in the Kyparissia Gulf (E6 + E7; 12.4 ng $g^{-1}$ dw) (Fig. 2). These coastal areas are known to receive riverine inputs from major watersheds, including the Acheloos and Louros rivers and the Messolonghi lagoon complex, which drain extensively cultivated and urbanized catchments (Parinos et al., 2026). Contaminants transported by these systems are deposited in coastal sediments, which act as long-term reservoirs of legacy POPs and potential secondary sources to the overlying water column (Parinos et al., 2026; SoHelME, 2005). The elevated DDT concentrations observed are consistent with the historical use of these compounds in the region, particularly during large-scale malaria eradication campaigns conducted between the 1940s and 1950s. During this period, substantial amounts of DDT were applied across the Peloponnese (up to 44,780 kg $year^{-1}$ between 1946 and 1950; Livadas and WHO Expert Committee on Malaria, 1952) and other rural districts (Hertig, 1950; Vine, 1947). These legacy applications may still influence environmental residues, although our data cannot directly confirm this linkage. Recent studies further confirm the persistence of DDT residues in agricultural soils within these areas, indicating that these regions continue to act as long-term sources of contamination (Tsiantas et al., 2023; Parinos et al., 2026).

Similarly, relatively high DDT concentrations were detected in zooplankton samples collected near Crete Island (E12 = 10 ng $g^{-1}$ dw), which may reflect the legacy of both agricultural applications and large-scale use during malaria eradication campaigns conducted between the 1940s and 1950s. During this period, substantial amounts of DDT were applied in Crete (up to 34,538 kg $year^{-1}$ between 1946 and 1950; Livadas and WHO Expert Committee on Malaria, 1952).

The persistence of DDT compounds in Mediterranean coastal environments contributes to their long-term retention and bioavailability in marine ecosystems, including zooplankton (Dolapsakis et al., 2001; Hertig, 1950; Parinos et al., 2026).

As observed for PCBs, elevated DDT levels were also recorded along the eastern coast of Sicily (E23 = 13.5 ng $g^{-1}$ dw). Atmospheric studies conducted in Sicily have reported the frequent occurrence of DDT and its degradation products, with ratios suggesting both historical residues and ongoing secondary emissions, supporting the role of regional re-volatilization processes in sustaining DDT presence in the marine environment (Pozo et al., 2016). Our observations are consistent with this hypothesis, although further targeted studies would be required to confirm such mechanisms.

