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Zooplankton as an indicator of the status of contamination of the Mediterranean Sea and temporal trends

G. Boldrocchi [a,b,*], B. Villa [a,b], D. Monticelli [c], D. Spanu [c], G. Magni [b], J. Pachner [b], M. Mastore [d], R. Bettinetti [a]

[a] Department of Human Sciences, Innovation and Territory, University of Insubria, Via Valleggio 11, Como, Italy
[b] One Ocean Foundation, Via Gesù 10, 20121 Milan, Italy
[c] Department of Science and High Technology, University of Insubria, Via Valleggio 11, Como, Italy
[d] Department of Theoretical and Applied Sciences, University of Insubria, 21100 Varese, Italy

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ABSTRACT

Zooplankton has been intensively used as bioindicators of water pollution at global level, however, only few comprehensive studies have been conducted from the Mediterranean Sea and manly dated back to the 1970s. To redress the urgent need for updated data, this study provides information on the presence and levels of contaminants in zooplankton from the Tyrrhenian Sea. Although banned, both PCBs (46.9 ± 37.2 ng g^{-1}) and DDT (8.9 ± 10.7 ng g^{-1}) are still present and widespread, but their contamination appears to be a local problem and to be declining over the past 50 years. Zooplankton accumulates high levels of certain TEs, including Zn (400 ± 388 ppm) and Pb (35.3 ± 45.5 ppm), but shows intermediate concentrations of other TEs, including Cd (1.6 ± 0.9 ppm) and Hg (0.1 ± 0.1 ppm), comparing with both strongly polluted and more pristine marine habitats, which may reflect a general improvement.

1. Introduction

Oceans function as sinks in which pollutants derived from human activities, such as persistent organic pollutants (POPs) and trace elements (TEs), are deposited (Elfes et al., 2010). Although levels of several contaminants, including polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT), have declined over time in multiple areas of the world, as a result of international regulatory bans (Albaiges et al., 2011; Apeti et al., 2010; Rigét et al., 2019), they persist in the environment due to their limited degradation and spread thanks to atmospheric transport. Thus, the continuous monitoring and assessment of marine pollutants in the biota remain necessary as both POPs and certain TEs have potentially significant impacts on human health and the environment, due to their ability to bioaccumulate in biota and biomagnify in food webs (Atwell and Hobson, 1998; Boldrocchi et al., 2021a, 2022; Borgå et al., 2001).

The use of naturally occurring bioindicators is an important tool to evaluate the status of contamination of marine environments and to detect possible changes. Spatiotemporal variations can be derived effectively from the comparison of contaminant levels measured in a selected indicator species, although at present only few studies have been conducted over a broad geographical scale (Boldrocchi et al., 2023; Chiesa et al., 2016; Elfes et al., 2010). In aquatic ecosystems, zooplankton plays an important role in evaluating the level of environmental pollution as it is considered an excellent bioindicator to determine the contamination of any water bodies (Parmar et al., 2016; Zannatul and Muktadir, 2009). Indeed, zooplankton constitutes a vital food source for fish and represents a crucial trophic link in the aquatic food webs, which transfers organic matter, energy, but also contaminants from phytoplankton to zooplanktivorous fish (Battuello et al., 2016; Bettinetti and Manca, 2014; Piscia et al., 2023). Moreover, bioaccumulation of pollutants, deriving from both water and food, takes place much more rapidly in zooplankton than in fish (Borgå et al., 2005): because of their short life span and rapid rate of reproduction, zooplankton responds more rapidly than zooplanktivorous fish to fluctuations of pollutants in the surrounding environment (Bettinetti et al., 2010). The combination of all these characteristics makes these organisms useful early warning detectors of pollution in food webs (Battuello et al., 2016; Bettinetti and Manca, 2014; Boldrocchi et al., 2018; Parmar et al., 2016).

* Corresponding author at: Department of Human Sciences, Innovation and Territory, University of Insubria, Via Valleggio 11, Como, Italy.
E-mail address: ginevra.boldrocchi@uninsubria.it (G. Boldrocchi).

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Among water bodies, high levels of contaminants are commonly found in estuaries, coastal areas, and "enclosed" basins such as the Mediterranean Sea (Ansari et al., 2004; Di Bella et al., 2006). The coasts of the Mediterranean Sea are not only one of the most densely inhabited in the world, with a population that grew from 276 million in 1970 to 412 million in 2000 and is expected to rise to 700 million by the end of 21st century, but also a worldwide hotspot for tourism, industries, and urbanization (UNEP/MAP, 2012). Moreover, the Mediterranean Sea is an economically important region, with both commercial fisheries and oil and gas extraction as extensive industries, as well as maritime transport, with the basin representing among the world's busiest waterways. Ultimately, the Mediterranean Sea is also shared by 22 countries, with different political situations, controls, and directives, which complicated the overall management of this area. All these factors, combined with its nature of semi-enclosed basin with a limited outflow of surface water to the Atlantic and slow turnover rate, result in a high anthropogenic impact with multiple sources of pollution through sewage, industrial and incidental discharges, continental runoff, and atmospheric deposition (Chemicals, 2002). In this context, the conservation of the Mediterranean marine environment has become a matter of growing concern as the basin represents an area of elevated risk of extinction for many species (Valsecchi et al., 2023; Walls and Dulvy, 2020), where data deficiency is a pervasive problem (Boldrocchi and Storai, 2021; Walls and Dulvy, 2020). However, only few comprehensive monitoring studies on the status of contamination of the Mediterranean Sea have been conducted in the basin and mainly from the 1970s and 1980s for what concerned organochlorine and elemental contaminants (Burns et al., 1985; Elder and Fowler, 1977; Fowler and Elder, 1978; Marchand, 1975; Sánchez-Pardo and Rovira, 1985). In more recent years, only Buckman et al. (2018) conducted a massive campaign over an extensive area to investigate the levels of methylmercury in the food web, including zooplankton organisms. Besides these works, most of the published studies are limited to specific areas of the basin and mainly restricted to certain contaminants, such as mercury (Hg), cadmium (Cd), zinc (Zn) and copper (Cu). Consequently, multi-contaminant studies that concurrently investigate extensive areas of the Mediterranean Sea are still limited, leaving a gap of knowledge on the current status of contamination of this important hotspot of biodiversity. While estimating the concentrations of pollutants is necessary for establishing limits and classifying the level of contamination of an ecosystem, quantifying the zooplankton repository of pollutants is the crucial step for estimating availability and uptake of these through the upper levels of the food web, more directly linked to human consumption and human health (Piscia et al., 2023).

To redress the urgent need for long time series data on the contamination of the Mediterranean Sea, this study aims to provide the first current comprehensive study of POPs and TEs in zooplankton samples collected over a wide geographic scale. Specifically, the presence and levels of legacy contaminants (PCBs and DDT) and 15 trace elements were determined in zooplankton collected along the Tyrrhenian Sea 1) to provide baseline information on the overall level of the present contamination in the western Mediterranean Sea; 2) to evaluate possible temporal trends on the levels of the selected contaminants from the 1970s up to date; 3) to determine the status of contamination of the basin by comparing the levels measured in this study with those from other areas of the world.

