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Heavy metal concentrations in sharks, rays and chimaeras from the western Mediterranean Sea

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A R T I C L E I N F O

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A B S T R A C T

The potential bioaccumulation of pollutants, such as heavy metals, may pose a threat to the western Mediterranean chondrichthyans and human consumers. Therefore, the first extensive assessment of cadmium (Cd), lead (Pb), and copper (Cu) concentrations in the muscle tissue of 17 species of sharks, rays, and chimaeras in this region was conducted *via* Microwave Assisted Extraction (MAE) and Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Significant differences between species were observed, particularly related to the rabbit fish (*Chimaera monstrosa*) and the velvet belly lantern shark (*Etmopterus spinax*), which exceeded the European Union (EU) Commission Regulation 2023/915 threshold of Cd. Overall, heavy metal concentrations correlated negatively with size and trophic level but positively with depth. Although the consumption of these species may entail minimal risk to adult humans, caution is advised, especially for children. These findings are important due to the widespread consumption of chondrichthyans in many western Mediterranean regions.

1. Introduction

Currently in the western Mediterranean Sea, there are several factors that contribute to the threat of biodiversity loss such as overexploitation, habitat loss, pollution and climate change (Bas, 2009; Consales and Marsili, 2021; Dulvy et al., 2021). In the case of pollution, heavy metals (*e.g.* cadmium (Cd), lead (Pb) and copper (Cu)), which are pollutants of an inorganic nature, are among the most common pollutants at a global scale, along with persistent organic pollutants (POPs) and marine debris (Colmenero et al., 2017; Tiktak et al., 2020). Given their long-term environmental persistence (Ali et al., 2019; Parker et al., 2023), heavy metals, similar to other pollutants, have the potential to bioaccumulate in tissues and organs such as the liver, muscle and gills (Eyckmans et al., 2013; Ali et al., 2019; Bezerra et al., 2019). The bioavailability of heavy metals, and consequently their uptake by marine biota, can be influenced by environmental variables including water chemistry and temperature (Molera-Arribas, 2018; Ghosn et al., 2020). The role of each species within food webs also influences their bioaccumulation rates, depending on their preferred preys (Pethybridge et al., 2010; McMeans et al., 2015; Le Croizier et al., 2016). Sharks, rays and chimaeras (chondrichthyans or cartilaginous fishes) are especially susceptible to bioaccumulation as they are considered keystone species, usually occupying high trophic levels as apical predators (Cortés, 1999; Ferretti et al., 2010; Barría et al., 2015), although some species may act as intermediate mesopredators (Fanelli et al., 2009; Barría et al., 2018; Coll-Calvo et al., 2020). Moreover, bioaccumulation rates are different among organs, thus, the concentration of heavy metals is different depending on the tissue analysed (Türkmen et al., 2013; Tiktak et al., 2020; Hauser-Davis et al., 2021).

Most of the described effects of heavy metals have on chondrichthyans are usually related to physiological alterations (Eyckmans et al., 2013; Bezerra et al., 2019; Wosnick et al., 2021). For instance, it is suggested that the presence of high concentrations of these pollutants could alter their reproductive physiology, as their presence could compromise and diminish the embryo development (Jeffree et al., 2008; Tiktak et al., 2020). This poses a significant health risk for developing embryos and pups, as they start their life cycle with higher concentrations of heavy metals, which potentially will continue to bioaccumulate

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throughout their lifespan, unless the concentrations are severe enough to threaten their survival (Bezerra et al., 2019; Tiktak et al., 2020; Veron et al., 2022). In the case of other heavy metals such as lead (Pb), it has been observed that its accumulation in gills, muscle, liver and rectal gland directly affects the osmoregulatory system, respiratory capacity and metabolism of chondrichthyans (Eisler, 2010; Eyckmans et al., 2013; Veron et al., 2022). Also, juveniles are more susceptible to lead poisoning compared to adults (Eisler, 2010; Yu et al., 2011). Nevertheless, chondrichthyans are a highly diverse group (Ferretti et al., 2010; Ebert et al., 2021). Therefore, it should be considered that the concentration and consequently the effects of heavy metals will vary between orders, families and species. This variation is due to differences in their habitats, behaviour, distribution, growth rates, age, trophic levels, and life strategies (Pethybridge et al., 2010; McMeans et al., 2015; Endo et al., 2016; Tiktak et al., 2020).

This high diversity is reflected in the Mediterranean Sea, which is considered a hotspot for chondrichthyans, as approximately 80 species are found (Dulvy et al., 2016; Serena et al., 2020; Ebert et al., 2021). However, due to their biological characteristics such as slow growth, low fecundity and late age of sexual maturity, these species are vulnerable (Ferretti et al., 2010; Dulvy et al., 2021). Although exploitation is currently the most important threat to chondrichthyans, pollution is considered an important emerging threat (Consales and Marsili, 2021; Dulvy et al., 2021). Despite being an understudied subject, it is estimated that approximately 6.9 % of the species assessed by the International Union for Conservation of Nature (IUCN) are threatened by pollution (Dulvy et al., 2021). It is considered under-examined, since the number of studies on pollutants at a global level are scarce (Tiktak et al., 2020; Consales and Marsili, 2021), this scarcity is proportionally reflected in the Mediterranean Sea, where approximately 9 % of the studies focus on pollutants (Consales and Marsili, 2021). Most of the studies in the Mediterranean Sea have been conducted particularly in regions of the Eastern basin, including the Aegean Sea and Levantine Sea (Hornung et al., 1993; Türkmen et al., 2013, 2014; Giovos et al., 2022), and the Adriatic Sea (Storelli et al., 2002a, 2002b). Other areas of the western basin, such as the Mediterranean Spanish coast, still remain unstudied, especially considering the fact that the coastal provinces of Spain exhibit a population density higher than the national average (Villacampa et al., 2017). Therefore, the lack of bibliography regarding pollutants, such as heavy metals, is a matter of concern considering the biological characteristics of chondrichthyans and current state of their populations (Tiktak et al., 2020; Consales and Marsili, 2021; Dulvy et al., 2021).

Nevertheless, the presence of pollutants can be a potential risk not just for chondrichthyans themselves but also to humans, mainly because of the importance of fishery resources as a food source (Dent and Clarke, 2015; Barría and Colmenero, 2019; Barone et al., 2021). In Spain, the fish consumption *per capita* stands at 46.2 $kg \cdot year^{-1}$, exceeding the European average (25.5 $kg \cdot year^{-1}$) (EUMOFA, 2021). Therefore, in order to ensure a safe human consumption of seafood, concentration thresholds of certain pollutants, such as some of the analysed heavy metals in this present study (*i.e.*, Cd and Pb), have been established by the European Union (EU) Commission Regulation 2023/915 and the Joint FAO/WHO Expert Committee on Food Additives (JEFCA). The maximum concentration allowed for cadmium and lead is 0.05 and 0.3 ($mg \cdot kg^{-1}$, wet weight) respectively (JEFCA, 2019; European Commission, 2023).

