Chemosphere 366 (2024) 143553

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Evaluation of the Adriatic Sea pollution using mesozooplankton as an environmental indicator

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HIGHLIGHTS

- This study evaluated the contamination status of the Adriatic Sea using zooplankton.
- PCB and DDT contamination appears to be declining over the past 50 years.
- DDT levels are comparable to pristine and less impacted areas worldwide.
- Data on trace elements pointed out some hotspots within the basin.
- Most TE levels in zooplankton are lower than renowned worldwide contaminated areas.

GRAPHICAL ABSTRACT

ARTICLE INFO

Handling editor: Milena Horvat

Keywords:
Plankton
Persistent organic pollutants
Trace elements
Marine pollution

ABSTRACT

The Adriatic Sea is an enclosed basin threatened by marine pollution due to its hydrographic features and anthropogenic pressure. Although zooplankton has been worldwide regarded as an immediate warning signal of contamination, limited information is available on the contamination of these organisms at the Adriatic level. Hence, this study provides comprehensive data on the presence and levels of multiple pollutants in zooplankton collected from 46 locations. With regards to legacy contaminants, both PCB and DDT levels have declined since the 1980s. Specifically, most samples were characterized by low DDT contamination (average of 3 ± 2.7 ng g^{-1} dry weight) and only few of these accumulated levels of concern for what concerns PCB, pointing out possible

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https://doi.org/10.1016/j.chemosphere.2024.143553
Received 19 July 2024; Received in revised form 14 October 2024; Accepted 15 October 2024

Mercury
metal(loid)s

hotspots of contamination in the central-eastern Adriatic Sea. As regards metal(loid)s, the Metal Pollution Index identified areas of concern in the north Adriatic Sea (Gulf of Venice) with high levels of Co, Cu, Hg, Cr and Pb; in the Central Adriatic Sea (Tremiti islands) with high levels of Co, Ni, Hg, Cr and Pb; in the Southern Adriatic Sea (Taranto and offshore Corfu), with high levels of most metal(loid)s, especially Cr, Ni and Zn. Certain metal(loid)s (e.g. Cd, Pb and Hg) have declined over time and most of them are lower than well-known contaminated worldwide marine ecosystems. Only Cu appears to be particularly high in the Mediterranean zooplankton. Overall, this work suggests a general improvement of the status of contamination of the Adriatic Sea.

1. Introduction

At European Union level, scientific knowledge has been largely recognized as fundamental for our understanding of the ocean and for effectively conserving and managing its resources (European Commission, 2024). For this reason, extensive water policies and actions are in place for protecting and restoring marine ecosystems. Among these, the Marine Strategy Framework Directive, issued to protect European marine environments, includes 11 Descriptors, with two of them namely “Descriptor 8: Contaminants” and “Descriptor 9: Contaminants in seafood” entirely focusing on the marine pollution issue with the aim of progressively mitigating it, avoiding adverse effects on marine ecosystems and humans (European Commission, 2024). In this context, assessing the presence and level of contaminants represents a priority especially considering that the marine environment has been deliberately used for long time as a disposal of our wastes, including chemical substances (Elfes et al., 2010). Therefore, comprehensive monitoring programs in environmental matrices, including biota, are necessary for the evaluation of the marine environmental status.

Today, a substantial amount of evidence showed that, despite regulatory bans, certain substances, including legacy pollutants, such as PCB and DDT, and metal(loid)s, are very persistent and still present in both the marine biotic and abiotic compartments. This is especially evident in semi enclosed basins like the Mediterranean Sea, whose hydrographic features enhance the accumulation of pollutants (Gómez-Gutiérreze et al., 2007; UNEP/MAP, 2001). Still, scientific data focusing on the Mediterranean Sea mostly originates from circumscribed studies, leaving a significant knowledge gap for most areas (Albaigés, 2005; Boldrocchi et al., 2023; UNEP/MAP, 2012). Furthermore, most research are limited to certain pollutants, especially those most harmful to human health (e.g. mercury), leaving other toxic contaminants poorly investigated (Albaigés, 2005). A further complication resides in the considerable heterogeneity in terms of methodologies and analytical procedures in the literature data concerning the Mediterranean Sea, limiting the reliability of data comparison (Albaiges et al., 2011; Bajt et al., 2019; Boldrocchi et al., 2023; UNEP/MAP, 2012). Consequently, it is difficult to use data coming from regional assessment and draw definite conclusions on the environmental status (Albaiges et al., 2011; Boldrocchi et al., 2023; UNEP/MAP, 2012), even for areas of special interest, like the Adriatic Sea, eastern Mediterranean Sea.

The Adriatic Sea, in fact, is threatened by marine contamination due to its hydrogeographic features. This basin, in fact, is a semi-enclosed, mostly river-dominated coastal system, and it collects approximately 1/3 of the freshwater inflow received by the entire Mediterranean (Danovaro and Boero, 2019). These features combined with high urbanization, population density especially in the northern part, and the intense maritime transportation led to multiple stressors from both land and maritime activities (Combi et al., 2016a,b; Danovaro and Boero, 2019; UNEP/MAP, 2012). Accordingly, several studies have monitored the levels of either POPs or specific metal(loid)s in marine bioindicators, especially mussels and clams, but mainly focusing on local areas (e.g. Bille et al., 2015; Di Leo et al., 2010; Giandomenico et al., 2013; Herceg-Romanić et al., 2014; Milun et al., 2016; Perugini et al., 2004), whereas only a few covered wider surfaces and multiple contaminants simultaneously (e.g. Bajt et al., 2019; Combi et al., 2016a,b). With regards to zooplanktonic organisms, which are largely used worldwide as early warning signal of aquatic contamination (e.g. Battuello et al., 2016; Bettinetti et al., 2012; Kahle and Zauke, 2003; Piscia et al., 2023), the number of studies from the Adriatic Sea is even more scarce and predominantly undertaken at a local level (e.g. Crisetig et al., 1982; Faganeli et al., 2023, 2003; Picer, 2000; Tronczynski et al., 2016; Vučetić and Dujmov, 1980). Indeed, previous studies are mainly focused on taxonomy characterization, reporting planktonic communities principally dominated by copepods (especially genus *Calanus, Acartia*, *Oithona*, and *Centropages*) and cladocerans (especially genus *Penilia*, *Podon* and *Evadne*) (Aubry et al., 2012; Bojanic et al., 2005; Fanelli et al., 2022; Fonda-Umani et al., 2005; Pierson et al., 2020; Vidjak et al., 2019). Other common crustaceans were hyperiids, decapod larvae, mysids, and euphausiids. Molluscs and Chaetognatha were locally abundant, and thaliaceans and calycophorans were the main representative of gelatinous zooplankton (Fanelli et al., 2022; Vidjak et al., 2019). The northern Adriatic Sea also experiences large, episodic blooms of scyphozoan medusae (e.g. *Chrysaora* spp. and *Pelagia noctiluca* and others such as *Aurelia* spp., *Rhizostoma pulmo*) (Pierson et al., 2020). Thus, up to date information on levels of pollutants in zooplankton is necessary since these organisms not only represent tracers of environmental contamination (Bettinetti et al., 2012), but also a fundamental link in transferring pollutants along the food web (Piscia et al., 2023).

Consistently, this study provides a first comprehensive geographical and temporal trend of elemental and organic contaminants, including PCB, DDT and 15 metal(loid)s, in zooplankton organisms from the Adriatic Sea and determines possible areas of concerns. Furthermore, this study aims to evaluate the level of contamination of the Adriatic Sea, mainly by comparison with the concentrations measured with in the Tyrrhenian Sea (Boldrocchi et al., 2023), as well as from comparable studies carried out at global level. Overall, this study contributes to fulfil the urgent gap of information about the time series of the Mediterranean Sea contamination.