2. Materials and methods

2.1. Field sampling

The study has been carried out in the Tyrrhenian Sea (western Mediterranean Sea), a deep basin delimited by the Italian coast to the east, by Corsica (France) and Sardinia (Italy) to the west, and by Sicily to the south. Zooplankton samples have been collected within 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/). This project consisted in a three-month expedition across the Tyrrhenian Sea between April to July 2022 onboard a 14 m catamaran, which covered 1500 miles, starting from the northeastern Sardinia Island, heading to Sicily, then to Calabria, and north up to Genoa (Liguria). From Genoa, the catamaran sailed south, back to the starting point in the northeastern coast of Sardinia Island. During the whole navigation, zooplankton samples were collected on an approximately daily basis, using a 200 μm mesh net, vertically at a depth of 20 m from the surface. A total of 53 zooplankton samples were collected (Fig. 1) and carefully preserved in order to avoid any cross-contamination, until lab analyses at the University of Insubria (Como). Once in laboratory, zooplankton samples were lyophilized for 72 h and then weighted. Aliquots of approximately 0.2 g dw (dry weight) were prepared for PCB and DDT analyses, while of 0.015 g dw for the determination of trace elements.

2.2. Chemical analyses

A total of 46 samples were analyzed for PCB and DDT determination, of these 20 were processed individually, while 26 were combined, based on their geographical proximity, to reach the quantity of 0.2 g dw. Extraction procedure with 50 ml of an acetone/*n*-hexane 1/1 mixture in a Whatman cellulose thimble in the Soxhlet apparatus followed Bettinetti et al. (2012). The lipid content was gravimetrically determined, then lipids were suspended in 2 ml of n-hexane and digested with 7 ml of H_2SO_4 (98 %, Carlo Erba, Italy). The *n*-hexane extracts were cleaned up on a Florisil® column, eluted by 25 ml of an 85:15 mixture of *n*-hexane and dichloromethane (pesticide analysis grade, Carlo Erba Reagents s.r.l, Cornaredo, Italy), then concentrated to 0.5 ml. The resulting samples were analyzed by gas chromatography (GC Carlo Erba, Top 8000) with a ^{63}Ni electron capture detector (Carlo Erba ECD 80), and a WCOT fused silica CP-Sil-8 CB column (50 m × 0.25 mm I.D., film thickness 0.25 μm, Varian, USA). For details on the temperature program see Bettinetti et al. (2012).

The concentrations of 15 trace elements (As, Cd, Cu, Fe, Mn, Mo, Hg, Se, Sr, Zn, Cr, V, Co, Ni, and Pb) were determined in zooplankton samples. The analysis followed our previous work (Boldrocchi et al., 2021b); briefly, approximately 15 mg were dissolved by microwave assisted digestion (Milestone ETHOS One) with 2 ml of ultrapure nitric acid produced by sub-boiling distillation (Monticelli et al., 2019). After mineralization samples were transferred to low-density polyethylene bottles and diluted with ultrapure water (produced by a Sartorius Arium mini UV Lab Water System). Samples were then analyzed by Thermo Scientific ICAP Q inductively coupled plasma mass-spectrometer (ICP-MS) using a He-collision cell in kinetic energy discrimination (KED) mode. The equipment was calibrated according to manufacturer suggested procedures, which include mass calibration and the optimization of the sensitivity while keeping interferences at the lowest possible level (oxide formation below 2 % as measured on the CeO^+/Ce^+ ratio and double charge species below 3 % as measured on the Ba^{2+}/Ba^+ ratio).

2.3. Quality assurance and quality control

Quantification of each sample was performed using external reference standards of pp.'DDT, pp.'DDE and pp.'DDD (Pestanal, Sigma-Aldrich, Germany) in isooctane (Carlo Erba, pesticide analysis grade), while Arochlor 1260 (Alltech, IL, USA) with the addition of PCB 28 and 118 for PCB quantification. The analyzed PCB congeners included: 18, 28, 31, 44, 101, 118, 138, 149, 153, 170, 180, 194 and 209. Total PCBs (ΣPCBs) were quantified as the sum of all congeners. Total DDTs (ΣDDTs) were calculated as the sum of the isomers op'DDT, pp.'DDT, op'DDD, pp.'DDD, op'DDE and pp.'DDE. The detection limit for all compounds was 0.1 ng g^{-1} dw. The results were expressed in ng g^{-1} dw.

With regards to trace elements, data quality was assured by analyzing the certified reference material BCR-414 during each analysis

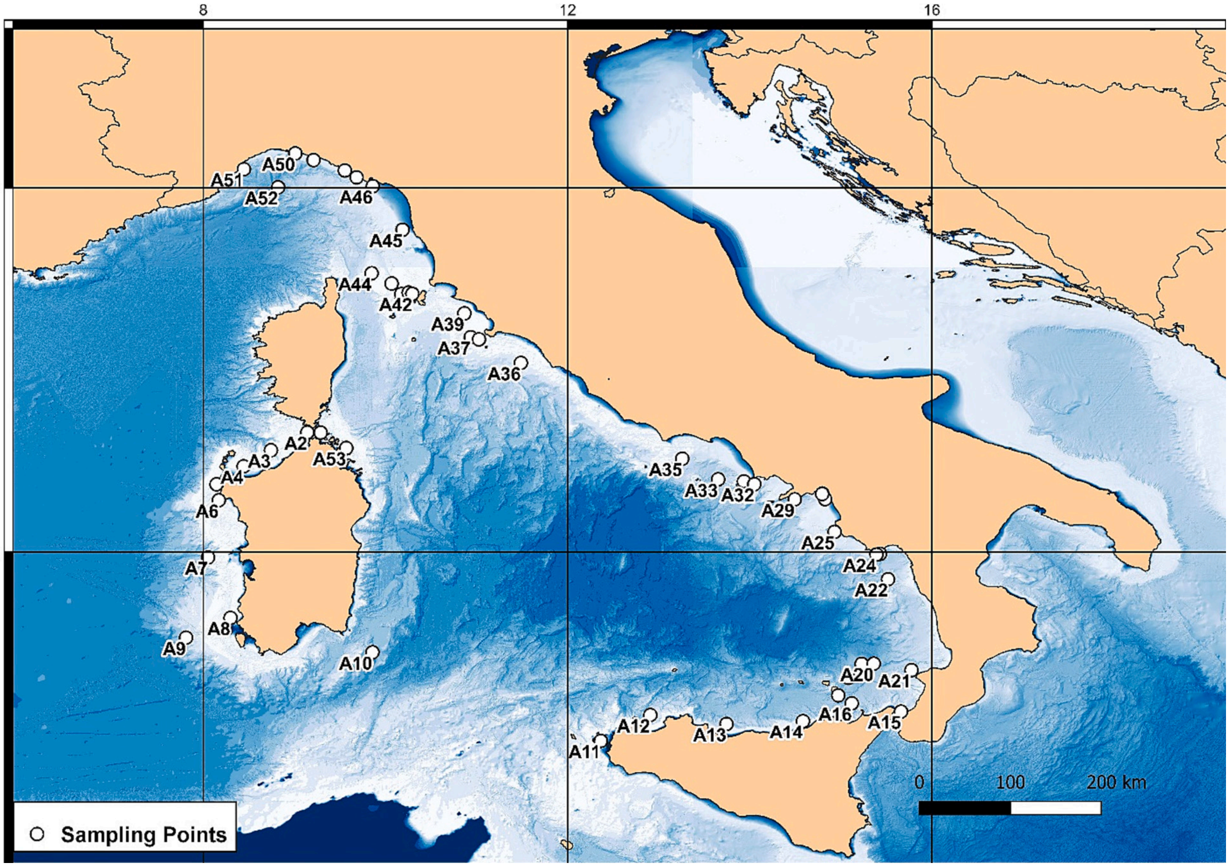

Fig. 1. Location of zooplankton sampling along the Tyrrhenian Sea in April–July 2022.

batch. The latter is a plankton reference material certified for trace elements issued by the Institute for Reference Materials and Measurements. As a result, the recovery of the 11 certified elements V, Cr, Mn, Ni, Cu, Zn, As, Se, Cd, Hg and Pb were in the range 99–127 %, showing no statistical difference from the certified values (average of 10 analysis batches). Limits of detection are as follows: As, Cd, Co, Cu, Cr, Hg, Mo, Mn, Pb, Se, Sr and V below 1 mg kg^{-1}, whereas Fe, Ni and Zn in the 1–10 mg kg^{-1} range. See also our previous work for additional information (Spanu et al., 2020). Results are reported as mg kg^{-1} dw.