Thus, knowing that chondrichthyans occupy high and intermediate trophic levels (Cortés, 1999; Fanelli et al., 2009; Barría et al., 2015, 2018; Coll-Calvo et al., 2020), in comparison to other marine organisms that may be consumed, this particular group could potentially accumulate higher concentrations of heavy metals among other xenobiotics, due to their biological characteristics (Pethybridge et al., 2010; Giovos et al., 2022). These characteristics make the description and quantification of the presence and concentration of pollutants, particularly heavy metals, significant for the health of these organisms as well as for humans (Storelli, 2009; Bosch et al., 2016; Giovos et al., 2022). Therefore, the aim of this study is to extensively assess for the first time the concentration of cadmium, lead and copper in the muscle tissue of chondrichthyan species from the western Mediterranean Sea.

2. Materials and methods

2.1. Sampling

In this study, a total of 116 muscle tissue samples extracted from 17 chondrichthyan species were analysed, obtained from different Spanish ports in Catalonia, Valencian Community and the Region of Murcia (Fig. 1; Table 1).

These samples were extracted primarily from adult individuals from bottom trawling and trammel netting, and in some cases from stranded individuals (only *Prionace glauca*) (Fig. 1). All of these samples were collected over several years (2013–2023) in western Mediterranean regions, including the Gulf of Lions, Catalan Coast, and the Balearic Sea. Ethical review and approval were not required for the animal study because the all chondrichthyan specimens analysed in the present work were obtained from commercial fisheries or from stranded individuals under the supervision of government environmental agencies.

2.2. Metal extraction and determination

All individuals underwent the same pre-treatment procedures as described in its respective section in the supplementary material. The extraction of Cd, Pb and Cu was performed *via* "Microwave Assisted Extraction" (MAE) with a microwave digestion system (CEM® Xpress MARS 6, USA) and Teflon (PTFE) tubes. Before the extraction, all the material underwent a cleaning regimen which involved cleansing the PTFE tubes with nitric acid (HNO_3) 10 % $v \cdot v^{-1}$ (Panreac Química S.L.U., Spain) (Hernández-Martínez, 2015; Molera-Arribas, 2018). Also, all non-sterile plastic and glass utensils were cleaned with HNO_3 10 % ($v \cdot v^{-1}$), followed by triple rinsing with ultrapure bidistilled H_2O (Ultramatic Plus Wasserlab S.L., Spain) to minimise the risk of contamination.

The MAE procedure involved weighing 0.2 g of the homogenised muscle sample into Teflon tubes. Into these, 0.4 mL of 30 % $v \cdot v^{-1}$ of H_2O_2 and 1.2 mL of 69 % $v \cdot v^{-1}$ hyperpure HNO_3 (Panreac Química S.L. U., Spain) were introduced (Mendil et al., 2010; Molera-Arribas, 2018). The MAE procedure followed the conditions outlined in Mendil et al.

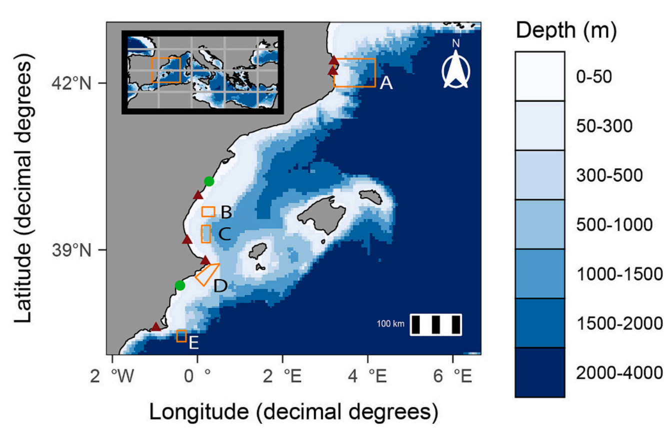

Fig. 1. Study area in the western Mediterranean Sea, depicting the sampling areas (orange polygons labelled with letters A to E) with their coordinates provided in Table S1 (Supplementary material). The map also indicates the locations of the home ports where the specimens were collected (red triangles) as well as the position of the stranded specimens used in this study (green circles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
List of the 17 chondrichthyan species analysed along with their respective sample size (N), and the autonomous communities where they were collected. These communities, from northernmost to southernmost, were: Catalonia (A), Valencian Community (B, C, D) and the Region of Murcia (E) (Fig. 1). The coordinates for sampling areas are located in Table S1 (Supplementary material). Notably, there is no sampling area and depth range for *Prionace glauca* (represented as NA) because its samples were obtained from stranded specimens (Fig. 1). Additionally, only the average catch depth was obtained for *Hexanchus griseus* and *Oxynotus centrina*.

Species (scientific name)	Sample size (N)	Sampling areas	Capture depth range (m)
Carcharhiniformes			
Blackmouth catshark (*Galeus melastomus*)	14	B, C	500–580
Blue shark (*Prionace glauca*)	3	NA	NA
Small-spotted catshark (*Scyliorhinus canicula*)	15	D	100–150
Chimaeriformes			
Rabbit fish (*Chimaera monstrosa*)	14	A, B	500–520
Hexanchiformes			
Bluntnose sixgill shark (*Hexanchus griseus*)	1	B	600
Myliobatiformes			
Bull ray (*Aetomylaeus bovinus*)	1	A	35–70
Common stingray (*Dasyatis pastinaca*)	2	A, C	125–150
Spiny butterfly ray (*Gymnura altavela*)	1	C	35–70
Rajiformes			
Longnosed skate (*Dipturus oxyrinchus*)	7	C, D	380–500
Blonde ray (*Raja brachyura*)	2	A	125–150
Thornback ray (*Raja clavata*)	2	A	125–150
Speckled ray (*Raja polystigma*)	15	A	125–150
Squaliformes			
Little gulper shark (*Centrophorus uyato*)	4	C, D, E	430–590
Kitefin shark (*Dalatias licha*)	5	B, C, D	475–675
Velvet belly lantern shark (*Etmopterus spinax*)	15	B, C, D	518–640
Angular roughshark (*Oxynotus centrina*)	1	B	520
Torpediniformes			
Marbled electric ray (*Torpedo marmorata*)	14	B	60–70

(2010) (Table S2, Supplementary material).

The resultant sample extract was filtered into a 10 mL glass volumetric flask using sterile 10 mL syringes and sterile 25 mm diameter syringe filters with a pore size of 0.45 μm. The sample volume was made up to volume with ultrapure bidistilled H_2O. Afterwards it was transferred into labelled 15 mL conical centrifuge tubes and stored in a refrigerator at 5 °C.

The determination of heavy metals (Cd, Pb and Cu) was accomplished *via* Graphite Furnace Atomic Absorption Spectrometry (GFAAS) (VARIAN® Model AA240Z, USA) (Hernández-Martínez, 2015; Molera-Arribas, 2018). Depending on the analyte, specific conditions based on Molera-Arribas (2018) were established in order to perform correctly the determination (Table S3, Supplementary material).

However, due to the requirement for high-resolution analysis of the samples, a calibration curve was necessary. Therefore, standard solutions of Cd, Pb and Cu (Panreac Química S.L.U., Spain), were prepared on a daily basis, with concentrations of 5 μg•L^{-1} for Cd, and 30 μg•L^{-1} for both Pb and Cu. Consequently, a minimum Pearson correlation coefficient (r) value of 0.9990 was established as the validation criterion for the curve.