2. Materials and method

2.1. Field sampling

Forty-two samples of zooplankton organisms were collected from the Adriatic Sea, while four from the Ionian Sea at the border of the Adriatic basin, daily, using a 200 μm mesh net: the first 20 m of the water column were sampled (Fig. 1). A total of 1235 miles were sailed between April and July 2023, starting from Taranto (Puglia – South of Italy), up to Trieste (Friuli Venezia Giulia – North of Italy), then sampling took place in Slovenian, Croatian, Montenegro, Albanian coastal waters, with the last sampling offshore Corfu Island (Greece) (Fig. 1). Zooplankton sampling took place in coastal waters and each sample, once collected, was stored frozen until laboratory analyses.

2.2. Chemical analyses

Once in the laboratory, samples were washed with MQ water, then lyophilized for 72 h and weighted. PCB and DDT analyses were performed on approximately 0.2 g dry weight (dw) aliquots. However, since the amount of sample was not sufficient to analyze both class of contaminants (organic and elemental pollutants), 27 samples only were processed for PCB and DDT quantification. Of these, 10 samples were pooled together based to their geographical closeness, allowing to reach the needed sample amount (0.2 g dw), leading to a total of 22 analyzed samples. A total of 13 PCB congeners were investigated: 18, 28, 31, 44, 101, 118, 138, 149, 153, 170, 180, 194 and 209. Total PCB (ΣPCBs) and DDT (ΣDDTs) concentrations were calculated as the sum of all PCBs congeners and the sum of the six DDT, DDE and DDD isomers (ortho, para and para, para). All the data were reported as ng g^{-1} dw.

The extraction procedure followed Bettinetti et al. (2012). Briefly, dried samples were extracted for 2 h using 50 ml of a 1:1 acetone/n-hexane solution, the solvent mixture evaporated, and the lipid content gravimetrically measured. The residual was then digested with 7 ml of sulfuric acid for 24 h (98%, Carlo Erba, Italy). The supernatant phase was passed through a Florisil® column and concentrated to 0.5 ml. Samples were analyzed by gas chromatography (GC Carlo Erba, Top 8000) coupled to an Electron Capture Detector (ECD). Quantification was performed by external calibration: standards for pp'DDT, pp'DDE and pp'DDD were acquired from Sigma-Aldrich (Pestanal standards), whereas PCB diluted standards were prepared from a concentrated (10 mg/L) PCB standard mixture. To ensure data quality, the standard reference materials BCR-598 and BCR-349 (Community Bureau of Reference, Brussels) for DDT and PCB residues were both analyzed. The percentages of recovery performed in triplicates were the following: 107.5 ± 4% (p,p'DDE), 106.2 ± 4% (p,p'DDD), and 106.2 ± 3% (p,p'DDT) for DDTs, and 91.3 ± 1.1% and 102.2 ± 1.6% for PCBs.

Fifteen metal(loid)s, namely Al, As, Cr, Cd, Cu, Co, Fe, Hg, Mn, Ni, Pb, Se, Sr, V and Zn were determined. The analytical procedure is explained in detail in Boldrocchi et al. (2021): a lyophilized zooplankton aliquot (approximately 20 mg) was mineralized by microwave assisted digestion (Milestone ETHOS One) with 0.5 ml ultrapure HCl and 0.5 ml ultrapure HNO_3 produced by sub-boiling distillation (Monticelli et al., 2019) in a multibatch system specially designed for small mass samples (Spanu et al., 2020). The samples were subsequently moved to low-density polyethylene bottles and diluted with ultrapure water. Solutions were analyzed by inductively coupled plasma mass-spectrometer (ICP-MS, Thermo Scientific ICAP Q) using kinetic energy discrimination (KED) mode to reduce interferences. Results are reported as mg kg^{-1} dw. To ensure data quality, one aliquot of the certified reference material BCR-414 for metal(loid)s in plankton was analyzed in each analysis batch. The recovery of certified elements (V, Cr, Mn, Ni, Cu, Zn, As, Se, Cd, Hg and Pb) were in the range of 83–110%, proving no statistical difference from the certified values (average of 4 analysis batches) (Table S1, Supplementary Materials). The limits of detection on the solid sample 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. In particular, the limit of detection for mercury was 0.03 mg kg^{-1}.

The Metal Pollution Index (MPI) was calculated as follows (AMA, 1992):

$$MPI = (Cf_1 \times Cf_2 \ldots Cf_n)^{1/n},$$

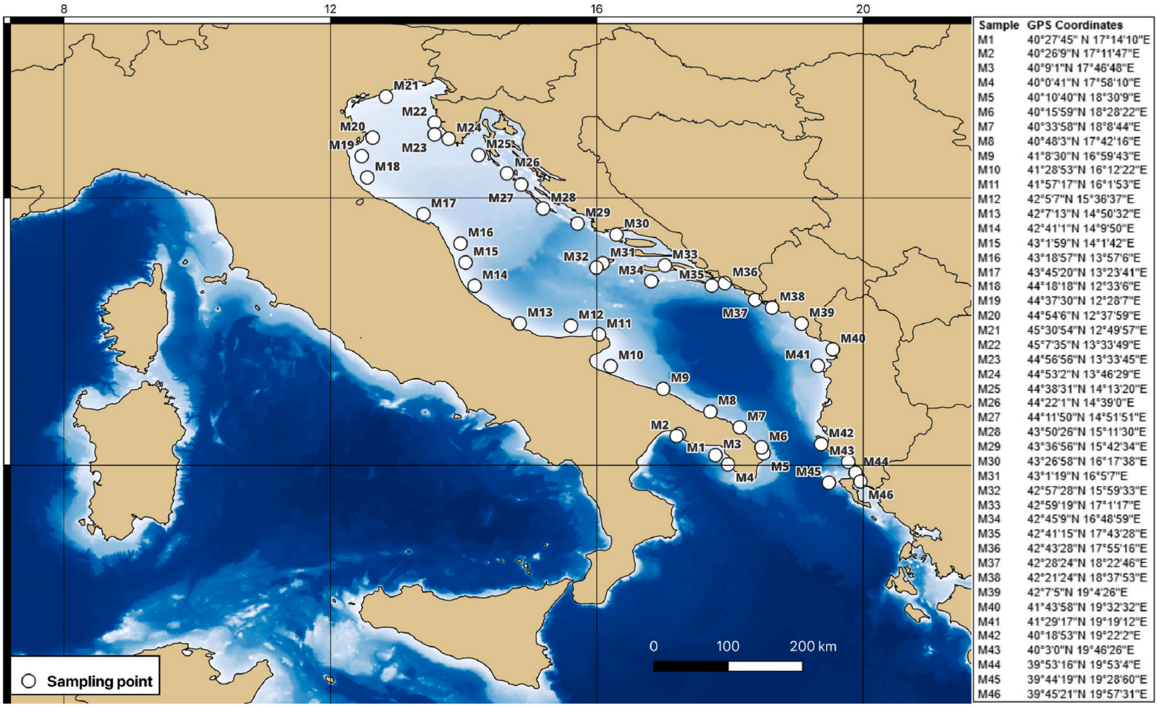
where Cf_i is the concentration of the metal(loid) *i* in the sample.

2.3. Statistical analysis

The significance level was set to α = 0.05. The Shapiro–Wilk test was used to assess normality, and Levene's test was employed to evaluate homogeneity of variance. The concentration of PCB 101 was used for comparison purposes, as this congener was the most ubiquitous congener in both the present and the Tyrrhenian basin. Since both assumptions were respected, *t*-test after square root transformation was employed to assess the equality of the concentrations in the two basins. The Welch version of Independent-samples *t*-test was used to compare DDT level, after a square root transformation, as the assumption of equal variances was not respected. Therefore, as this does not assume that the variances in the two groups are equal. To test any statistical differences in the levels of metal(loid)s measured in zooplankton from the Adriatic and the Tyrrhenian Sea, an Independent-samples *t*-test was performed when assumptions of normality and homogeneity of variance were confirmed, otherwise the non-parametric Wilcoxon signed-rank test was used.