2.4. Statistical analyses

All data were evaluated for normality using a Shapiro-Wilk test prior to analysis, while assumptions of homogeneity of variance was tested using Bartlett's and Levene's test. Since assumptions of normality and homogeneity of variance were both violated, the non-parametric Wilcoxon/Kruskal-Wallis (Rank Sums) test and the Post hoc Steel-Dwass methods were used to evaluate any difference in the mean concentration of PCB congeners in zooplankton samples. Pearson correlation was applied in order to investigate possible correlation trace metals found in zooplankton samples. Finally, linear regression trendlines were fitted to the data to determine any change in the levels of selected TEs over the sampling period. Significance level used in the present study was set at $p > 0.05$.

3. Results and discussion

At global level, zooplankton has been intensively used as an early warning indicator of water pollution. However, in the Mediterranean Sea studies on zooplankton have been mainly focused on community and abundance, reporting a composition mostly dominated during spring and summer by copepods such as *Clausocalanus* spp., *Paracalanus parvus*, *Oithona* spp. and *Acartia spp;* cladocerans such as *Penilia avirostris*, *Evadne spinifera* and *Pseudoevadne tergestina*; tunicates (appendicularians); meroplankton (decapod larvae); cnidarians; chaetognaths and ostracods (Battuello et al., 2016; Mazzocchi et al., 2011). Our study provided updated baseline data, useful for future research, on the presence and levels of multiple contaminants in zooplankton organisms over a wide geographical sampling area within the Mediterranean Sea.

3.1. PCBs and DDT

The mean concentration of the sumPCB$_{13}$ in zooplankton samples was 46.9 ± 37.2 ng g^{-1} dw and varied substantially between samples, from a minimum value of 8.4 ng g^{-1} dw up to 164.9 ng g^{-1} dw (Table 1).

Among PCBs, PCB 101 was the most abundant and ubiquitous congener detected in all samples, while PCBs 194 and 209 were never detected (Table 1). The profile of PCB congeners in zooplankton samples was dominated by the more volatile Tri-, Tetra- and Penta-PCBs congeners. Tri- and Penta-CB accounted alone for 92.7 % of all congeners (Fig. 2). In particular, Penta-CB was the predominant congener accounting on average for 66.5 % of total PCBs (Fig. 2) with PCB 101 (31.8 ± 29.2 ng g^{-1} dw) comprising 65.5 % of all congeners. Tri-CB also occurred in high concentrations and accounted on average for 26.2 % of total PCBs with PCB 18 (10.3 ± 8.8 ng g^{-1} dw) contributing for 21.8 % of all congeners (Fig. 2). Tetra-, Hexa- and Hepta-CB, showed intermediate concentrations, accounting for 3.8 %, 2.5 % and 1 % of total PCBs, respectively (Fig. 2). Finally, high-chlorinated PCBs, including Octa- and Deca-CB were not detected. The Wilcoxon Kruskal-Wallis Test and the Post Hoc Test Steel-Dwass showed a statistical difference in the mean concentration of congeners in all samples ($\chi^2 = 104.56$, df = 2, $p < 0.0001$), with Penta- and Tri-CB statistically higher than all the other groups ($p < 0.0001$). In line with this finding, previous studies found similar results: congener specific analysis of Antarctic krill samples

Table 1
Mean concentrations, maximum and minimum values of PCBs and DDTs (ng g^{-1} dw) in zooplankton samples collected from the Mediterranean Sea. "N.d." – "Not detected".

Organochlorine Compound	N	CONGENER	Mean ± SD	Min	Max
PCB	31	18	10.3 ± 8.8	0.34	37.3
		28 + 31	1.93 ± 1.5	0.01	5.85
		44	3.93 ± 3.6	0.04	11.13
		101	31.8 ± 29.2	4.97	139.1
		149	1.13 ± 1.5	0.08	3.26
		118	2.01 ± 1.4	0.74	4.77
		153	1.90 ± 3.0	0.03	11.2
		138	0.89 ± 0.6	0.06	1.62
		180	1.31 ± 0.8	0.32	2.27
		170	1.23 ± 0.6	0.82	1.64
		194	n.d.		
		209	n.d.		
		TOT	46.9 ± 37.2	8.41	164.9
DDT	31	o,p' DDE	3.84 ± 3.0	n.d.	11.0
		p,p'DDE	2.42 ± 4.2	n.d.	14.4
		o,p'DDD	4.88 ± 9.0	n.d.	35.5
		p,p'DDD	1.06 ± 0.9	n.d.	2.37
		o,p'DDT	1.10 ± 0.9	n.d.	3.31
		p,p'DDT	5.33 ± 3.3	n.d.	13.3
		TOT	8.9 ± 10.7	n.d.	48.1

found a dominance of Penta-chlorinated PCBs primarily contributed to by PCB 101, which was the most ubiquitous congener (Nash et al., 2008). Similarly, Corsolini et al. (2003) found the lower chlorinated PCBs to dominate at low trophic levels (e.g. silver fish and krill), and Berrojalbiz et al. (2011) reported a predominance of Penta-CB in the Mediterranean zooplankton, and of Tri- congeners in the particulate phase of surface water samples. The dominance of Penta-chlorinated congeners in zooplankton could be due to the natural abundance of these congeners in the surrounding environment, their hydrophobicity and partitioning pathways into zooplankton mainly through surface adsorption and organic phase partitioning (Yeo et al., 2020).

DDT in zooplankton samples showed a mean concentration of 8.9 ± 10.7 ng g^{-1} dw, ranging from "not detected" (n.d.) to 48.1 ng g^{-1} dw (Table 1). The relative contribution to the total DDT content was the following: o,p'DDE and o,p'DDT were the predominant metabolite in the majority of samples (29.0 %), followed by p,p'DDT (22.6 %) > p,p'DDE and o,p'DDD (9.7 %) and > p,p'DDD (0 %). An insight to the aging process of pesticides in the environment can be gained by comparing isomer patterns in zooplankton to the original compositions in technical mixtures. Technical grade DDT is composed by 65–80 % of p,p'DDT, 15–21 % of o,p'DDT, up to 4 % of p,p'DDD, and other compounds in very small amounts. DDT isomers degrade into DDE and DDD under aerobic and anaerobic conditions, respectively. Therefore, a ratio DDE/DDT > 1 indicates that most of the active substance (DDT) has been degraded to DDE there are no recent inputs to the environment. Overall, the mean ratio of DDE/DDT across samples was 1.3 ± 3.3, suggesting that the exposure of the marine species to DDT is likely due to the large amount used in the coastal areas before the ban. Actually, DDT is typically persistent in the agricultural soil and it is re-mobilized through evaporation and runoff. However, in few samples, the p,p'DDT was the predominant component of the total DDT burden. Therefore, more recent DDT input to the environment might not be ignored, as already speculated for certain Italian areas such as the Sarno River and its estuary, which represent an important contribution source of OCs into the Tyrrhenian Sea (Montuori et al., 2014), and some locations of the central and southern Italy (Estellano et al., 2012; Pozo et al., 2016; Qu et al., 2016; Thiombane et al., 2018). Moreover, while European countries have banned the production and use of persistent pesticides, potential dispersion into atmosphere from neighboring countries might not be dismissed (Pozo et al., 2016).