Subsequently, the limit of detection (LOD) and limit of quantification (LOQ) were calculated following the formulae established by the EU Commission Regulation 333/2007 (European Commission, 2007):

$$\mathrm{LOD} = \mathrm{X} + 3\sigma$$

$$\mathrm{LOQ} = \mathrm{X} + 10\sigma$$

Being X, the mean heavy metal concentration of the samples analysed, and σ the standard deviation of the mean of blank determinations (European Commission, 2007). The values of LOD (μg•L^{-1}) were 0.003 for Cd, 0.01 for Pb, and 0.09 for Cu. In the case of LOQ (μg•L^{-1}), the values were 0.01 for Cd, 0.09 for Pb, and 0.3 for Cu.

Certified reference material was used to validate the methodology, in particular the dogfish (*Squalus acanthias*) liver material NRCC-DOLT-5 (Yang et al., 2014), and mussel (*Mytilus edulis*) tissue ERM®-CE278k (Snell et al., 2012). The obtained mean recovery was of 97–110 % for all the studied analytes (Cd, Pb and Cu).

To facilitate the comparison with the concentrations of heavy metals permitted for human consumption established by the EU Commission Regulation 2021/1323 and 2023/915 as well as the FAO/WHO *Codex Alimentarius* JEFCA (2019), it was necessary to convert the dry weight of the muscle tissue sample to wet weight to match the regulations guidelines. This conversion was done using the formula using the formula of Gaion et al. (2016):

$$\mathrm{WW} = \mathrm{DW} \times [1 - (\mathrm{WP})]$$

where WW corresponded to the wet weight (w.w.), DW to the dry weight of each sample and WP to the water percentage in the muscle tissue calculated for each sample.

2.3. Risk from consumption

The risk associated with the consumption of chondrichthyans was determined through the calculation of the Estimated Daily Intake (EDI) and the Target Hazard Quotient (THQ) for both adults and children (US-EPA, 2021, 2023).

To facilitate the comparison between the Estimated Daily Intake (EDI) (μg•kg^{-1}•bw^{-1}) and the oral reference dose (RfDo) (μg•kg^{-1}•bw^{-1}•day^{-1}) established by the U.S. Environmental Protection Agency (US-EPA) (US-EPA, 2023), the calculation of EDI involved utilizing the following formula (Giovos et al., 2022):

$$\mathrm{EDI} = (\mathrm{C} \times \mathrm{IR})/\mathrm{BW}$$

where C represented the concentration of the heavy metal in the muscle tissue sample (μg•kg^{-1}, w.w.); IR corresponded to the daily average consumption of fish in the study area (0.1265 kg *per capita*•day^{-1}) (EUMOFA, 2021; US-EPA, 2021, 2023); and BW was the body weight, for which the average weight of an adult and a child was used, being 70 and 16 kg respectively (Copat et al., 2013; US-EPA, 2021, 2023; Giovos et al., 2022). The oral reference dose values used for the comparison with the estimated daily ingestion (RfDo) (μg•kg^{-1}•bw^{-1}) were: 1 for Cd, 3.5 for Pb, and 40 for Cu (US-EPA, 2021, 2023; Giovos et al., 2022).

Furthermore, the Target Hazard Quotient (THQ) was used to assess the potential health risks associated with the consumption of the chondrichthyan species analysed in this present study. A THQ value below 1, suggests that the exposure level is lower than the oral reference dose (RfDo), implying that a daily exposure of this concentration would not pose harm to human health (US-EPA, 2023). The calculation of THQ followed the formula (Giovos et al., 2022; US-EPA, 2023):

$$\mathrm{THQ} = (\mathrm{C} \times \mathrm{IR} \times \mathrm{EF} \times \mathrm{ED})/(\mathrm{RfDo} \times \mathrm{BW} \times \mathrm{AT})$$

where C denoted the concentration of the heavy metal in the muscle tissue sample (mg•kg^{-1}, w.w.); IR corresponded to the daily average consumption of fish in the study area (0.1265 kg *per capita*•day^{-1}) (EUMOFA, 2021; US-EPA, 2021, 2023); EF represents the frequency of exposure (350 days•year^{-1}); ED was the exposure duration, which according to Copat et al. (2013) and Giovos et al. (2022) was 26 years for adults and 6 for children; RfDo was the oral reference dose (mg•kg^{-1}•bw^{-1}) established by US-EPA (2023); BW stood for the body weight for adults (70 kg) and children (16 kg) (Copat et al., 2013); and AT referred to the average time of exposure to noncarcinogens (350 days•year^{-1}•ED) (Copat et al., 2013; Giovos et al., 2022; US-EPA, 2021, 2023).

The oral reference dose (RfDo) (mg•kg^{-1}•day^{-1}) values that have been used were: 0.0035 for lead (Pb), 0.001 for cadmium (Cd) and 0.04 for copper (Cu) (US-EPA, 2021, 2023; Giovos et al., 2022).

2.4. Biological information revision

For subsequent statistical analysis, a bibliographic review was conducted using Web of Science and Google Scholar with specific keywords (Table S4, Supplementary material) to gather information about the diet type and trophic level of the analysed chondrichthyan species, as depicted in Table S5 (Supplementary material). To reduce potential geographical differences, preference was given to studies conducted in the Mediterranean Sea or nearby regions. Particularly, the diet type was determined based on the taxonomic group of the most significant prey category. To achieve this, the %IRI (Index of Relative Importance) (Cortés, 1999) was employed as a reference metric.

2.5. Statistical analysis

The statistical analysis and the elaboration of figures were performed using R Statistical Software v4.2.1 (R Core Team, 2022) and GraphPad Prism v8.0.2. The normality of each dataset was assessed using the Shapiro-Wilk, Anderson-Darling, D'Agostino & Pearson, and Kolmogorov-Smirnov tests (Gaion et al., 2016; Le Croizier et al., 2016; Molera-Arribas, 2018). As the datasets exhibited non-normal distributions, non-parametric Kruskal-Wallis test and *post hoc* Dunn's test were conducted.

The Spearman rank correlation coefficient (r_s) (Le Croizier et al., 2016; Molera-Arribas, 2018; Hauser-Davis et al., 2021; Shipley et al., 2021), was employed for the determination of the correlation between heavy metal concentrations and continuous biological characteristics such as the trophic level, recorded capture depth (m) and measured total length (cm), although in the case of chimaeras the pre-supracaudal fin length (PSCL) was used instead of the total length. The correlations were calculated based on adult specimens; therefore, to maintain this condition, *Dalatias licha* and *Oxynotus centrina* were excluded from this