3. Results and discussion

Our study provides comprehensive background levels of target chemical loads in zooplankton samples in the coastal areas of the



Sample	GPS Coordinates
M1	40°27'45" N 17°14'10"E
M2	40°26'9"N 17°11'47"E
M3	40°9'1"N 17°46'48"E
M4	40°0'41"N 17°58'10"E
M5	40°10'40"N 18°30'9"E
M6	40°15'59"N 18°28'22"E
M7	40°33'58"N 18°8'44"E
M8	40°48'3"N 17°42'16"E
M9	41°8'30"N 16°59'43"E
M10	41°28'53"N 16°12'22"E
M11	41°57'17"N 16°1'53"E
M12	42°5'7"N 15°36'37"E
M13	42°7'13"N 14°50'32"E
M14	42°41'1"N 14°9'50"E
M15	43°1'59"N 14°1'42"E
M16	43°18'57"N 13°57'6"E
M17	43°45'20"N 13°23'41"E
M18	44°18'18"N 12°33'6"E
M19	44°37'30"N 12°28'7"E
M20	44°54'6"N 12°37'59"E
M21	45°30'54"N 12°49'57"E
M22	45°7'35"N 13°33'49"E
M23	44°56'56"N 13°33'45"E
M24	44°53'2"N 13°46'29"E
M25	44°38'31"N 14°13'20"E
M26	44°22'1"N 14°39'0"E
M27	44°11'50"N 14°51'51"E
M28	43°50'26"N 15°11'30"E
M29	43°36'56"N 15°42'34"E
M30	43°26'58"N 16°17'38"E
M31	43°1'19"N 16°5'7"E
M32	42°57'28"N 15°59'33"E
M33	42°59'19"N 17°1'17"E
M34	42°45'9"N 16°48'59"E
M35	42°41'15"N 17°43'28"E
M36	42°43'28"N 17°55'16"E
M37	42°28'24"N 18°22'46"E
M38	42°21'24"N 18°37'53"E
M39	42°7'5"N 19°4'26"E
M40	41°43'58"N 19°32'32"E
M41	41°29'17"N 19°19'12"E
M42	40°18'53"N 19°22'2"E
M43	40°3'0"N 19°46'26"E
M44	39°53'16"N 19°53'4"E
M45	39°44'19"N 19°28'60"E
M46	39°45'21"N 19°57'31"E

Fig. 1. Sampling sites of zooplankton organisms in the Adriatic Sea (April–July 2023).

Adriatic Sea from 46 locations, which might be used as reference values for future comparison on the bioaccumulation of contaminants in zooplankton at the Mediterranean and global level.

3.1. Persistent organic pollutants

The concentration of legacy pollutants in zooplankton samples ranged from 3.2 to 113.1 ng g^{-1} dw (mean 31.1 ± 29.6 ng g^{-1} dw for the sumPCB$_{13}$), while from 1.28 to a maximum of 9.6 ng g^{-1} dw (mean 3.1 ± 2.7 ng g^{-1} dw) for DDT (Table 1). Excluding those samples (M1-M4) collected in the Gulf of Taranto, in the Ionian Sea, the mean values were 32.2 ± 29.9 ng g^{-1} dw for the sumPCB$_{13}$, and 3.2 ± 2.8 ng g^{-1} dw for DDT.

With regards to PCBs, PCB 101 (19.3 ± 27.2 ng g^{-1} dw) shows the highest concentration, accounting for 45% of all PCBs on average. It was detected in all samples except for one. Octa- and Deca-CB were instead not detected. This finding is in line with what previously reported from middle latitudes (e.g. Mediterranean Sea; Boldrocchi et al., 2023), higher latitude (e.g. British Columbia coastal waters; Desforges et al., 2014) and polar regions (e.g. Antarctic; Nash et al., 2008), where zooplankton preferentially bioaccumulated PCB 101, the most ubiquitous PCB. Other congeners presented in significant amounts included PCB 44, 149 and 18 (approx. for 9–10% of all PCBs: Table 1).

With regard to DDT, the most ubiquitous metabolites were p,p'DDD and p,p'DDE found in 95% and 75% of the samples, respectively. The less frequent metabolites were o,p'DDT and p,p'DDT found in only 45% and 36% of total samples, respectively.

DDE and DDD are well known degradation products of DDT commercial formulations under aerobic and anaerobic conditions, respectively. Therefore, the ratio pp'DDE/pp'DDT has served as an index of the aging process of pesticides in the environment: a ratio greater than 1 reflects past DDT contamination, otherwise fresh input in the environment. In our study, despite the pp'DDE/pp'DDT ratios showed a wide range of variation (0.02–12), DDE concentrations were higher compared to DDT, leading to a pp'DDE/pp'DDT ratio higher than 1. Ratios below 1 were observed in a small number of samples, which is expected since occasional inputs of this compound into coastal areas might originate from atmospheric deposition, agricultural soil leaching, or sediment resuspension (UNEP/MAP, 2012).

Table 1

Concentrations of metal(loid)s (mg kg^{-1} dw), PCB and DDT compounds (ng g^{-1} dw) in zooplankton samples of the Adriatic Sea in 2023. "N.d." – "Not detected".

Pollutant	Congener	Mean ± SD	Median	Min	Max
PCB	PCB 18	2.5 ± 2.2	2.2	<LOD	9.0
	PCB 28 + 31	1.9 ± 1.7	1.6	0.18	6.2
	PCB 44	5.2 ± 3.8	4.3	<LOD	14.1
	PCB 101	19.3 ± 27.2	8.2	0.23	100
	PCB 149	3.4 ± 2.8	3.6	<LOD	7.5
	PCB 118	1.8 ± 1.4	1.3	0.33	4.2
	PCB 153	2.3 ± 2	1.5	<LOD	6.9
	PCB 138	2.3 ± 1.9	1.5	0.12	5.9
	PCB 180	n.d.			
	PCB 170	n.d.			
	PCB 194	n.d.			
	PCB 209	n.d.			
	TOT	**31.1 ± 29.6**	**21.9**	**3.2**	**113**
DDT	o,p' DDE	1.18 ± 1.7	0.27	0.06	5.7
	p,p' DDE	1.1 ± 2.0	0.44	<LOD	8.1
	o,p' DDD	0.41 ± 0.28	0.40	<LOD	0.81
	p,p' DDD	0.32 ± 0.27	0.23	<LOD	0.94
	o,p' DDT	0.52 ± 0.49	0.34	0.11	1.8
	p,p' DDT	2.5 ± 2.1	2.03	0.2	5.7
	TOT	**3.1 ± 2.7**	**2.11**	**1.3**	**9.6**
Sr		6206 ± 7118	4187	65.5	25837
Al		2190 ± 3286	1042	165	18091
V		5.19 ± 6.29	3.36	0.3	33.3
Cr		13.4 ± 39.2	4.03	0.52	245
Mn		53.5 ± 74.1	22.8	4.3	334
Fe		1976 ± 2719	907	87.7	13892
Co		1.57 ± 1.51	0.89	0.14	6.03
Ni		9.81 ± 18.6	4.78	1.1	114
Cu		135 ± 283	38.0	10.6	1598
Zn		149 ± 88.6	128	30.1	541
As		11 ± 4.97	11	3.7	27.1
Se		3.48 ± 1.07	3.73	1.1	5.14
Cd		1.46 ± 0.85	1.41	0.26	3.42
Hg		0.06 ± 0.03	0.05	0.02	0.20
Pb		3.05 ± 3.61	1.65	0.21	14.4