Although both DDT and PCBs have been banned since the mid 1970's in several EU countries, this study shows that they are still widespread in the Mediterranean marine environment. However, the contamination of POPs appears to be a local problem, more than a widespread issue (Fig. 3). This is in line with what observed with the Mediterranean sediments analyzed for the Mediterranean Marine Pollution Assessment and Control Program inventory report, in which both PCBs and DDT appear not uniformly distributed throughout the basin, but with some hotspot locations. Moreover, high PCB levels were reported not only in sediments, but also in marine biota in the northwestern Mediterranean coastal areas, in the vicinity of industrial and urban sites, especially close-by cities such as Genoa and Barcelona or Marseille, where PCB levels reached concentration up to 1500 ng g^{-1} dw. High PCB levels were recorded also in the marine biota sampled from both the Italian and French coastline, from Livorno to Nice, and close by the mouths of rivers (UNEP/MAP, 2012). Consistently, in this study high PCB concentrations in zooplankton were recorded for instance in the area between Livorno and Piombino (Tuscany), and close-by Gaeta and Naples (Campania), although never reaching comparable levels of those of Barcelona or Marseille. Both areas not only represent industrial and important coastal sites, but they are located in proximity of Italian river

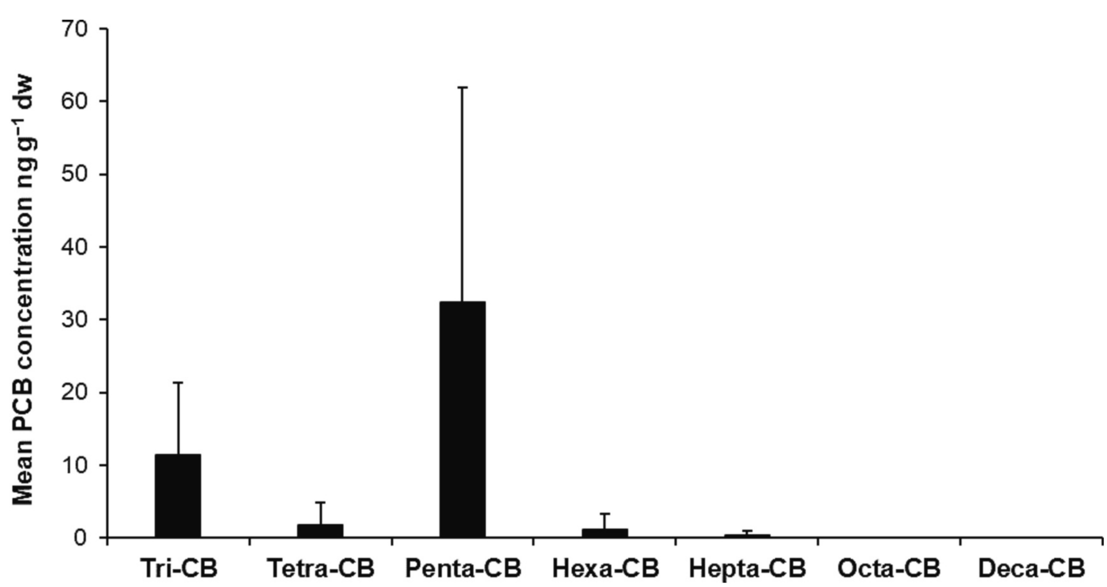

Fig. 2. Mean (±SD) of PCB, grouped according to the chlorine content of each PCB congener of all samples (ng g^{-1} dw).

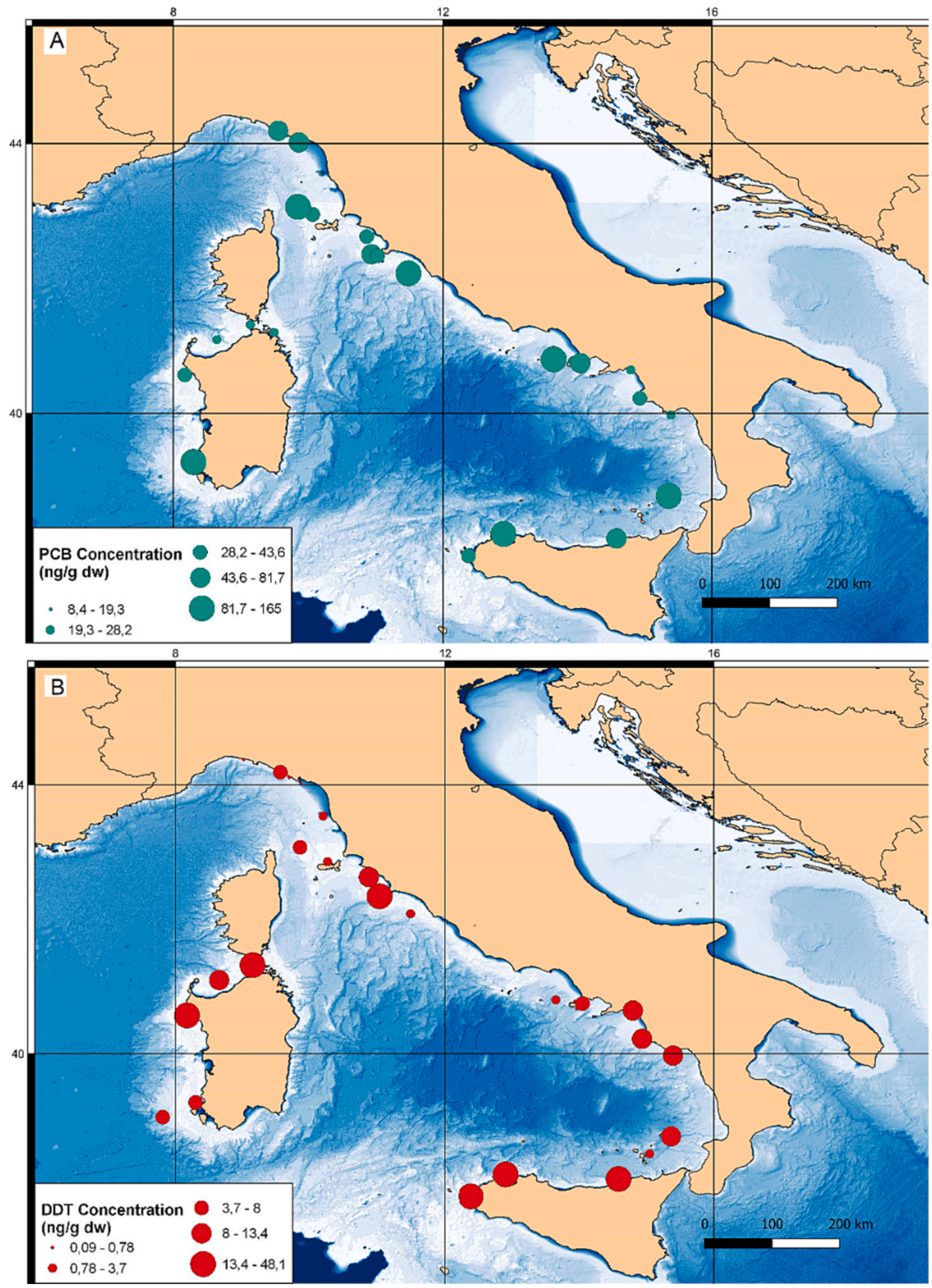

Fig. 3. Levels (ng g^{-1} dw) of PCBs (A) and DDT (B) measured in zooplankton samples collected in the Tyrrhenian Sea in 2022.

discharges, including Arno and Volturno, respectively. In general, river mouths are considered major sources of pollutants in the marine environment, including the Mediterranean Sea (UNEP/MAP, 2012). Moreover, Merhaby et al. (2019), who reviewed 194 studies concerning POPs in the Mediterranean basin, reported an important contribution of PCBs in the Tyrrhenian Sea derived from Naples Bay, one of the most highly industrialized and densely populated areas in southern Italy, with maximum PCB concentrations that reached in the past up to 3200 μg kg^{-1} dw.