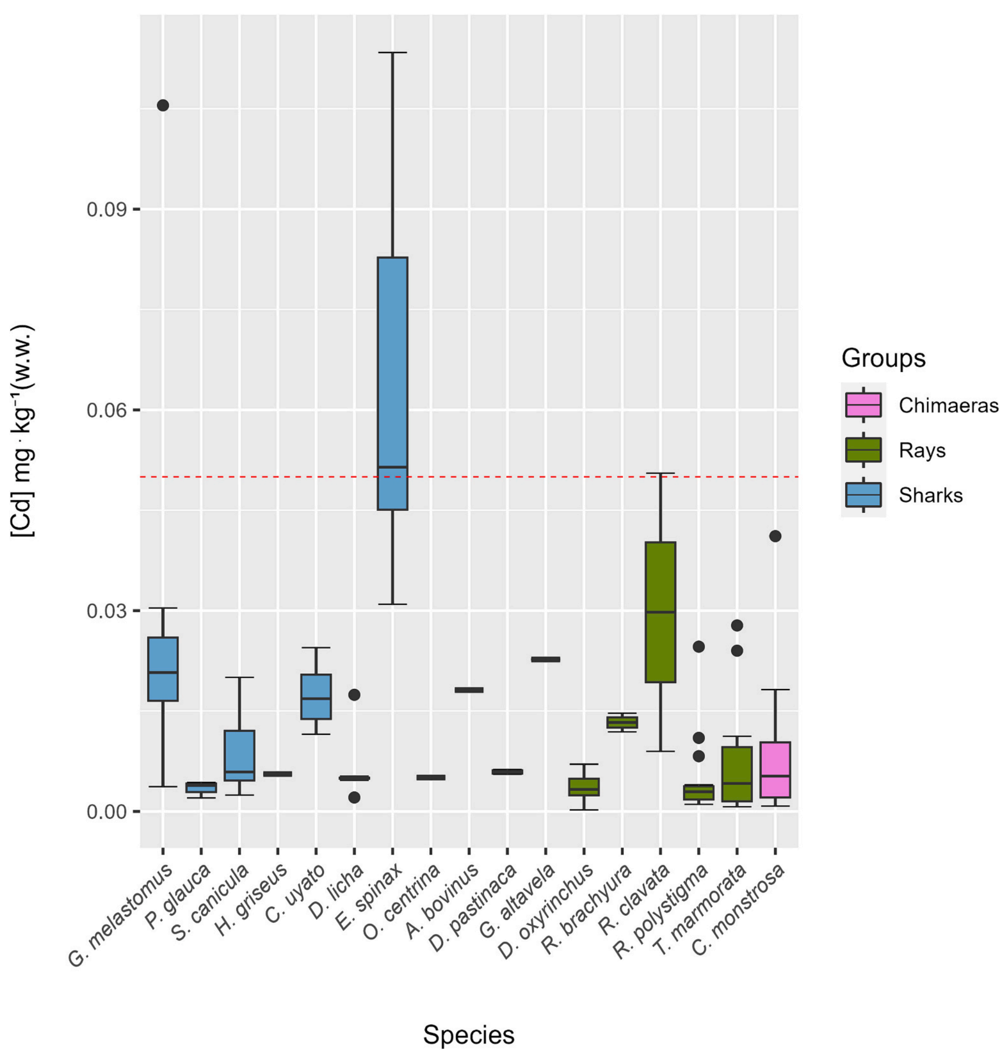

Fig. 2. Box plots depicting the concentration of Cd (mg•kg^{-1}, w.w.) in muscle tissue of 17 species. The dashed line represents the maximum concentration threshold for Cd (0.05 mg•kg^{-1}, w.w.) established by the EU Commission Regulation 2023/915.

particular analysis, as only juveniles were available. When the p-value (P) < 0.05 was met, significance was considered.

3. Results

3.1. Heavy metal concentrations

Overall, sharks had a higher Cd concentration than rays and chimaeras, namely the Squaliformes order. At the species level, the velvet belly lantern shark (*Etmopterus spinax*) had the highest Cd concentration (0.0622 ± 0.0244 mg•kg^{-1}, w.w.), and it was the only species to exceed the threshold delimitated by the EU Commission Regulation 2023/915 (Fig. 2). Conversely, the species with the lowest concentration was the blue shark (*Prionace glauca*) with 0.0034 ± 0.0012 mg•kg^{-1} (w.w.).

Significant differences (Dunn's test) were observed among the species, with the majority of these differences being associated with the velvet belly lantern shark (*E. spinax*). These differences were noted between: *E. spinax* and *R. polystigma* (P < 0.0001), *E. spinax* and *C. monstrosa* (P = 0.0001), *E. spinax* and *T. marmorata* (P < 0.0001), *E. spinax* and *D. oxyrinchus* (P = 0.0002), *E. spinax* and *P. glauca* (P = 0.0306), *G. melastomus* and *R. polystigma* (P = 0.0131), *E. spinax* and *S. canicula* (P = 0.0021).

In the case of Pb, chimaeras had a higher concentration than sharks and rays, specifically the rabbit fish (*C. monstrosa*), being the only species of chimaera analysed. This species had an average Pb concentration of 0.2924 ± 0.1689 (mg•kg^{-1}, w.w.), and in some individuals, the concentration surpassed the threshold of 0.3 (mg•kg^{-1}, w.w.) delimitated by the Commission Regulation (EU) 2023/915 (Fig. 3). On the other hand, the species with the lowest concentration was the angular roughshark (*Oxynotus centrina*) (0.0208 mg•kg^{-1}, w.w.).

Significant differences (Dunn's test) were found between: *E. spinax* and *G. melastomus* (P = 0.0031), *E. spinax* and *R. polystigma* (P = 0.0328), *G. melastomus* and *C. monstrosa* (P < 0.0001), *R. polystigma* and *C. monstrosa* (P < 0.0001), *C. monstrosa* and *T. marmorata* (P = 0.0074), *C. monstrosa* and *D. oxyrinchus* (P = 0.0008), *C. monstrosa* and *P. glauca* (P = 0.0408), *C. monstrosa* and *S. canicula* (P = 0.0022).

Regarding Cu, similar concentrations were observed among sharks and chimaeras, while rays exhibited the lowest concentrations. Similar to cadmium, the highest Cu concentrations were found within the Squaliformes order, and this same similitude also occurred at the species level. Since, the species with the highest concentration of Cu was the velvet belly lantern shark (*E. spinax*) (0.8179 ± 0.4167 mg•kg^{-1}, w.w.) (Fig. 4). On the contrary, the species with the lowest concentration was the bluntnose sixgill shark (*Hexanchus griseus*) (0.0432 mg•kg^{-1}, w.w.).

Significant differences (Dunn's test) between the copper concentrations of the analysed species were found, all of which were related to the velvet belly lantern shark (*E. spinax*). These differences were: *E. spinax* and *R. polystigma* (P < 0.0001), *E. spinax* and *C. monstrosa* (P = 0.0198), *E. spinax* and *D. oxyrinchus* (P = 0.0006), *E. spinax* and *D. licha* (P = 0.0006), *G. melastomus* and *P. glauca* (P = 0.0232).