3.2. Spatial and temporal variability

Data on zooplankton showed that PCB contamination is not uniformly distributed throughout the Adriatic Sea, ranging from 3.2 ng g^{-1} dw in Puglia (southern Italy) to 113 ng g^{-1} dw in Croatia (eastern Adriatic) (Fig. 2). With regards to DDT, besides a few punctual locations that showed levels in the range of 6–9 ng g^{-1} dw, the rest was below 5 ng g^{-1} dw suggesting, nowadays, a low contamination in the Adriatic Sea. However, few areas of concern have been identified, where zooplankton accumulated higher levels of both pollutants. Starting from the north of the basin, the Gulf of Venice presented high PCB levels, which are likely linked to the discharge of the Po River in the Northern Adriatic (e.g. Combi et al., 2016a,b). The Po River receives input from densely populated and industrialized zones, representing one of the main PCB sources to the Adriatic Sea (Combi et al., 2016a, 2016b; Galassi et al., 1994; Viganò et al., 2023). Zooplankton samples collected south of the Po River delta, as well as in front of Rijeka (Croatia), showed also higher DDT levels compared with other sampling stations (Fig. 2), likely attributed to the inputs from past contamination transported by Po River (Viganò et al., 2019). Going southern, the area of Šibenik-Split (Croatia) and the Gulf of Drin (Albania) showed the highest PCB levels, exceeding 70 ng g^{-1} dw (Fig. 2). Multiple studies have documented high levels of PCBs in the Šibenik-Split area (e.g. Bajt et al., 2019; Herceg-Romanić et al., 2014; Kožul et al., 2011), especially in the Krka River estuary, that likely receives an anthropogenic input of untreated wastewaters from the city of Šibenik (Cukrov et al., 2008). Indeed, PCB levels in the Krka River estuary are highly influenced by wastewaters discharge and run off from the mainland into the coastal area (Bajt et al., 2019). Other possible PCB sources in this area include the capacitors and transformers damaged in the war, as well as uncontrolled landfills, and the proximity to the yacht marina and big ship route (Herceg-Romanić et al., 2014; Kožul et al., 2011). In addition, the area of Split is highly urbanized and industrialized, with Kaštela Bay already reported as a hotspot of pollution for multiple contaminants (e.g. metalloids) from many different sources (Kožul et al., 2011; UNEP/MAP, 2012). Therefore, results from this study corroborate the data previously reported for other organisms, e.g. blue mussels and red mullets, in both coastal areas (UNEP/MAP, 2012), including the PCB peak measured in the Gulf of Drin, which is likely ascribable to the Drin River and its tributaries (Nuro and Marku, 2012). Finally, isolated points of DDT contamination can be found in the southern basin, likely attributed to surface runoff from agricultural and urban land, and local discharges (Thiombane et al., 2018).

Overall, in this study, zooplankton sampled in semi-enclosed gulfs and close the estuary or delta of major rivers (Po, Krka and Drin) appear to accumulate higher PCB and DDT concentrations. Major rivers, in fact, are considered a passive source of riverborne materials and associated pollutants which can be dispersed in the basin driven by the general water circulation (Combi et al., 2016a). During winter, the northern Adriatic is subject to intense cooling associated with Bora wind, resulting in the formation of the North Adriatic Dense Water, which travels south following the Po River plume. This southerly flow is responsible for a high delivery of particles, and associated contaminants, to the southern areas of Adriatic Sea (Langone et al., 2016). The general surface circulation in the Adriatic Sea consists also of a northerly flow along the eastern coast (Orlic et al., 1992), which might spread particles and associated pollutants deriving from Albanian rivers along the basin

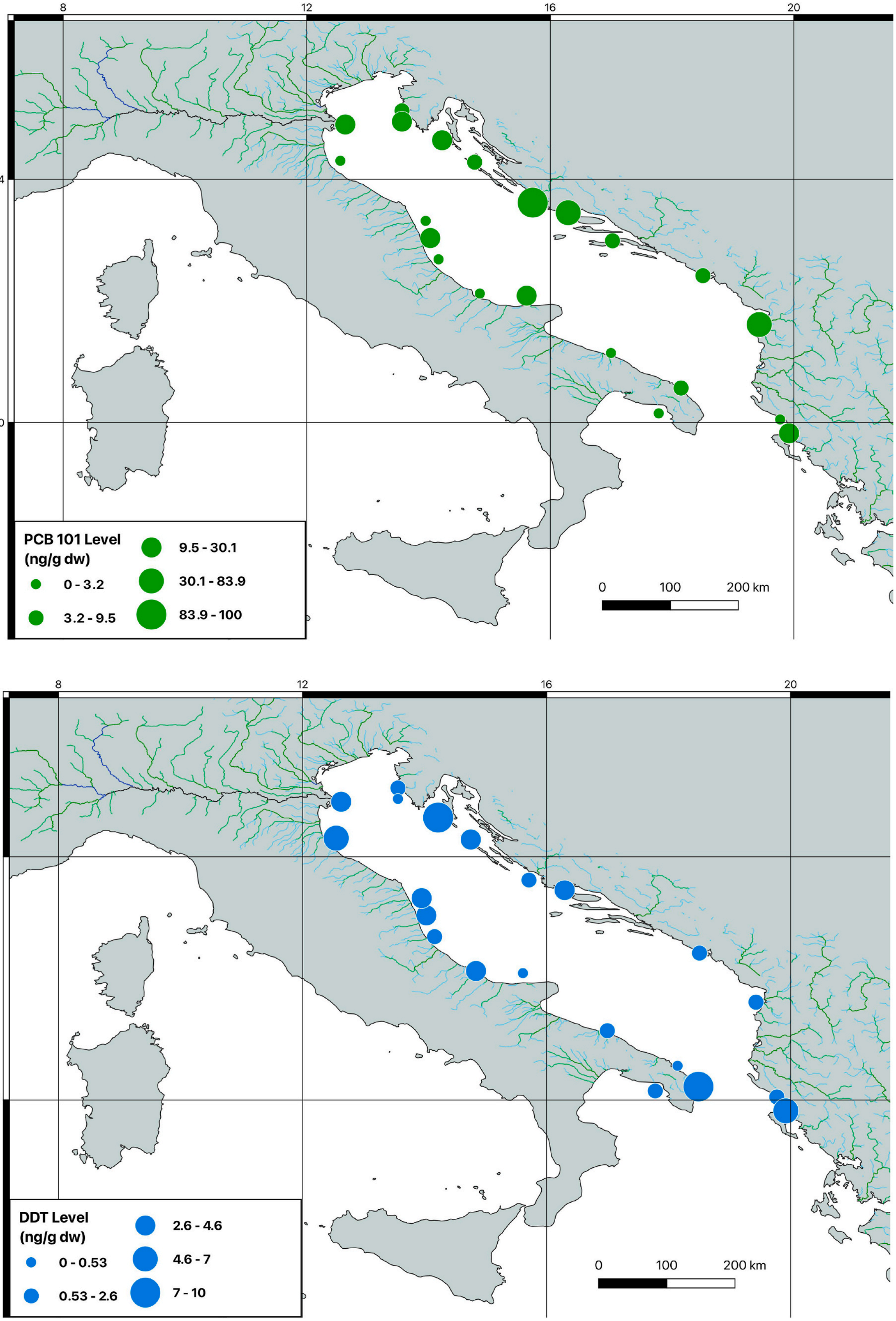


Fig. 2. Concentrations (ng g^{-1} dw) of PCBs and DDT determined in zooplankton samples from the Adriatic Sea in 2023.

(Vlachogianni et al., 2018). Thus, while some sources of contamination highlighted in this study are punctual and surely derive from local origins, others might be attributed to the general water circulation and oceanographic conditions.

Possible temporal trends were investigated by comparing OC levels to those previously published on zooplankton organisms from the Adriatic Sea. The bioaccumulation of DDT and PCBs in zooplankton from the western Mediterranean is scarcely reported (Boldrocchi et al., 2023), and even less from the Adriatic Sea where most studies dated back to 1977–1999 (Table S2, Supplementary Material). Despite the paucity of information, it appears that both PCB and DDT levels have been decreasing from the 1970s, when ecotoxicology studies reported mean PCB levels of from 38 ng g^{-1} dw for the sum of five congeners (Fowler and Elder, 1980) up to 126 ng g^{-1} dw (Vučetić and Dujmov, 1980) (Table S2). Similarly, studies from the 1980s (Fowler and Elder, 1980; Vučetić and Dujmov, 1980) reported DDT levels in zooplankton were much higher than the current study (Table S2, Supplementary Materials). These results align with the prohibition of production and use of both compound classes since the mid-1970s in several EU countries. Similar decreasing trend have been also described for other marine bioindicators, such as mussels, but also for sediments, whose levels have decreased since 1971, with a decline more evident for DDT than for PCBs in accordance with the current study (UNEP/MAP, 2012).