Concentrations of DDT in the Mediterranean zooplankton from this study appear to be not uniformly distributed as well (Fig. 3). This is in line again with previous reports on both sediment and marine biota samples (UNEP/MAP, 2012). Understanding the regional variability found in this study for PCBs and DDT is made difficult by the multiple sources of pollutant release into the marine environment. As a matter of facts, anthropogenic impacts over the Mediterranean basin may result from direct and/or indirect emissions from disposal of industrial and domestic wastes, river discharges, shipping activities, atmospheric emissions, but also re-suspension of contaminated bed sediments and other non-point sources by which pollutants reach the sea (Gómez-Gutiérrez et al., 2007). For instance, PCBs can be also unintentionally released in the Mediterranean Sea as a result of combustion processes or as by-products in some industrial processes related to cement and metal industry, while DDT might be linked to organic chemical industry, or to wastewater treatment plants, which may collect pesticides contained in sewage and runoff waters (UNEP/MAP, 2012).

To determine the status of pollution, OC levels from this study were compared to those reported in other studies focused on the Mediterranean Sea. Despite an intensive monitoring program of POPs at the global level, only 14 articles reported levels of either DDT or PCBs in the Mediterranean zooplankton over the past 50 years (1974–2023) (Table S1, Supplementary Material) and among these studies, only few (n = 7, including the current one) were published after the 2000s. Considering the most recent reports, it appears that PCB values measured in zooplankton samples during our campaign (47 ± 37.2 ng g^{-1} dw) are in line with those reported from the Bay of Marseille in 2017 (Castro-Jiménez et al., 2021) and Ligurian Sea in 2017–2018 (Rivoira et al., 2022), but not with those published in the past, between the 1970s and 1980s, which were of the order of thousands ng g^{-1} dw (Table S1, Supplementary Material). This suggests a possible declining trend of PCB level in marine biota over the last 50 years. Studies reporting DDT values in zooplankton are even fewer than PCBs and mainly concentrated in the 1970s (Table S1, Supplementary Material). Those studies reported range of 40–76 ng g^{-1} dw and 35–135 ng g^{-1} dw in zooplankton collected using 60 and 300 μm mesh nets, respectively, from the northwestern Mediterranean Sea (Marchand, 1975), and a mean value of 143 ± 87.1 ng g^{-1} dw from the Alboran Sea (Sánchez-Pardo and Rovira, 1985). In recent years (2012-2013), de Lucia et al. (2014) reported values ranging from 112.0 ng g^{-1} dw to 665.3 ng g^{-1} dw in neuston-plankton collected using a 500 μm mesh net. All mentioned studies, from the 1970s, 1980s and 2010s, reported DDT levels much higher than what measured in the current research from the Tyrrhenian Sea (8.9 ± 10.7 ng g^{-1} dw) suggesting a declining trend in the presence of DDT in the marine biota over the past years. This finding is consistent with the banning of production and use of these compounds and is in line to what reported also for other indicator organisms, such as mussels, whose levels of chlorinated pesticides have declined since the 1990s (UNEP/MAP, 2012). In addition, two surveys conducted along the Mediterranean coast of France and Italy, in 1973–1974 and in 1988–1989, showed that DDT levels decreased by a factor of approximately 5 in 15 years (Villeneuve et al., 1999). However, evaluating a possible statistical temporal trend is impossible due to the low number of published studies. Moreover, concerning PCBs, each research analyzed a different number and type of congeners, and therefore, reliable comparisons cannot be performed. To overcome this limitation, only a specific congener was considered to evaluate possible temporal trends. However, this choice appears to be not an option at this stage as PCB 153, the most frequently measured (29.4 %) congener among 209, was found only in 4 published studies from 2004 to 2023 (Table S1, Supplementary Material).

Finally, to determine the status of pollution of the Mediterranean Sea, OC levels from this study were compared to those reported from other areas of the world, in recent years. $SumPCB_{13}$ levels in zooplankton samples collected for this study (46.9 ± 37.2 ng g^{-1} dw) appear to be much lower than those measured in Japanese coastal waters from 2013 to 2017 ($SumPCB_{13}$: 107 ± 299 ng g^{-1} dw; Yeo et al., 2020), and in Djibouti (Gulf of Aden), which is considered a heavily polluted area ($SumPCB_{13}$: 336 ± 254 ng g^{-1} dw; Boldrocchi et al., 2018). On the contrary, when comparing the Tyrrhenian Sea with less urbanized areas, such as the polar regions, it appears that the Mediterranean Sea is still much more impacted in terms of PCBs (Svalbard fjords, $sumPCB_7$ in copepods: 2.95 ± 8.79 ng g^{-1} dw; and $sumPCB_7$ all zooplankton taxa: 4.17 ± 6.42 ng g^{-1} dw) (Pouch et al., 2022). However, due to different number of analyzed congeners (current study: $N = 13$; Pouch et al., 2022: $N = 7$), it is impossible to drive solid conclusions.

With regard to DDT levels, zooplankton collected in the Tyrrhenian Sea appears to accumulate higher DDT levels (8.9 ± 10.7 ng g^{-1} dw) compared to tropical coral reef ecosystem from the northern South China Sea (0.77 ± 0.20 ng g^{-1} dw; Kang et al., 2022). However, the levels reported in this study are much lower than those measured in well recognized polluted ecosystems such as Sagar Island (India) (6.3–87.6 ng g^{-1} dw; Basu et al., 2021) and Djibouti (Gulf of Aden) (44.7 ± 27.4 ng g^{-1} dw; Boldrocchi et al., 2018). With regards to polar regions, there are few data on POP concentrations in Arctic zooplankton, mainly due to their low concentrations (Fisk et al., 2001). However, it appears that the pollution levels measured in the Tyrrhenian Sea are only slightly higher than both the Alaskan and Canadian Arctic (e.g. 5.33 ± 0.94 and 5.57 ± 0.58 ng g^{-1} dw; Hoekstra et al., 2002) as well as the North Water Polynya (4.74 ± 0.74 ng g^{-1} dw; Fisk et al., 2001).

3.2. Trace elements

The concentration of 15 trace elements in zooplankton samples from the Tyrrhenian Sea are presented in Fig. 4. As expected, essential TEs (e.g. Fe, Cu, Zn, and Ni) were found in high content as these play a significant role in the physiological metabolism of zooplankton organisms. Iron, for instance, is an essential trace element for the biological requirements of marine plankton and often acts as a limiting parameter of growth; Cu is normally accumulated by crustacean species as a component of enzymes related to egg production and growth (Achary et al., 2020). Zinc in plankton is essential for many metabolic roles as it is involved in a plethora of cell functions, in the synthesis of tRNA and in the synthesis of single-stranded DNA from RNA (Twining and Baines, 2013). Therefore, Sr and Fe had the highest concentrations in zooplankton (6812 ± 6261 and 1818 ± 4867 mg kg^{-1}, respectively), followed by Zn (400 ± 388 mg kg^{-1}), Cu (120 ± 207 mg kg^{-1}), Mn (110 ± 593 mg kg^{-1}), Pb (35.3 ± 45.5 mg kg^{-1}) and Ni (24.7 ± 23.8 mg kg^{-1}) (Table S2, Supplementary Material). TEs such as Cr, As, V, Mo, Se were found to have lower concentrations and ranged from a maximum of 9.2 ± 11.4 mg kg^{-1} for Cr to a minimum of 3.3 ± 1.5 mg kg^{-1} for Se (Table S2, Supplementary Material). On the contrary, the non-essential elements, such as Cd and Hg, were found in lowest concentrations: Cd (1.6 ± 0.9 mg kg^{-1}), Co (1.1 ± 1.0 mg kg^{-1}) and Hg (0.1 ± 0.1 mg kg^{-1}) which showed the lowest concentrations (Table S2, Supplementary Material).