Concentrations were compared on a dry-weight basis to ensure consistency with previously published Mediterranean zooplankton datasets. Although lipid content may influence POP accumulation, the use of a common reporting basis facilitates inter-basin comparisons across available studies. Nevertheless, comparisons among Mediterranean sub-basins should be interpreted with some caution, as differences in sampling period, zooplankton community composition, analytical methodologies, limits of detection, pooling strategies, and data normalization approaches may contribute to variability among studies. Mean PCBs and DDT levels measured in central-southern Mediterranean zooplankton were compared to those recorded from other Mediterranean sub-basins: Tyrrhenian (Boldrocchi et al., 2023a), Adriatic (Villa et al., 2024) and Western (Boldrocchi et al., 2026), considering only the same congeners analyzed in all studies (e.g. PCBs:18, 28 + 31, 101, 118 + 123, 138, 149, 153, 170). Starting with PCBs, a One-way ANOVA showed a statistically significant difference among the mean PCBs concentrations between the four areas [$F$ (3, 95) = 8.149, $p \leq 0.0001$]. When considering only shared congeners, the post-hoc Tukey test showed that the mean PCB concentration measured in the central-southern (11.4 ± 10.3 ng $g^{-1}$) was statistically lower than Tyrrhenian Sea (45.0 ± 36.4 ng $g^{-1}$), but not than Western (34.0 ± 52.1 ng $g^{-1}$) and Adriatic Sea (28.1 ± 27.4 ng $g^{-1}$) (Fig. 3). Similarly, the non-parametric Kruskal-Wallis test showed a statistically significant difference among the mean DDTs concentrations between the four areas [$\chi^2 = 11.03$, df = 3, $p = 0.0116$]. The post hoc Steel-Dwass test showed that the mean DDT levels measured in the central-southern (3.3 ± 5.6 ng $g^{-1}$) was statistically lower than Tyrrhenian Sea (8.9 ± 10.7 ng $g^{-1}$), but not than Western (5.1 ± 6 ng $g^{-1}$) and Adriatic Sea (3.1 ± 2.7 ng $g^{-1}$) (Fig. 3). The higher PCB and DDT levels observed in the Tyrrhenian Sea compared to the central-southern basin, and particularly to the Aegean Sea, could be influenced by the combined effects of more intense historical industrialization, higher urbanization pressure, and substantial riverine inputs along the Italian coasts. Global PCB production has been estimated to exceed 1.3 million tons, with Italy accounting for more than 2% of total consumption (Breivik et al., 2007). This extensive use is consistent with the country's industrial history, including domestic PCBs production between 1930 and 1984 in Brescia, northern Italy (Di Guardo et al., 2017). In contrast, lower PCBs levels have been reported in Greece compared to other European countries, reflecting the fact that Greece has never produced PCBs and has experienced a lower degree of industrialization (Papadopoulos et al., 2004). These differences are consistent with previous studies highlighting a longitudinal gradient in the distribution of POPs across the Mediterranean basin, driven by spatial variability in emissions and atmospheric transport processes (Berrojalbiz et al., 2014). In particular, higher concentrations are generally associated with regions more directly influenced by continental European sources, while lower levels are observed toward the eastern Mediterranean, where low population density, limited industrial activity, and efficient dispersal and dilution associated with open-sea circulation and enhanced atmospheric dilution contribute to decreased contaminant burdens (Berrojalbiz et al., 2014; Parinos et al., 2026). In this context, the Tyrrhenian Sea has been consistently identified as one of the most contaminated sub-basins of the Mediterranean, particularly for PCBs, with sediment and coastal records indicating elevated concentrations relative to other Western and central Mediterranean areas, reflecting its role as a major accumulation zone for legacy industrial pollutants (Merhaby et al., 2019). This interpretation is further supported by previous studies in zooplankton and marine food webs, which have highlighted the central role of riverine inputs as a dominant pathway for contaminant transfer from land to sea in the Northwestern Mediterranean, including the Tyrrhenian system, where rivers act as key vectors of both legacy and current-use pollutants, enhancing their bioavailability and incorporation into lower trophic levels (Boldrocchi et al., 2023a; Boldrocchi et al., 2026).

With regards to DDTs, historical use to fight malaria in Italy was particularly severe, especially along both the Tyrrhenian and Ionian coasts with the presence of *falciparum* malaria. Indeed, during the second World War DDT was used extensively by the military to control malaria carrying mosquitoes, and after the war a massive use of DDT was applied in Italy to stop the proliferation of malaria vectors (Majori, 2012). The campaign for the eradication of malaria from the whole national territory began in 1947 and ended virtually in 1948, with the total interruption of transmission of *falciparum* malaria. Indoor treatment with DDT (2 g of active ingredient per $m^2$) of houses, stables, shelters, and all other rural structures continued into the mid-1950s and even later in some hyperendemic areas (Majori, 2012).