3.3. *Comparison with Mediterranean and worldwide levels*

The levels of contamination by POPs measured in this study were compared to the ones determined in the Tyrrhenian Sea. The comparison was carried out using the published data in Boldrocchi et al. (2023) as these are the most recent and comprehensive of the whole Tyrrhenian Sea. The mean concentration of PCB 101 showed a significant difference between the two basins, with the Adriatic Sea showing lower mean levels ($t(39.2) = 2.25$, $p = 0.03$). Similarly, the Welch version of the *t*-test found that Adriatic Sea showed a statistically significantly lower DDT levels (Welch's *t*-test, $t(41.7) = 2.54$, $p = 0.0149$). High levels of both PCBs and DDT were measured in multiple bioindicators in the Tyrrhenian Sea, including zooplankton (Boldrocchi et al., 2023) and mussels (UNEP/MAP, 2012), highlighting several areas of concern. The Adriatic Sea has recently experienced a general improvement of the quality of most its rivers input: for instance, Viganò et al. (2023) showed that most of the investigated chemicals, including DDT and PCBs, are decreasing with time in the Lambro River, which is affluent to the Po River. The Lambro River, in fact, is historically responsible for a significant load of contaminants and for the impairment of water and sediment quality of the Po River (Viganò et al., 2015). A general decreasing trend has been also found in the sediments sampled in the Western Adriatic Sea (Combi et al., 2016a, 2020). Since the Adriatic Sea receives high river inputs (Danovaro and Boero, 2019), an improvement in the quality of its rivers affects the overall status of the basin (Combi et al., 2016a, 2016b). Indeed, the major mitigating factors, including the improvement of wastewater treatments, have reduced the inputs to rivers and ameliorated the environmental quality (Combi et al., 2016a, 2020; Viganò et al., 2023) leading to lower OC levels in the Adriatic zooplankton.

Finally, the DDT and PCB levels were compared to the ones recently determined in different marine basins (Table S2, Supplementary Materials). With regard to PCB concentration in zooplankton, levels measured in the Adriatic Sea (31.2 ± 29.6 ng g^{-1} dw) appears to be at intermediate levels between strongly affected areas, such as the Japanese coasts (107 ± 299 ng g^{-1} dw, Yeo et al., 2020) and Djibouti (336 ± 254 ng g^{-1} dw, Boldrocchi et al., 2018), and more pristine locations, such as the Svalbard Islands in the Greenland Sea (4.2 ± 6.4 ng g^{-1} dw, Pouch et al., 2022). On the contrary, DDT levels recorded in zooplankton from the Adriatic Sea (3.1 ± 2.7 ng g^{-1} dw) were much lower than what reported in areas particularly contaminated, such as the Black Sea (34.4 ng g^{-1} dw, Malakhova et al., 2023), and the Gulf of Aden (44.7 ± 27.4 ng g^{-1} dw, Boldrocchi et al., 2018), but comparable to what recorded from the Arctic Ocean (e.g. from 1.2 ± 0.95 to 5.6 ± 0.58 ng g^{-1} dw; Fisk et al., 2001; Hoekstra et al., 2002) and rural tropical coral reef ecosystems (0.8 ± 0.2 ng g^{-1} dw, Kang et al., 2022).

3.4. *Metal(loid)s*

Fifteen metal(loid)s were determined in zooplankton samples collected along the Adriatic basin. Sr, Al and Fe had the highest concentrations in zooplankton, followed by Zn, Cu and Mn (Table 1). Most of these metal(loid)s represent essential metals for the functioning of the organism (e.g. Fe, Zn, Cu and Mn) and therefore are likely to be found in higher concentrations. Aluminium is the most abundant metallic element in the Earth's crust, and it is widespread in the environment (Exley and Mold, 2015). Cr, As, Ni, V, Se, and Pb showed lower concentrations, ranging from a maximum of 13.4 ± 39.2 mg kg^{-1} for Cr to a minimum of 3.1 ± 3.6 mg kg^{-1} for Pb. On the contrary, Co (1.58 ± 1.51 mg kg^{-1}), Cd (1.46 ± 0.85 mg kg^{-1}) and Hg (0.06 ± 0.03 mg kg^{-1}) were determined in at much lower levels (Table 1). Excluding samples collected in the Ionian Sea (M1-M4), Sr, Al and Fe presented highest levels in the Adriatic basin (6662 ± 7226 mg kg^{-1}; 2334 ± 3382 mg kg^{-1} and 2068 ± 2802 mg kg^{-1}, respectively), followed by Zn, Cu and Mn (139 ± 63.4 mg kg^{-1}; 137 ± 294 mg kg^{-1}; 57 ± 76 mg kg^{-1}, respectively). Arsenic (11.1 ± 5.1 mg kg^{-1}), Ni (7.2 ± 7.6 mg kg^{-1}), Cr (7.3 ± 9.6 mg kg^{-1}), V (5.5 ± 6.5 mg kg^{-1}) and Se (3.5 ± 1.1 mg kg^{-1}) presented intermediate levels, while Pb (3.1 ± 3.7 mg kg^{-1}), Co (1.6 ± 1.5 mg kg^{-1}), Cd (1.5 ± 0.9 mg kg^{-1}) and Hg (0.06 ± 0.04 mg kg^{-1}) the lowest concentrations.

3.5. *Spatial and temporal variability*

Major anthropogenic sources of non-essential, toxic metal(loid)s include wastewater discharges, atmospheric deposition and transport from contaminated sites (UNEP/MAP, 2012). Although metal(loid) levels showed significant spatial variations, and each element showed some degree of specificity in its spatial distribution within the Adriatic Sea, this study identified possible hotspots of contamination. In these locations, zooplankton accumulated high levels of most of the analyzed metal(loid)s, with MPI >20 (range 3–59) (Fig. 3). The MPI provides a composite picture of the aggregate impact of each metal on the overall water quality. Starting from the north, the highest MPI was recorded in the Gulf of Venice (M20-21-22), followed by Pola (M24) and Lastovo Island (M34), both located in Croatia, Tremiti Islands (M12), the Gulf of Taranto (M1-2), located in the Italian Ionian side, and offshore Corfu Island (M45) (Fig. 3). These hotspots of contamination are even more evident when focusing solely on the most toxic metal(loid)s. For instance, zooplankton accumulated highest levels of Pb, Co, Ni, and Cr in Gulf of Venice (M20-21-22), Tremiti islands (M12), Taranto (M1-2), and Mathraki Island (M45) (Fig. S1, Supplementary Materials). Another group of toxic metal(loid)s, including Cu, Zn, Cd, Se and As, showed highest concentration in zooplankton sampled in proximity of Lastovo Island (M34) (Fig. S1, Supplementary Materials). Some of these locations have already been identified as areas of concern for chemical pollution; starting from the north of the basin, the first hotspot of chemical pollution is the Gulf of Venice (M20-21-22) which hosts Porto Marghera. This industrial district hosts multiple industrial activities including oil refining and storage, chemistry, shipbuilding, energy production and distribution, metal extraction and metallurgy, wastewater treatment, and hazardous waste incineration (Bellucci et al., 2002; Regione del Veneto and Comune di Venezia, 2003; Zonta et al., 2007): it is considered a high environmental hazard site (Italian law 426/1998). The Gulf of Venice also receives Po River discharges well renowned to be the source of contamination in the basin not only for organochlorine compounds, but also for metal(loid)s (Farkas et al., 2007; Viganò et al., 2019, 2023). Studies conducted on sediments (Lopes-Rocha et al., 2017; Riminucci et al., 2022), mussels (Camusso