Urban and industrial wastewaters, atmospheric deposition, which is the main pathway for TEs to enter open-water areas, and run-off from metal contaminated sites constitute the major sources of toxic TEs. Aside from direct discharges from urban and industrial sources, also rivers and streams are important contributors of TEs of anthropogenic and natural origin to coastal areas, although metal enhancements in local geology may also influence sediment TE content (UNEP/MAP, 2012). In general, according to the National Baseline Budget inventory, in the Mediterranean countries, atmospheric emissions of Hg and Cu are mostly related to the cement industry; As, Cd, Ni to the production of energy; while Pb and Zn to the metal industry. Water releases appear to be mostly related to the fertilizer industry for Hg, As, Pb; metal industry for Ni and Zn; wastewater treatment plants for Cd, Cu; while oil refining is the main source of Cr releases, both to air and water (UNEP/MAP, 2012). Overall, contamination by TEs has both natural and human sources of emissions, consequently a great variability exists in levels of TEs among different areas. As a matter of facts, besides one samples collected in the northwest of Sardinia (40°44'31.3"N 8°08′32.8″E) in front of an abandoned coastal mine, which showed very high concentrations of all the analyzed TEs, all others sampling sites showed great spatial variability in term of TE levels (Fig. 5). Certain TEs, such as Cu and Zn, showed similar spatial distribution with lowest levels in A33 (3.7 mg kg^{-1} and 38.9 mg kg^{-1}, respectively) and A41 samples (5.8 mg kg^{-1} and 22.8 mg kg^{-1}) and very high concentration in A28 (1114 mg kg^{-1} and 1098 mg kg^{-1}, respectively), A49 (775 mg kg^{-1} and 624 mg kg^{-1} respectively) and A52 (673 mg kg^{-1} and 1605 mg kg^{-1}, respectively) (Fig. S1, Supplementary Material). Others, such as Hg and Cr, a completely different pattern, not only between each other, but also between each sample site (Fig. S1, Supplementary Material). In general, V, Ni, Mn, Co, Zn and Fe were significantly positively correlated with most TEs (Pearson correlation analysis; $p < 0.05$, Table S3, Supplementary Material), whereas Mo, Se, Sr, Cr, and Hg were poorly correlated with other trace elements (Table S3, Supplementary Material). The strongest correlations among studied TEs accumulated by zooplankton were observed among Fe, Mn, V, and Co suggesting a common source (Strogyloudi et al., 2021). Similarly, significant correlations between essential, such as Fe, Mn, Co, Ni, Cu and Zn, and non-essential, like Cd and Pb (Table S3, Supplementary Material), may indicate synergistic interactive effects with similar polluting origin, suggesting common routes of uptake from their interaction with the habitat (Biju and George, 2021). Mercury levels were particularly high around Milazzo (0.75 mg kg^{-1}), Aeolian Islands

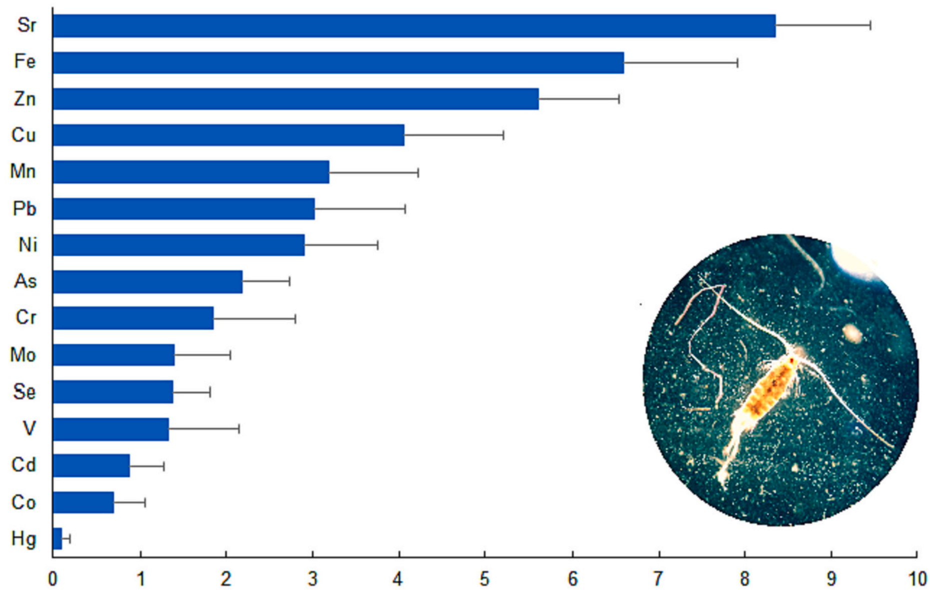

Fig. 4. Mean (±s.d.) concentration of trace elements (ppm dw) in zooplankton samples collected in the Tyrrhenian Sea in 2022. Data were log(x + 1) transformed.

(0.19 mg kg^{-1}) and Palermo (0.17 mg kg^{-1}) in Sicily, potentially influenced by natural contributions from volcanic and geothermal sources of the southern Tyrrhenian Sea, as already reported for marine sediments (UNEP/MAP, 2012). With regards to Cd, previous studies found relatively high levels of Cd in biota along the western coast of Italy (Naples), the southern shores of the Tyrrhenian Sea (Messina and Palermo), and western Sardinia (UNEP/MAP, 2012). In this study, Cd showed relatively high levels in zooplankton collected in the north-western Sardinia (from 2.75 to 3.47 mg kg^{-1}), around Palermo (3.24 mg kg^{-1}) and Milazzo in Sicily (2.89 mg kg^{-1}), and Salerno in Campania (3.23 mg kg^{-1}) (Fig. S1, Supplementary Material). On the contrary, Cr showed low levels in most samples, besides those collected between Civitavecchia (Lazio) and Piombino (Tuscany) as well as around Genoa port (Liguria) (Fig. S1, Supplementary Material).

To determine the status of pollution, TE levels from this study were compared to those reported in other studies from the western Mediterranean Sea focusing on zooplankton organisms. To the best of the author knowledge, Table S4 (Supplementary Material) comprises all published studies on trace elements over the last ~45 years. Due to the limited number of research at the Mediterranean level, temporal variation in TE levels was investigated only for Cu, Zn, Cd, Hg and Pb. The results showed a general significant decreasing trend for both Zn and Cd ($R^2 = 0.28$, $n = 18$, $P = 0.0256$ and $R^2 = 0.53$, $n = 19$, $P = 0.0004$, respectively), although current levels appear to be comparable to certain studies back to 1970s (Fig. 6). These decreasing trends are probably the result of a reduced supply of both TEs into the marine environment as a consequence of the application of the Law 319/76 in Italy, which contains the guidelines to regulate the concentrations of chemicals in wastewaters (Romano et al., 2013). Moreover, according to the European Environment Agency, the effectiveness of the restrictions implemented in the EU has been reflected on the declines on the air emission recorded from 1990 to 2016 for several metals, including Cd and Zn. On the contrary, not significant trends were found for Cu, Pb and Hg. This is in line with what reported for blue mussel, where Pb concentration is consistently high at locations with Pb-contaminated sediments, such as the western coast of Italy from the Gulf of Genoa to Naples at different locations in the Tyrrhenian Sea, along the Italian west coast, along the coast of northern Sicily (Palermo) and in the southern part of Sardinia (Portoscuso). These high levels were attributed to discharges and non-point sources of pollution from mining, industry and sewage (UNEP/MAP, 2012). However, it is worth to say that time series and differences among sampling conditions mean that most available pollution data for the Mediterranean are not yet adequate for robust trend analysis (UNEP/MAP, 2012).