Based on the obtained Cd, Pb and Cu concentrations of the analysed species (Figs. 2–4), and their continuous biological characteristics including depth, trophic level and total length, the Spearman's correlation coefficient (r_s) and the subsequent correlations were obtained

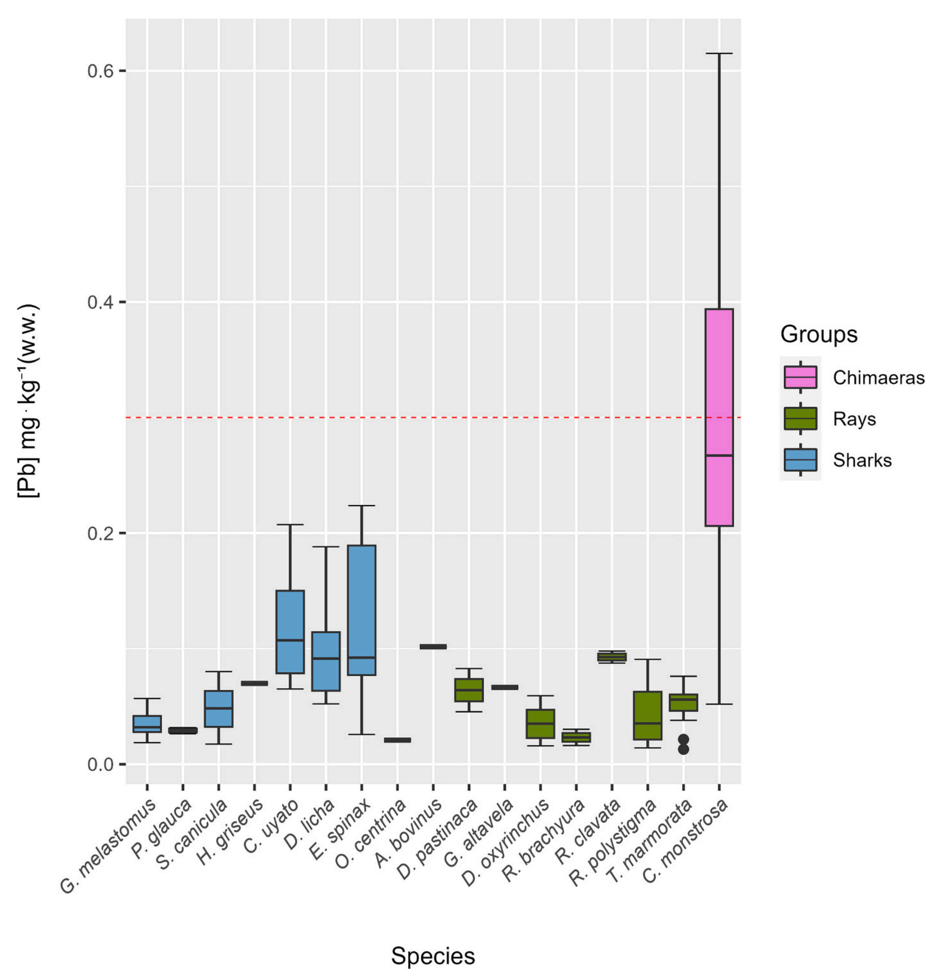

Fig. 3. Box plots depicting the concentration of Pb (mg•kg^{-1}, w.w.) in muscle tissue of 17 species. The dashed line represents the maximum concentration threshold for Pb (0.3 mg•kg^{-1}, w.w.) established by the EU Commission Regulation 2023/915.

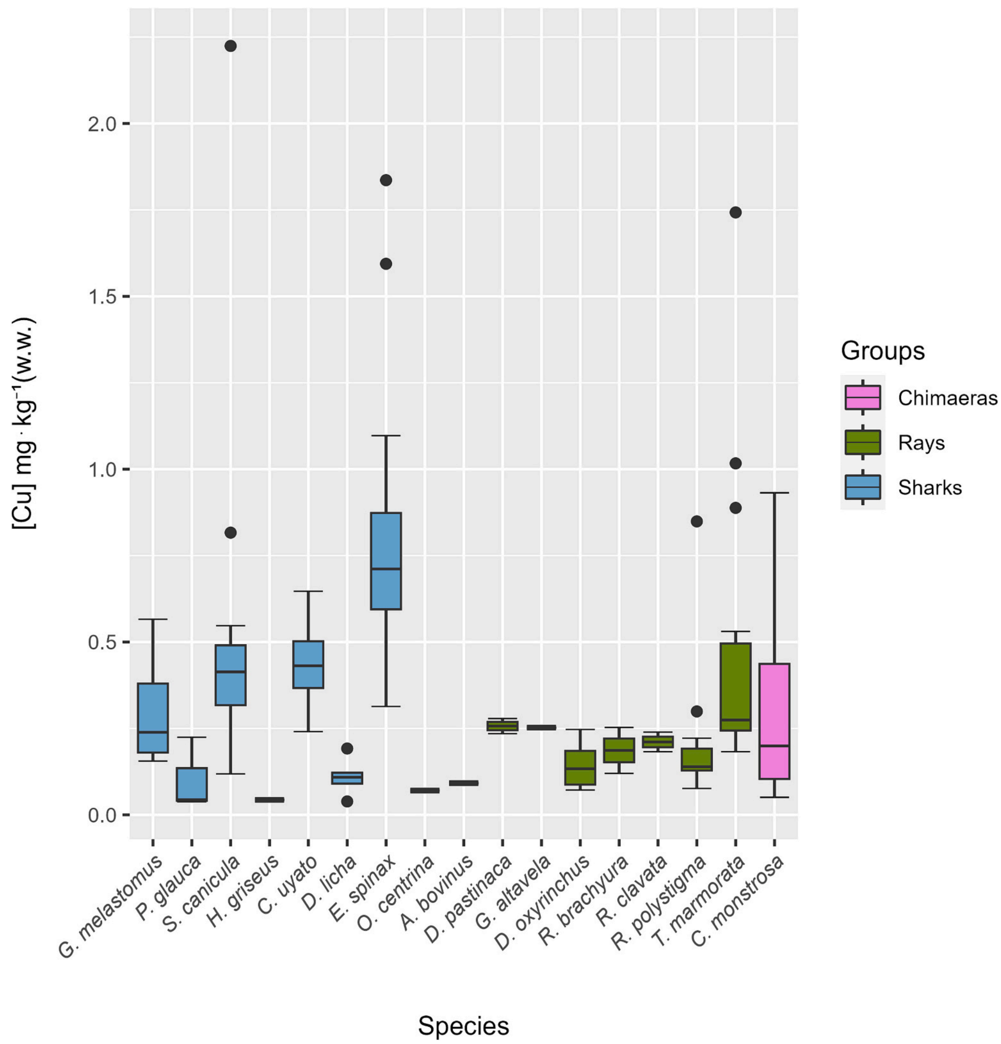

Fig. 4. Box plots depicting the concentration of Cu (mg•kg^{-1}, w.w.) in muscle tissue of 17 species.

(Fig. 5).

For Cd, a moderate positive correlation ($r_s = 0.52$; $P < 0.0001$) was observed between Cd concentration and depth. However, in relation to total length, the correlation was weak and negative ($r_s = -0.36$; $P = 0.0002$). Despite exhibiting a weak negative correlation with the trophic level, this correlation was not statistically significant ($r_s = 0.031$; $P = 0.714$).

Regarding Pb, a weak positive correlation ($r_s = 0.30$; $P = 0.0027$) was identified between Pb concentration and depth. However, in the case of the total length of the species, the correlation was notably weak and negative, and statistically not significant ($r_s = -0.057$; $P = 0.669$). Conversely, a weak and negative correlation was observed in relation to the trophic level ($r_s = -0.27$; $P = 0.0135$).