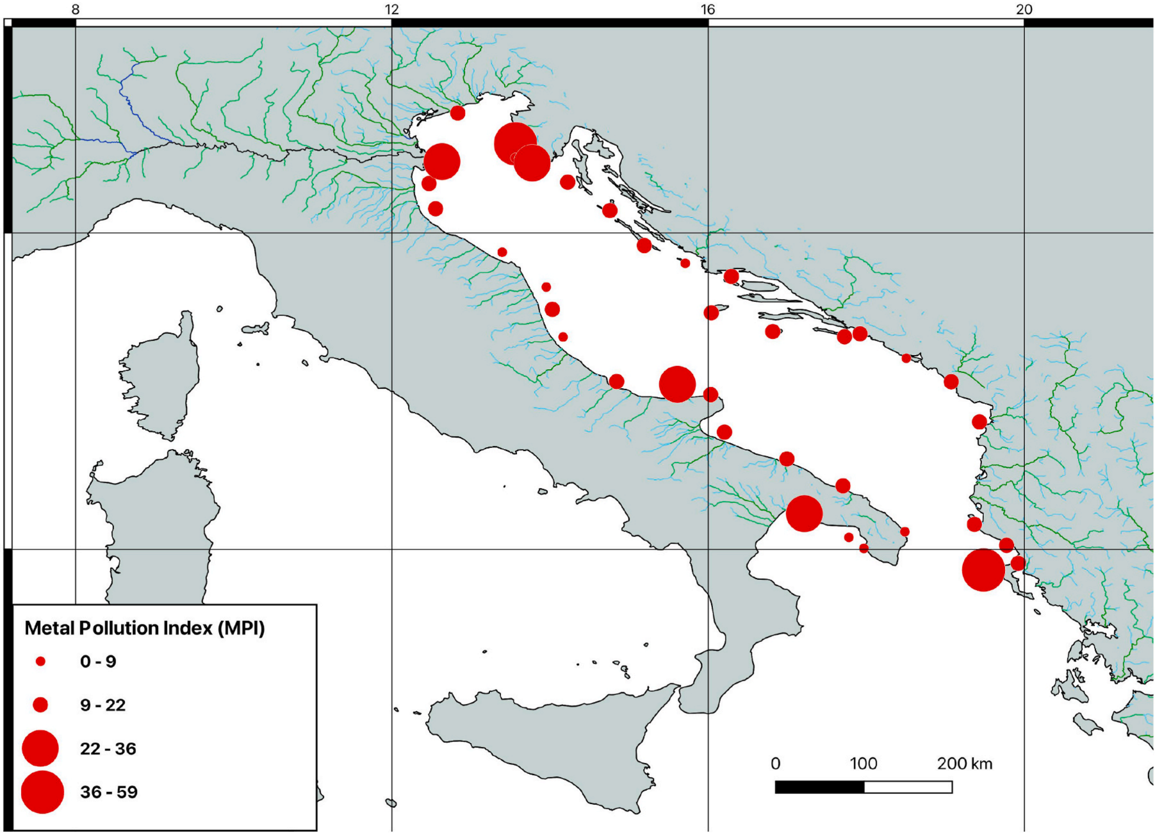


Fig. 3. Metal Pollution Index class map of zooplankton samples collected from the Adriatic Sea in 2023. Categories were presented using the Jenks natural breaks classification method in QGIS software.

et al., 1994; Fattorini et al., 2008) and seawater (Illuminati et al., 2019; Penezić et al., 2022) have shown that metal(loid) variability in the northern Adriatic basin is mainly influenced by the Po River runoff. Consistently, high levels of Pb, V, Co, Ni, Cr, Mn, Fe, Cu, Zn and Hg were registered in the zooplankton sampled in this sub-basin (Fig. S1, Supplementary Materials). Besides the Po River, the Gulf of Venice receives inputs from other multiple rivers, including the Italian Isonzo, Tagliamento, Piave, Adige; the Croatian Mirna and Raŝa; the Slovenian Dragonja and Rižana (Dolenec et al., 1998). The Isonzo River, for instance, contributes Hg to the Adriatic Sea as it drains the cinnabar-rich area close to the Idrija mine (Faganeli et al., 2003). Moreover, Isonzo River might be also a source of Pb and Zn into the Adriatic Sea as it transports metal contents deriving from the mining activity of the closed-mine of Raibl (Barago et al., 2021, 2023; Pavoni et al., 2020). Similarly, Piave River discharges water into the Adriatic Sea deriving from the extraction site of Salafossa (Pavoni et al., 2018). These two mines represent the largest mineral deposit of Pb and Zn (Covelli et al., 2020) and a well-known source of these elements to the environment. Besides rivers discharge that surely represents a major source of contamination, the Gulf of Venice is also a very densely urbanized area that bears the highest human pressure among the basin, which includes intense marine traffic, fisheries, untreated wastewater, coastal and offshore aquaculture and oil and gas exploration (Danovaro and Boero, 2019). Thus, the high levels of most contaminants found in this area are not surprising. Going southern, this study highlighted two more hotspots in the central Adriatic Sea, one in the Tremiti Islands (Italian side) and one in proximity of Lastovo Island (Croatian side). In the Tremiti, high levels of Pb, Ni, Co, Cr, Al, Fe, and Hg have been recorded which appears to be peculiar as these islands lack industrial activities (Fig. S1, Supplementary Materials). However, this site is known for presenting high element concentrations. For instance, Bajt et al. (2019) carried out a comprehensive study on chemical contamination in the Adriatic Sea using *Mytilus galloprovincialis* and found very high V levels in the Tremiti Islands, attributed to the gas platforms situated northward. Similarly, Spada et al. (2013) found very high levels of Cr in mussels which were linked to natural sediment enrichment. On the eastern side, the area of Lastovo and Mljet islands showed high levels of Cu, Zn, and As (793, 326 and 27.1 mg kg^{-1} dw, respectively). Both islands are exposed to the northwest sea current, which brings water, including pollutants, from the southern Adriatic. It is important to consider, in fact, that coastal zooplankton consists also of inorganic and organic particles containing metal(loid)s (Libes, 2009; Faganeli et al., 2023). Indeed, the plankton fraction include a variable amount of metal(loid)s associated with mineral particles, originating, for instance, from the bottom sediment resuspension and deposition of riverine particles in the river delta, as showed in the northern Adriatic Sea (Faganeli et al., 2023; Ogorelec et al., 1991). The area of Lastovo and Mljet islands has been demonstrated to accumulate high levels of plastic debris likely originated from transboundary litter from southern shores (Vlachogianni et al., 2018). Together with plastic debris, the northerly current brings also metal(loid)s originating from the south Adriatic Sea, in particular from Albanian rivers that carry high concentrations of metal(loid)s, primarily Cr, Zn and Ni, into the marine system and then transported north and deposited in the coastal area of the Mljet Island (Sondi et al., 2017). The geomorphology of the islands, then, with narrow bays and small beaches in the end, acts like a ‘funnel’ that favors the accumulation of litter and pollutants (Vlachogianni et al., 2018). Similarly to what concerns organochlorine compounds, also the eastern Adriatic rivers represent a “passive” source of metal(loid)s which can transported over wide distance driven by ocean currents (Lopes-Rocha et al., 2017).