Contrary to OC compounds, several studies have quantified the zooplankton TE concentrations in different areas worldwide. Therefore, to determine the status of pollution of the Mediterranean Sea by TEs, levels from this study (excluding sample A5 collected in front of the abandoned mine) were compared to those reported from other areas of the world over the past 15 years (Table S5, Supplementary Material). Zinc, for instance, is an essential trace element, however it is known to cause toxic effects such as low survival rates in crustaceans in polluted environment (Rieuwerts, 2017). Levels of Zn content in zooplankton from the Tyrrhenian Sea are far below the published values of 2000 μg g^{-1} from the Gulf of Bengala (Rejomon et al., 2008) but comparable to those reported from the same location in more recent years (356.8 mg kg^{-1} dw; Achary et al., 2020) and to other well-renowned polluted locations such as the Arabian Sea (374 mg kg^{-1} dw; Rejomon et al., 2008) and Red Sea (416 ± 375 mg kg^{-1} dw; Cai et al., 2022). Copper is another essential TEs for metabolic activities of organisms but becomes toxic when exceed a certain threshold level beyond optimal. In the Mediterranean Sea, atmospheric deposition related to cement industry and wastewater treatment plant are important sources of Cu pollution in aquatic environments. Among all TEs investigated in this study, Cu content (122 ± 209 mg kg^{-1} dw) appears to be the highest recorded, when compared to other worldwide locations: it is double than most polluted locations, such as the Gulf of Aden, Bay of Bengala and Taiwan (Table S5, Supplementary Materials). Nickel acts as an important metal for many organisms and occurs in aquatic systems as soluble salts adsorbed on clay particles or organic matter or associated with organic ligands viz. humic acids, fluvic acids and proteins (Thomson, 1982). However, Ni has been proved to exert long-term harmful effects to aquatic organisms (Attig et al., 2010). Major sources of Ni pollution include industrial discharges from electroplating, smelting, mining and refining operations and other industrial emissions (Vijayavel et al., 2009). Similar to Zn, Ni levels reported from the Tyrrhenian Sea are in line with well-renowned polluted locations such as the Djibouti (25.1 ± 18.6 mg kg^{-1} dw; Boldrocchi et al., 2020) and Taiwan (20.7 ± 7.73 mg kg^{-1} dw; Albarico et al., 2022), and higher than less impacted areas such as the Arctic Ocean (1.79 ± 0.16 and 7.44 mg kg^{-1} dw; Lobus et al.,

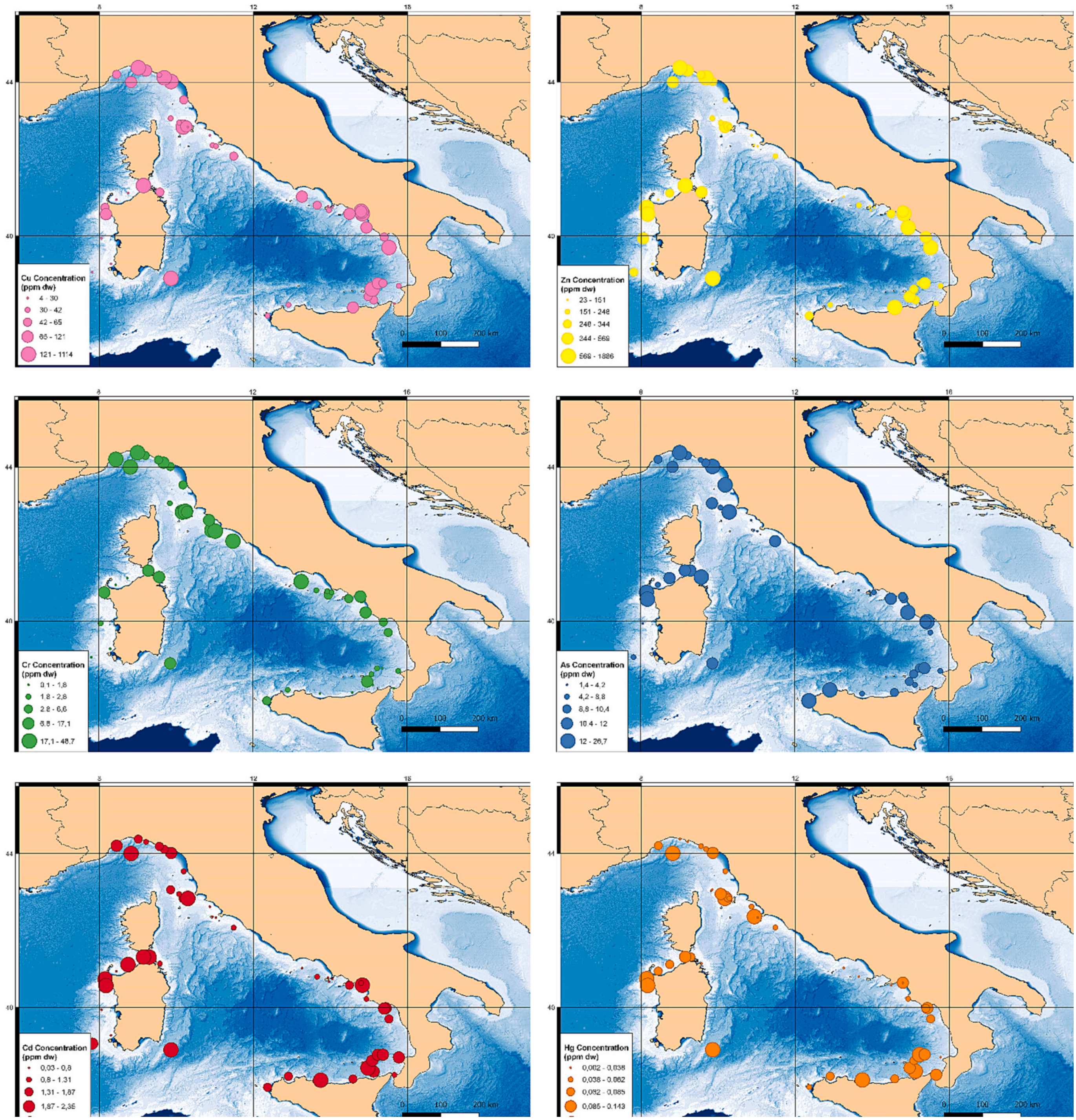

Fig. 5. Levels (mg kg^{-1} dw) of Cu, Zn, Cr, As, Cd and Hg measured in zooplankton samples collected in the Tyrrhenian Sea in 2022.