In the case of Cu, a notably weak and negative correlation was observed with depth, although it did not reach statistical significance ($r_s = 0.092$; $P = 0.387$). However, concerning the total lengths of the species, the correlation was moderate and negative ($r_s = -0.55$; $P < 0.0001$). Analogous to depth, a weak and negative correlation was noted with the trophic level, but this correlation was not statistically significant ($r_s = -0.042$; $P = 0.871$).

Furthermore, according to other biological characteristics of the species analysed, such as their type of diet (Table S5, Supplementary material), significant differences were only observed in relation to Pb, rather than Cd or Cu. In the case of this particular heavy metal (Pb), the species that mainly fed on Reptantia crustaceans had a higher concentration of Pb (0.2639 ± 0.1756 mg•kg^{-1}, w.w.) (Fig. 6). Additionally, significant differences were observed between species that mainly fed on Natantia crustaceans and Reptantia crustaceans ($P < 0.0001$), as well as between Reptantia crustaceans and Teleostei feeders ($P = 0.0006$).

3.2. Risk derived from the consumption of chondrichthyans

Overall, the calculated average values for sharks, rays and chimaeras for both adults and children of Estimated Daily Intake (EDI) (μg•kg^{-1}•bw^{-1}) (Table 4) for Cd, Pb and Cu did not surpass the oral reference dose (RfDo) (μg•kg^{-1}•bw^{-1}•day^{-1}) established by the US-EPA (2021, 2023).

However, in the particular instance of *C. monstrosa* (Table 4), even though the average Pb value did not exceed the oral reference dose established by the US-EPA (2021, 2023), certain specimens exceeded this dose (3.5 μg•kg^{-1}•bw^{-1}), specifically among children, due to their lower body weight in comparison to adults.

Furthermore, the potential risks to human health from the consumption of sharks, rays and chimaeras (Table 5), were assessed by estimating the Target Hazard Quotient (THQ).

Much like EDI, the overall THQ values of the analysed heavy metals remained below 1, indicating that they did not surpass the RfDo (mg•kg^{-1}•bw^{-1}) (US-EPA, 2021, 2023). Nevertheless, the case of *C. monstrosa* should be highlighted (Table 5). Because, as well as EDI,

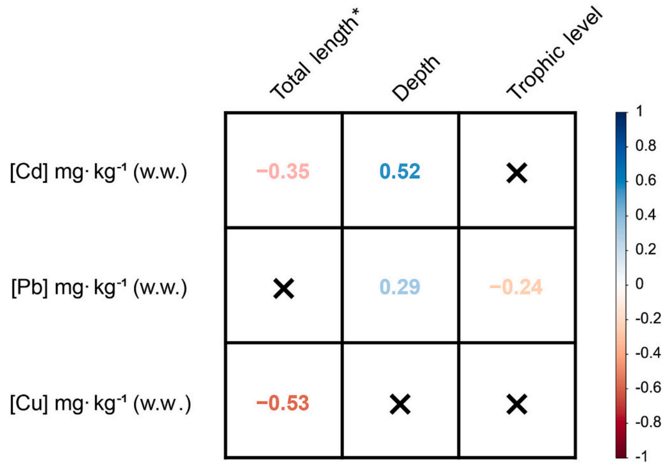

Fig. 5. Spearman's correlation coefficient (r_s) values between the concentrations of cadmium (Cd), lead (Pb) and copper (Cu) (mg•kg^{-1}, w.w.), and continuous biological characteristics such as total length (cm), capture depth (m), and trophic level. Crossed cells represent non-significant correlations ($P > 0.05$). The red colour indicates negative correlations, while the blue colour indicates positive correlations. The asterisk (*) next to length specifies that in the case of chimaeras, the pre-supracaudal fin length (PSCL) was used instead of total length. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

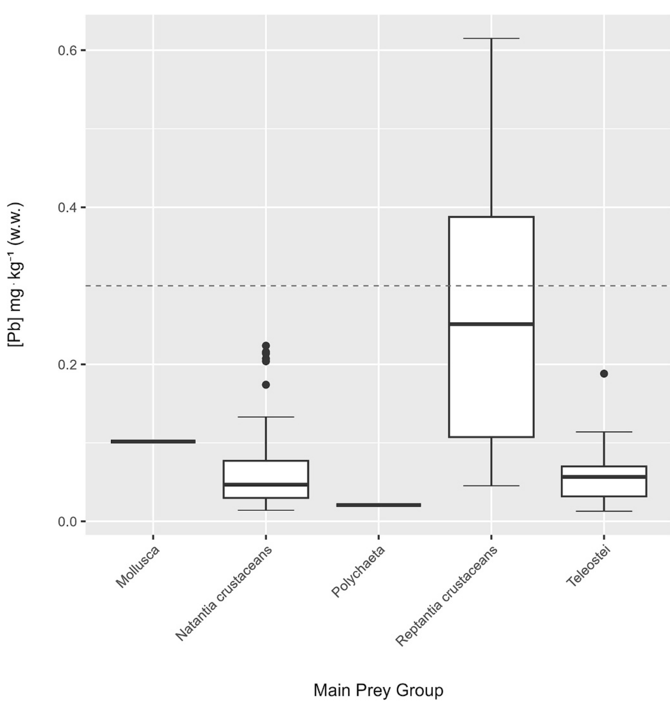

Fig. 6. Box plots depicting the concentration of Pb (mg•kg^{-1}, w.w.) in muscle tissue of chondrichthyans based on their main prey group. The dashed line represents the maximum concentration threshold for Pb (0.3 mg•kg^{-1}, w.w.) established by the EU Commission Regulation 2023/915.

despite its average value, certain specimens had THQ values above 1, specifically among children.

4. Discussion

This present study stands out as the first assessment performed on the Spanish coasts of the western Mediterranean Sea, analysing the largest number of chondrichthyan species (N = 17). In comparison with the species analysed in other studies conducted in the Mediterranean Sea (Hornung et al., 1993; Storelli et al., 2002a, 2002b; Türkmen et al., 2013, 2014; Giovos et al., 2022), there are several recurrent species, particularly those that are abundant in the catches within the study area, such as the small-spotted catshark (*Scyliorhinus canicula*), blackmouth catshark (*Galeus melastomus*), thornback ray (*Raja clavata*) and the marbled electric ray (*Torpedo marmorata*) (Follesa et al., 2020; Ruiz-García et al., 2023). Furthermore, this present study also delves into the analysis of species that are not commonly examined, regardless of their abundance in catches. These include species such as the velvet belly lantern shark (*Etmopterus spinax*), rabbit fish (*Chimaera monstrosa*) and the angular roughshark (*Oxynotus centrina*), which, despite not being commercially valuable, also need a focused assessment, especially in the case of critically endangered species such as the angular roughshark (Dulvy et al., 2016; Walls and Dulvy, 2020).

The differences between the determined heavy metal concentrations can likely be attributed to the diversity inherent to these species (*e.g.*

Table 4
Average EDI values (μg•kg^{-1}•bw^{-1}) of the analysed shark, ray and chimaera species calculated for adults and children; italicized values indicate the presence of individuals that exceeded the RfDo value.