Another well-renowned area of concern within the Mediterranean Sea is the Gulf of Taranto (southern Italy), located in the Ionian Sea, at the border of the Adriatic basin. This location is characterized by strong human pressures including a massive industrial settlement (Fe and steel factory, petroleum refinery and shipyard): the industrial area of Taranto has been classified as "Site of National Interest" (Ministry Decree 426/1998) that requires detailed characterization and remediation programs (Ministry Decree 468/01). All ecotoxicology studies conducted in the area, on different contaminants and different matrices (e.g. atmosphere, biota or sediments), identified this area as a polluted location, especially in proximity to the ILVA iron and steel factory (Annicchiarico et al., 2011) and the Navy Harbor (Cardellicchio et al., 2016). Considering the multiple sources of contamination in the area, high values of Cr (245 mg kg^{-1} dw), Co (2.64 mg kg^{-1} dw), Ni (113.6 mg kg^{-1} dw), Zn (541 mg kg^{-1} dw), Cu (181 mg kg^{-1}), Hg (0.1 mg kg^{-1} dw), and Pb (6.56 mg kg^{-1} dw) reported in this location, compared to the other sampling points from the Adriatic Sea, are in line with previous findings. Finally, zooplankton showed particularly high levels of Cu (1598 mg kg^{-1} dw), Al (6981 mg kg^{-1} dw), V (18.1 mg kg^{-1}), Cr (36.5 mg kg^{-1}), Ni (38.4 mg kg^{-1} dw), Pb (9.85 mg kg^{-1} dw), offshore Corfu Island. Those levels might be linked to the intense maritime traffic as the area of Otranto Strait represents the entrance into the Adriatic Sea. Moreover, illegal dumping of toxic waste through ship sinking causes a significant contamination in the southern Adriatic, especially along the coast of the Puglia Region (Danovaro and Boero, 2019).

Table S3 (Supplementary Materials) compares the data here collected to the ones reported in the literature: a limited number of studies investigated the concentration of metal(loid)s in zooplankton organisms and the majority of these dates back to the 2000s and focus on very specific pollutants (e.g. Hg, Cd and Pb). Starting with Hg, levels from the present study (0.06 ± 0.03 mg kg^{-1} dw) were definitively lower than those from studies before 1980s (e.g. 1.63 mg kg^{-1} dw, Vučetić et al., 1974; 0.21 mg kg^{-1} dw, Kosta et al., 1978; 1.8 ± 1.4 and 2.6 ± 2.1 mg kg^{-1} dw, Crisetig et al., 1982) (Table S3, Supplementary Materials), suggesting a decreasing trend, as reported by Živković et al. (2017). Nevertheless, the use of different analytical techniques and the advancements in analytical procedures (sample treatment, contamination control …) may have played a role in determining this apparent decrease. Mercury levels reported in this study were in line with more recent data from samples collected in the entire Adriatic basin, that ranged from 0.02 to 0.14 mg kg^{-1} dw (Ferrara and Maserti, 1992). All other studies reported much higher Hg levels: e.g. 530 ± 220, 202 ± 71 and 21 ± 6 mg kg^{-1} dw, respectively, in mesozooplankton from 1995 to 1996 (Faganeli et al., 2003); 0.25 mg kg^{-1} dw in 1999 (Horvat et al., 1999) and 0.17 mg kg^{-1} dw in 2016 (Faganeli et al., 2023) (Table S3, Supplementary Materials). It is worth noting that all these studies focused on the Gulf of Trieste (northeastern sub-basin), which is renowned for its pollution. Only one localized study reported Hg mean values in mesozooplankton collected in 2014 (Živković et al., 2019) comparable to our study, further suggesting no strong changes since the 1990s. With regards to Cd and Pb, the mean level reported from this study (1.46 ± 0.85 mg kg^{-1} dw and 3.05 ± 3.61 mg kg^{-1} dw, respectively) were both much lower than studies reported in the 1980s, which were at least 2.5 and 3 folders higher for Cd and Pb, respectively (Table S3, Supplementary Materials).

3.6. Comparison with Mediterranean and worldwide levels

Analogously to legacy contaminants, metal(loid) concentrations were compared to the ones measured from the Tyrrhenian Sea (Boldrocchi et al. (2023): no significant differences were found for most of metal(loid)s, except for As, after square root transformation ($t(88.39) = -2.13$, $p = 0.036$), Ni ($\chi^2 = 26.66$, $df = 1$, $p < 0.0001$), Zn ($\chi^2 = 20.61$, $df = 1$, $p < 0.0001$), Hg ($\chi^2 = 6.05$, $df = 1$, $p = 0.0139$) and Pb ($\chi^2 = 50.49$, $df = 1$, $p < 0.0001$). Specifically, levels of Ni, Zn, Hg and Pb were all statistically lower in the Adriatic Sea compared to the Tyrrhenian basin, except for As (Fig. 4). The Western Mediterranean, including the Tyrrhenian Sea, has been already reported as an area characterized by

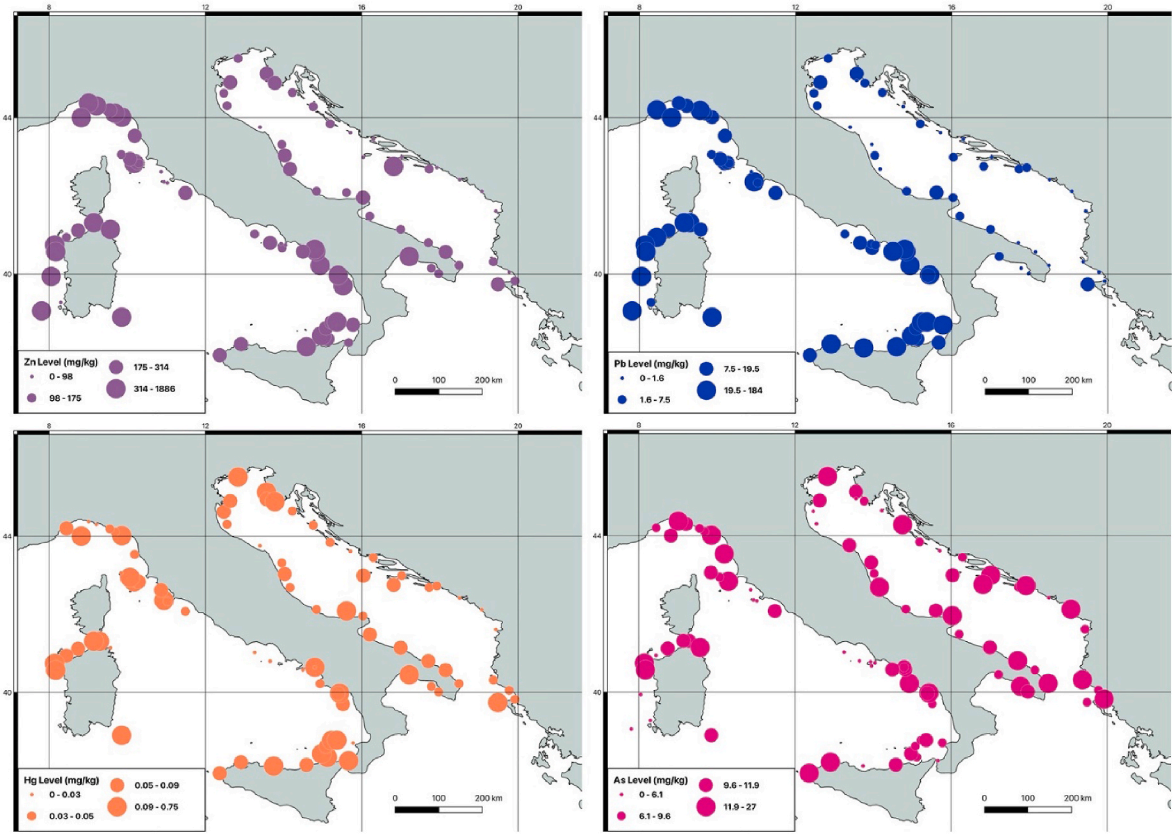


Fig. 4. Levels (mg kg^{-1} dw) of Zn, Pb, Hg, and As determined in zooplankton samples from the Tyrrhenian Sea in 2022 (Boldrocchi et al., 2023) and in the Adriatic Sea in 2023.

multiple sources of pollution and several areas of concern, chronically polluted with metal(loid)s (Boldrocchi et al., 2023; Lafabrie et al., 2007; Leoni et al., 1991; Lofrano et al., 2016; Tranchina et al., 2008; UNEP/MAP, 2012). For instance, Pb was found consistently high in biota from the western of Italy in multiple locations (Boldrocchi et al., 2023; UNEP/MAP, 2012) as well as in other areas of the western Mediterranean Sea (UNEP/MAP, 2012). Hg concentrations were high in sediments and biota from the southern Tyrrhenian Sea, possibly due to natural contributions from geothermal and volcanic sources, but also in the northwest Italian coast (Boldrocchi et al., 2023; UNEP/MAP, 2012). The different sources of anthropogenic pollution, basin characteristics, natural TE levels might be linked to different contamination levels recorded for certain metal(loid)s among the two areas.