2019; Mohan et al., 2019) (Table S5, Supplementary Material). Concentrations of Pb are generally low in natural waters because of its low solubility and strong binding in soils, but they increase in surface seawater, reflecting anthropogenic atmospheric inputs (Rieuwerts, 2017). Lead bioaccumulates in aquatic organisms, including zooplankton organisms, and generate toxic effects even at low concentrations. Similar to Zn, Ni and Cu, Pb levels in this study were comparable to extremely polluted areas such as the coastal waters of southern Taiwan, adjacent the highly industrialized city of Kaohsiung (24.9 ± 23.1 mg kg^{-1} dw; Albarico et al., 2022) or the Bay of Bengala (38.6 mg kg^{-1} dw; Achary et al., 2020) (Table S5, Supplementary Material).

On the contrary, for what concerned highly toxic TEs, such as Cd, Co, Hg and Cr, zooplankton from the Tyrrhenian Sea appears to accumulate intermediate concentrations between areas that face extreme pollution pressures due to domestic wastes, agricultural run-offs, harbor and industrial effluents, marine outfall diffusion, and other more pristine such as the Arctic Ocean. As a matter of facts, Cr and Cd levels from this study (9.2 ± 11.5 mg kg^{-1} dw and 1.6 ± 0.9 mg kg^{-1} dw, respectively) were much lower than those recorded in Djibouti (Boldrocchi et al., 2020), Taiwan (Albarico et al., 2022), the Bay of Bengala (Achary et al., 2020) or Bohai Bay, but not for Cd (Zhang et al., 2016) (Table S5, Supplementary Materials). Cr was more in line with levels reported from Svalbard (Mohan et al., 2019), while Cd was even in lower content (Table S5, Supplementary Materials). Mercury is among the most highly

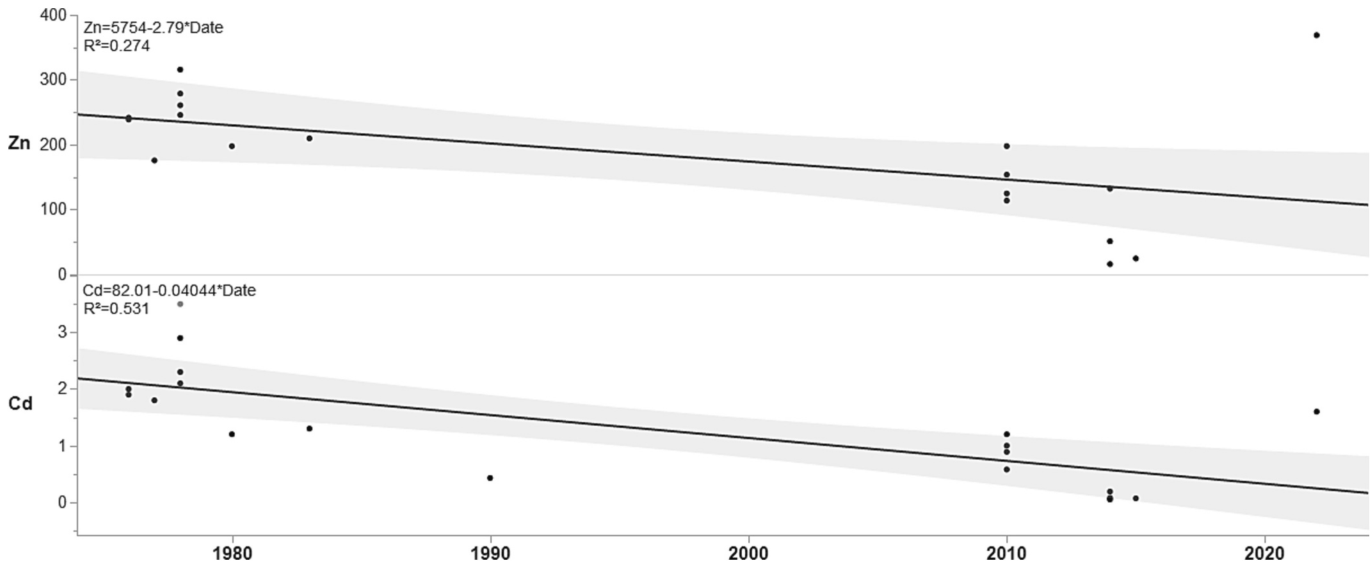

Fig. 6. Temporal trends in Cd and Zn (R^2 = 0.28, n = 18, P = 0.0256 and R^2 = 0.53, n = 19, P = 0.0004, respectively) levels in zooplankton samples from the Mediterranean Sea in 1976–2023.

toxic TEs. The Mediterranean Sea has been considered strongly impacted from Hg pollution with elevated concentrations in marine biota. For instance, the Mediterranean fish appear to accumulate twice levels than the same species living in the Atlantic Ocean (UNEP/MAP, 2012), due to the geomorphology of the basin, but also to the strong anthropogenic emissions from various land-based sources, which are transported to the sea through river, as well as through atmospheric precipitation (Cossa and Coquery, 2005). However, Hg levels in zooplankton from this study (0.1 ± 0.1 mg kg^{-1} dw) appear to be not so alarming, but at intermediate concentrations between the Gulf of Aden (Boldrocchi et al., 2020), 10 times lower, and the Laptev Sea in the Arctic Ocean (Lobus et al., 2019), 10 times higher.

4. Conclusion

Zooplankton is an early warning indicator of aquatic pollution, fundamental for the monitoring of marine pollution. The collection of zooplankton samples on a regular basis over wide geographical coverage has proven to be a reliable tool for providing baseline information of the environmental pollution in the marine environment. The Mediterranean has been longed referred as a hotspot of marine biodiversity, but also of chemical pollution. However, studies on the contamination of zooplankton organisms by both trace elements and organochlorine compounds mainly came from the 1970s and 1980s leaving a gap of information over the past 40 years. Despite the limited data on both DDT and PCB levels in zooplankton and a lack of uniformity in the expression of data (e.g. fresh, dry or lipid weight), results presented here showed that the levels of persistent organic pollutants in the basin not only appear to be decreased over the last 50 years, but they are far lower than well-renowned contaminated areas with poor management of waste water, with chronic release of industrial pollutants, mostly untreated, and where the use of pesticides is still scarcely controlled (e.g. the Gulf of Aden). On the contrary, pollution of TEs is more complicated to be evaluated due to the multiple sources of contamination, both natural and anthropogenic. However, by comparing TE levels in zooplankton from the Mediterranean Sea with those from other marine bodies, it appears that certain elements such as Zn, Cu, Pb and Ni show comparable concentrations to those measured in zooplankton from strongly polluted coastal areas such as Taiwan, Bay of Bengala and Gulf of Aden. Other TEs, especially those much toxic like Cd, Co, and Hg, however, were at intermediate levels between strongly affected and more pristine areas. Overall, the level of TEs in zooplankton are locally heterogeneous and do not show a consistent pattern. This is due to the fact that accumulation of TEs in biota depends on many factors such as the natural and human source of pollution, the assimilation potential, life cycle, and elimination/uptake rates, which is variable among planktonic groups. Despite this, certain TEs showed a general pattern of stable to declining trends in the Mediterranean Sea, which may reflect a general improvement in areas with particularly high levels of TE pollution.

CRediT authorship contribution statement

G. Boldrocchi: Conceptualization, Methodology, Visualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration. **B. Villa:** Investigation, Formal analysis, Writing – original draft, Visualization. **D. Monticelli:** Validation, Investigation, Resources, Formal analysis, Writing – review & editing. **D. Spanu:** Validation, Resources, Writing – review & editing. **G. Magni:** Resources, Project administration, Funding acquisition. **J. Pachner:** Resources, Project administration, Funding acquisition. **M. Mastore:** Resources, Writing – review & editing. **R. Bettinetti:** Validation, Resources, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors did not use any generative AI and AI-assisted technologies in the writing process.

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Appendix A. Supplementary data

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