Species	Target consumer	Cd	Pb	Cu
Carcharhiniformes				
Galeus melastomus	Adults	4.63E−02	6.17E−02	5.32E−01
	Children	2.03E−01	2.70E−01	2.33E+00
Prionace glauca	Adults	6.15E−03	5.26E−02	1.86E−01
	Children	2.69E−02	2.30E−01	8.12E−01
Scyliorhinus canicula	Adults	1.53E−02	8.83E−02	9.22E−01
	Children	6.70E−02	3.86E−01	4.03E+00
Hexanchiformes				
Hexanchus griseus	Adults	1.01E−02	1.26E−01	7.81E−02
	Children	4.41E−02	5.53E−01	3.42E−01
Squaliformes				
Centrophorus uyato	Adults	3.15E−02	2.20E−01	7.91E−01
	Children	1.38E−01	9.62E−01	3.46E+00
Dalatias licha	Adults	1.24E−02	1.84E−01	1.99E−01
	Children	5.44E−02	8.06E−01	8.71E−01
Etmopterus spinax	Adults	1.12E−01	2.21E−01	1.48E+00
	Children	4.92E−01	9.68E−01	6.47E+00
Oxynotus centrina	Adults	9.15E−03	3.76E−02	1.27E−01
	Children	4.00E−02	1.64E−01	5.55E−01
Chimaeriformes				
Chimaera monstrosa	Adults	1.57E−02	5.28E−01	5.31E−01
	Children	6.88E−02	*2.31E+00*	2.33E+00
Myliobatiformes				
Aetomylaeus bovinus	Adults	3.28E−02	1.84E−01	1.66E−01
	Children	1.43E−01	8.04E−01	7.24E−01
Dasyatis pastinaca	Adults	1.07E−02	1.16E−01	4.64E−01
	Children	4.67E−02	5.06E−01	2.03E+00
Gymnura altavela	Adults	4.10E−02	1.20E−01	4.56E−01
	Children	1.80E−01	5.26E−01	1.99E+00
Rajiformes				
Dipturus oxyrinchus	Adults	6.48E−03	6.44E−02	2.58E−01
	Children	2.84E−02	2.82E−01	1.13E+00
Raja brachyura	Adults	2.40E−02	4.19E−02	3.37E−01
	Children	1.05E−01	1.83E−01	1.47E+00
R. clavata	Adults	5.38E−02	1.68E−01	3.81E−01
	Children	2.35E−01	7.33E−01	1.67E+00
R. polystigma	Adults	8.82E−03	7.60E−02	3.66E−01
	Children	3.86E−02	3.33E−01	1.60E+00
Torpediniformes				
Torpedo marmorata	Adults	1.34E−02	9.32E−02	8.66E−01
	Children	5.85E−02	4.08E−01	3.79E+00

Table 5
Average THQ values of the analysed shark, ray and chimaera species calculated for adults and children; italicized values indicate presence of individuals with THQ values above 1.

Species	Target consumer	Cd	Pb	Cu
Carcharhiniformes				
Galeus melastomus	Adults	4.63E−02	1.76E−02	1.33E−02
	Children	2.03E−01	7.71E−02	5.82E−02
Prionace glauca	Adults	6.15E−03	1.50E−02	4.64E−03
	Children	2.69E−02	6.57E−02	2.03E−02
Scyliorhinus canicula	Adults	1.53E−02	2.52E−02	2.31E−02
	Children	6.70E−02	1.10E−01	1.01E−01
Hexanchiformes				
Hexanchus griseus	Adults	1.01E−02	3.61E−02	1.95E−03
	Children	4.41E−02	1.58E−01	8.54E−03
Squaliformes				
Centrophorus uyato	Adults	3.15E−02	6.28E−02	1.98E−02
	Children	1.38E−01	2.75E−01	8.65E−02
Dalatias licha	Adults	1.24E−02	5.26E−02	4.98E−03
	Children	5.44E−02	2.30E−01	2.18E−02
Etmopterus spinax	Adults	1.12E−01	6.32E−02	3.70E−02
	Children	4.92E−01	2.76E−01	1.62E−01
Oxynotus centrina	Adults	9.15E−03	1.07E−02	3.17E−03
	Children	4.00E−02	4.70E−02	1.39E−02
Chimaeriformes				
Chimaera monstrosa	Adults	1.57E−02	1.51E−01	1.33E−02
	Children	6.88E−02	*6.61E−01*	5.81E−02
Myliobatiformes				
Aetomylaeus bovinus	Adults	3.28E−02	5.25E−02	4.14E−03
	Children	1.43E−01	2.30E−01	1.81E−02
Dasyatis pastinaca	Adults	1.07E−02	3.31E−02	1.16E−02
	Children	4.67E−02	1.45E−01	5.07E−02
Gymnura altavela	Adults	4.10E−02	3.43E−02	1.14E−02
	Children	1.80E−01	1.50E−01	4.99E−02
Rajiformes				
Dipturus oxyrinchus	Adults	6.48E−03	1.84E−02	6.44E−03
	Children	2.84E−02	8.05E−02	2.82E−02
Raja brachyura	Adults	2.40E−02	1.20E−02	8.42E−03
	Children	1.05E−01	5.23E−02	3.69E−02
R. clavata	Adults	5.38E−02	4.79E−02	9.53E−03
	Children	2.35E−01	2.09E−01	4.17E−02
R. polystigma	Adults	8.82E−03	2.17E−02	9.15E−03
	Children	3.86E−02	9.50E−02	4.00E−02
Torpediniformes				
Torpedo marmorata	Adults	1.34E−02	2.66E−02	2.17E−02
	Children	5.85E−02	1.17E−01	9.47E−02

diet, habitat, behaviour, distribution, growth rates, age, trophic level and life strategies) (Pethybridge et al., 2010; McMeans et al., 2015; Endo et al., 2016; Tiktak et al., 2020). As observed with the velvet belly lantern shark (*E. spinax*), being the species with the highest concentration of Cd, even surpassing the concentration threshold delimitated by the EU Commission Regulation 2023/915 of 0.05 mg•kg^{-1} (w.w.). The obtained Cd concentration was akin to the value determined by Hornung et al. (1993) in the Levantine Sea (0.08 mg•kg^{-1}, w.w.), which was higher than the obtained concentration in this present study, and therefore it also exceeded the current legal concentration threshold for cadmium (European Commission, 2023). It is noteworthy that the high Cd concentration in *E. spinax* could be linked to its preference for cephalopods in its diet, particularly in areas with high cephalopod prey availability (Doubleday et al., 2016; Barría et al., 2018). Additionally, the ability of cephalopods to transfer Cd to higher trophic levels may explain its high concentration (Bustamante et al., 1998, 2002). However, as this species predominantly fed on Natantia crustaceans (*e.g.* shrimps and prawns), the high Cd concentration it exhibited might be attributed to the scavenging behaviour of these species, potentially exposing them to heavy metals (Drava et al., 2004; Cartes et al., 2008; Nouar et al., 2011; Olgunoglu, 2015).

As reported by Tiktak et al. (2020), the Cd concentration showed a negative correlation with the trophic level. However, in the present study, despite having a negative correlation, it was not statistically significative. The negative correlation between the Cd concentration and trophic level arises due to the high concentrations found in low-trophic level species such as *E. spinax*. This pattern has also been observed in the study of Hornung et al. (1993), where high-trophic level species, such as the little sleeper shark (*Somniosus rostratus*) and the bluntnose sixgill shark (*Hexanchus griseus*), had lower concentrations than *E. spinax*, similar to the results obtained in this study.