Table S3 (Supplementary Materials) reports metal(loid) concentrations in zooplankton sampled in different basins worldwide. However, since pollution by metal(loid)s is element specific and caused by several different sources, the comparison is limited to the elements with major ecotoxicological interest (Cr, Co, Ni, Cu, Zn, As, Cd, Pb and Hg) (Ali et al., 2019). Starting with Cr, this study found that levels in zooplankton from the Adriatic (13.4 ± 39.2 mg kg^{-1}) were lower than areas of major concern for metal pollution, such as the east coast of India (60 mg kg^{-1}; Achary et al., 2020), Taiwanese coast (26.7 ± 10.9 mg kg^{-1}; Albarico et al., 2022), Bohai Bay in the northeastern China (39.4 mg kg^{-1}; Zhang et al., 2016), but more similar to the ones reported for zooplankton from Svalbard (4.4 mg kg^{-1}; Mohan et al., 2019), Red Sea (9.2 ± 5.5 mg kg^{-1}; Cai et al., 2022), and Moroccan coasts (10.3 ± 18.3 mg kg^{-1}; Bouthir et al., 2023). Similar findings can be drawn for Co, Ni, Cd and Pb which all appear to be lower than highly contaminated areas (e.g. Taiwan, Albarico et al., 2022; Kalpakkam coast, Achary et al., 2020; Djiboutian coast, Boldrocchi et al., 2020), and comparable to the concentrations reported for Svalbard (Mohan et al., 2019), Red Sea (Cai et al., 2022), and Moroccan coasts (Bouthir et al., 2023). All metal(loid)s, beside Co, showed concentrations even lower than what reported by Mohan et al. (2019) for two fjords located in Svalbard. Zinc was also found to be lower than most polluted locations (e.g. Achary et al., 2020; Albarico et al., 2022; Boldrocchi et al., 2020; Singaram et al., 2023), but similar to what reported from Moroccan waters (Bouthir et al., 2023) and from Arctic ecosystems (Lobus et al., 2019). The mean As level from this study (11 ± 5 mg kg^{-1} dw) is definitively higher than renowned polluted area mentioned above which are characterized by concentrations in the range 2–5 mg kg^{-1} dw (e.g. Albarico et al., 2022; Boldrocchi et al., 2020), but surprisingly similar to more pristine area such as the Arctic Ocean (Lobus et al., 2019). However, As concentration shows marked variability in sea basins. Indeed, Neff (1997) reported a worldwide As concentrations in zooplankton of 0.2–24.4 mg kg^{-1} dw, which is consistent with As levels from the Adriatic Sea. Regarding Hg, levels measured from the Adriatic Sea (0.06 ± 0.03 mg kg^{-1}) appear to be in line with different areas of the world, such as the Arctic Ocean (from 0.021 ± 0.018 mg kg^{-1} to 0.068 ± 0.066 mg kg^{-1}, Pomerleau et al., 2016), Antarctic Ocean (from 0.003 to 0.08 mg kg^{-1}, Korejwo et al., 2023), and copepods and euphausiids collected from the Southwest Atlantic Ocean (0.09 ± 0.07 and 0.110 ± 0.108 mg kg^{-1}, respectively, Fioramonti et al., 2022).

Zooplankton from the Adriatic Sea showed Cu content (148.9 ± 88.6 mg kg^{-1} dw) among the highest documented worldwide, being twice as high as in most contaminated locations, such as the Gulf of Aden, the east coast of India and Taiwan (Achary et al., 2020; Albarico et al., 2022; Boldrocchi et al., 2020), and even higher than the extreme high levels reported from the Tyrrhenian Sea in the Mediterranean (Boldrocchi et al., 2023). Previous studies (e.g. Boyle et al., 1985; Yang et al., 2019) have already highlighted that the basin is characterized by very high concentrations of dissolved Cu in coastal areas compared to other places. Those levels have been attributed to multiple Cu sources both natural, such as river input (Baconnais et al., 2019; Tankere and Statham, 1996), atmospheric aerosol deposition (e.g. Sahara Desert; Baconnais et al., 2019), but also anthropogenic (Baconnais et al., 2019; Jordi et al., 2012), like the use of Cu in agricultural practices that release Cu in their watershed (Guasch et al., 2002), atmospheric deposition from industrial sources (Jordi et al., 2012), and the worldwide use of copper-based antifouling paints which are considered a major source of Cu pollution (e.g. Cima and Varello, 2022). Overall, all the mentioned sources combined might likely explain the levels measured in this study.

4. Conclusion

This study highlighted multiple locations within the Adriatic Sea that raise concerns regarding contamination by both POPs and metal(loid)s. With regards to PCBs, the central-eastern (Šibenik-Split) and the southern side (Gulf of Drin) represent important site of marine contamination. With regard to metal(loid)s, in the north Adriatic Sea high levels of Co, Cu, Hg, Cr and Pb were found in the Gulf of Venice; in the Central Adriatic Sea high concentrations of Co, Ni, Hg, Cr and Pb were reported in Tremiti Islands and Cu, Zn, As, Se, Cd in Lastovo Island. In the Southern Adriatic Sea, high levels of most metal(loid)s, especially Cr, Ni and Zn, were found in Taranto and offshore Corfu. Overall, the Metal Pollution Index was higher in anthropogenically impacted areas (dense population, industrial activities, maritime traffic) as well as in proximity to the estuaries of the major rivers. On the contrary, contamination by DDT appears to be low within the whole basin.

Comparing both groups of pollutants with previous studies from the Adriatic Sea, this study highlighted a lack of knowledge on the contamination of zooplankton. Still, levels of both POPs and metal(loid)s appear to have decreased over time, at least since the 1970s. Although considered vulnerable to marine pollution due to its shallowness, the semi-enclosed and river-dominated nature, the status of contamination of zooplankton suggests a general improvement of the Adriatic ecosystem for certain pollutants. In fact, concentrations of both groups of pollutants are lower than well-renowned polluted locations around the world, besides Cu that was the only pollutant found in very high concentrations.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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.

CRediT authorship contribution statement

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

Acknowledgement

This research was conducted under the "*Marine Adventure for Research and Education*" Initiative, a project by Fondazione Centro Velico Caprera E.T.S. with One Ocean Foundation as Scientific Partner and patronized by Marina Militare (Italian Navy), Ministero della Transizione Ecologica (Italian Ministry of the Environment), Guardia Costiera (Italian Coast Guard) and Regione Autonoma della Sardegna (Autonomous Region of Sardinia). The authors, along with the One Ocean Foundation, wish to extend their gratitude to Mr. Enrico Bertacchi (General Secretary – Fondazione Centro Velico Caprera) and Mr. Paolo Bordogna (Former President – Fondazione Centro Velico Caprera) for their role in promoting the *Marine Adventure for Education and Research* Initiative. The authors also express their appreciation to the project sponsors, especially Yamamay (https://www.yamamay.com) as the main sponsor, as well as Sorgenia (https://www.sorgenia.it/), TOIO (https://www.toio.com/), and Polaroid (https://www.polaroid.com/). The authors would like to express their deep gratitude to Ms. Rossella Perna (University of Insubria) and all the students who contributed. They also thank Eleonora Moretta from CodeZero Digital Communication (www.codezerodigital.com) for her work on the graphical abstract. Additionally, the authors appreciate the support of the project guests during the zooplankton sampling. Finally, the authors are deeply grateful to Professor Jadran Faganeli (Marine Biology Station, National Institute of Biology, Slovenia) and the other unknown reviewers for the effort spent in revising this manuscript and for the precious suggestions that contributed to improve this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143553.

Data availability

Data will be made available on request.

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