In conclusion, this study provides the first basin-scale assessment of

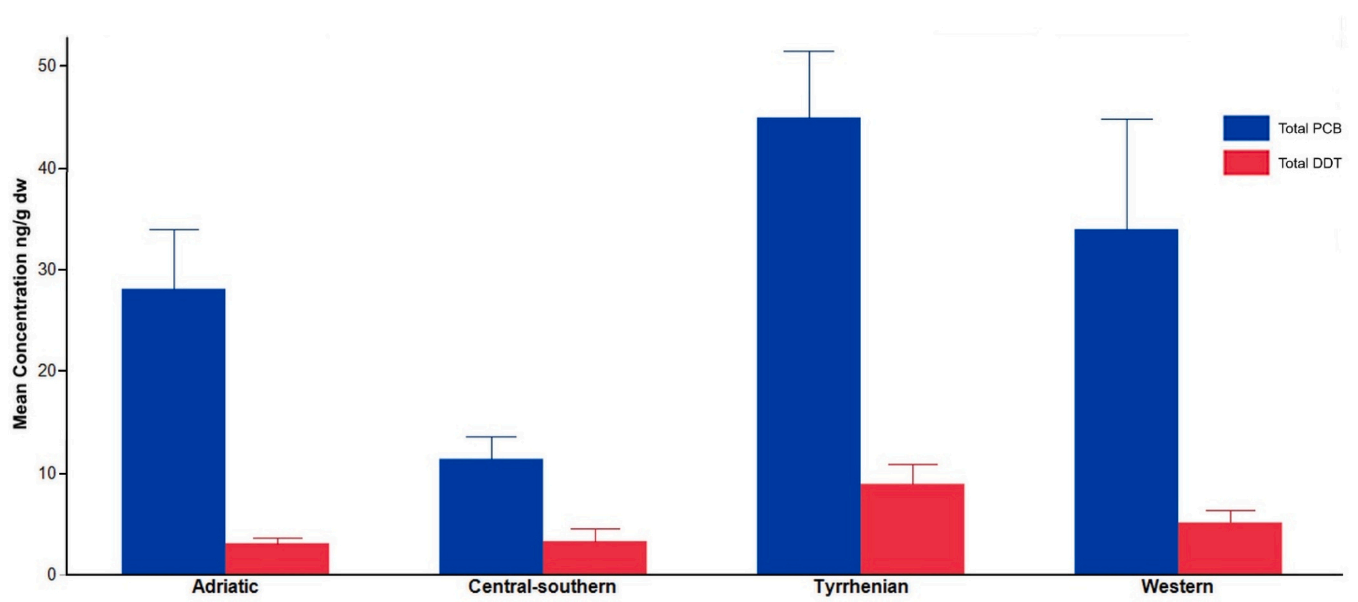


**Fig. 3.** Mean (±SD) of PCB (18, 28 + 31, 101, 118 + 123, 138, 149, 153, 170) and DDT levels (ng $g^{-1}$ dw) in zooplankton samples from four different sub-basins of the Mediterranean Sea.

PCB and DDT contamination in zooplankton from the central–southern Mediterranean Sea, establishing an important baseline for future monitoring efforts. Zooplankton proved to be an effective bioindicator for basin-scale POP assessments due to its wide distribution, rapid turnover, and central role in contaminant transfer across marine food webs. The spatial variability observed across scattered and remote stations highlights the ability of zooplankton to capture both localized coastal inputs and offshore dilution patterns, providing a coherent picture of basin-scale POP distribution. The inclusion of geographically dispersed sampling stations further demonstrated the value of zooplankton-based monitoring for distinguishing local contamination hotspots from broader regional contamination patterns across the Mediterranean Sea. The use of pooled samples was necessary to obtain sufficient biomass for chemical analyses. Although pooling may have reduced the ability to detect fine-scale differences between neighbouring stations, the procedure was restricted to geographically adjacent locations and is therefore unlikely to affect the interpretation of broader basin-scale contamination patterns. Although the basin-scale transect provided extensive spatial coverage, the sampling design represents a temporal snapshot and therefore cannot account for potential seasonal or interannual variability in POP concentrations. Consequently, the present dataset should be considered a baseline assessment of contamination patterns, to be complemented by future long-term monitoring efforts. Future work will extend temporal coverage, include additional legacy and emerging contaminants, integrate chemical analyses with eDNA-based biodiversity data, and explore multivariate approaches to further investigate spatial contamination patterns across Mediterranean sub-basins.

## CRediT authorship contribution statement

**B. Villa:** Writing – original draft, Visualization, Investigation, Formal analysis. **G. Zeri:** Writing – original draft, Investigation. **M. Pè:** Writing – original draft, Investigation, Formal analysis. **L. Pedini:** Writing – original draft, Investigation. **G. Liguori:** Writing – original draft, Investigation. **J. Pachner:** Resources, Project administration, Funding acquisition. **C. Santolini:** Writing – original draft, Investigation. **R. Bettinetti:** Writing – original draft, Validation, Resources. **G. Boldrocchi:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2026.120252.

## Data availability

Data will be made available on request.

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