In the case of Pb, the rabbit fish (*C. monstrosa*) was the species that had the highest concentration. Note that there are no studies containing Pb concentration data on *C. monstrosa* in the Mediterranean Sea, as the only study on heavy metals related to *C. monstrosa* is about methylmercury (MeHg), being the species with the highest concentration of MeHg (Storelli et al., 2002b). Also, a weak and negative correlation was found between the Pb concentration of species and their trophic level, this trend is also evident in the absence of a significant correlation with the total length of the species. This phenomenon closely resembles the findings in Cd (Tiktak et al., 2020), primarily attributed to the high concentrations found in relatively small-sized species and low-trophic level species such as *C. monstrosa* and *E. spinax*. Species like the rabbit fish, that primarily fed on Reptantia crustaceans (*e.g.* crabs and lobsters) (Tamayo et al., 2021), had the highest Pb concentrations. Similar to Natantia crustaceans, the observed Pb concentration could be due to the high scavenging activity of Reptantia crustaceans (Padilla et al., 2022). Therefore, due to their physiological characteristics and feeding behaviour, they could potentially bioaccumulate heavy metals such as Pb (Botté et al., 2022).

Copper concentrations were generally higher than those of Cd and Pb in all the analysed species, and also it was negatively correlated with the size of the species analysed. The species with the highest Cu concentrations were not only carcinophagous, but also small-sized demersal species like *E. spinax*, which had the highest concentration. This species was followed by the small-spotted catshark (*S. canicula*), marbled electric ray (*T. marmorata*), little gulper shark (*C. uyato*) and the blackmouth catshark (*G. melastomus*). In comparison to other studies conducted in the Mediterranean Sea, the velvet belly lantern shark had a higher concentration than Hornung et al. (1993) in the Levantine Sea. This high concentration could be attributed to the presence of haemocyanin, a copper-based oxygen-transport protein present in significant prey groups such as molluscs and crustaceans (Vas, 1991; Taylor and Anstiss, 1999; Barría et al., 2015, 2018; Kato et al., 2018; Tamayo et al., 2021).

As with diet, the habitat of the analysed species may also play a fundamental role in the transfer of heavy metals (Pethybridge et al., 2010; Company et al., 2012). In the western Mediterranean Sea, depth is the main determining factor in the assemblage of chondrichthyan species, hosting a higher diversity of species (Ruiz-García et al., 2023), which elucidates the positive correlations observed between depth and heavy metal concentrations. In this region, submarine canyons are habitats of great importance for predatory species such as chondrichthyans (Company et al., 2012; Fernandez-Arcaya et al., 2017). These habitats, where species such as *C. monstrosa* and *E. spinax*, as well as their potential prey (*e.g.* crustaceans) can be found (Sion et al., 2004; Company et al., 2012; Fernandez-Arcaya et al., 2017; Ríos et al., 2022; Ruiz-García et al., 2023), are susceptible to anthropogenic impacts such as trawling and pollution (Company et al., 2012; Koenig et al., 2013; Fabri et al., 2014; Paradis et al., 2017). Due to their funnel-like shape and location, submarine canyons act as passageways for the transfer of sediments as well as organic and particulate matter, which can potentially include anthropogenic pollutants such as heavy metals (Company et al., 2012; Dumas et al., 2014; Palanques et al., 2022). Therefore, canyon-dwelling species could potentially be more exposed to heavy metals and other pollutants in comparison to species from other habitats (Solé et al., 2009; Company et al., 2012; Koenig et al., 2013; Nos et al., 2021).

Due to the different levels of hazard and toxicity of each heavy metal, as reflected in various parameters such as the oral reference dose (RfDo) (US-EPA, 2021, 2023) or the concentration thresholds of Cd (0.05 mg•kg^{-1}, w.w.) and Pb (0.3 mg•kg^{-1}, w.w.) established by the EU Commission Regulation 2023/915 (European Commission, 2023), as well as the biological characteristics and ecological role of chondrichthyans, their consumption may pose risks to human health (Storelli et al., 2002a, 2002b; Storelli, 2009; Bosch et al., 2016; Tiktak et al., 2020; Giovos et al., 2022). As observed in *E. spinax*, it was a species that exceeded the limit concentration of Eu Commission Regulation 2023/915 (European Commission, 2023) and therefore its consumption is not recommended, as well as some specimens of *C. monstrosa* in the case of Pb. Similar to another study conducted recently in the Mediterranean Sea, such as the one performed by Giovos et al. (2022) in the Aegean Sea, it can be generally observed how neither sharks nor rays and chimaeras exceeded the oral reference dose (RfDo) (μg•kg^{-1}•bw^{-1}•day^{-1}) values for the Estimated Daily Intake (EDI) (μg•kg^{-1}•bw^{-1}•day^{-1}) and the THQ threshold (THQ above 1) calculated for the analysed heavy metals (Cd, Pb and Cu). However, as it was observed with Pb in *C. monstrosa*, at lower body weights, such as those of children, the same heavy metal concentration increases the value of EDI and THQ, leading to a greater potential exposure to pollutants present in the chondrichthyan muscle (Copat et al., 2013; Barone et al., 2021; US-EPA, 2021, 2023). Consequently, the regular consumption of this species would not be recommended for children and pregnant people (Schoeters et al., 2006; Herreros et al., 2008). The mislabelling of chondrichthyan-derived products destined for human consumption is an important issue to be considered for future studies, as it also occurs in the same regions of the western Mediterranean as in this study (Barría and Colmenero, 2019; Pardo and Jiménez, 2020). In the particular case of rays, 55 % of their landings are labelled as *Raja sp.*, which encompasses not only species of the same genus, but also different species and different families as well (Barría and Colmenero, 2019). Thus, given the observed heavy metal concentrations differences between the analysed ray species, which are species of commercial interest, it can be assumed that there is a potential risk to human health stemming from the unintentional ingestion of mislabelled chondrichthyan products (Bornatowski et al., 2013; Hasan et al., 2023).

Additionally, the determined concentrations of heavy metals pose a potential risk not only to humans but also to Mediterranean chondrichthyans themselves (Consales and Marsili, 2021; Dulvy et al., 2021). This is because the potential negative effects of heavy metals and other pollutants (Bezerra et al., 2019; Tiktak et al., 2020; Wosnick et al., 2021) categorise pollution as a passive threat (Dulvy et al., 2021), which can be an aggravating factor due to the biological characteristics of chondrichthyans (Tiktak et al., 2020; Dulvy et al., 2021). This is especially concerning since certain species can accumulate high concentrations of pollutants, as has been the case with Cd in *E. spinax* and Pb in *C. monstrosa*. Despite having a similar sample size to other studies carried out in the Mediterranean Sea (Türkmen et al., 2013, 2014; Giovos et al., 2022), it would be desirable for future studies to have larger sample sizes, similar to other studies such as Storelli et al. (2002a, 2002b) This would allow for a more accurate description of the potential effects of pollutants, such as heavy metals, on species and consequently ecosystems, as well as providing appropriate guidelines and recommendations for the safe consumption of these species.

5. Conclusions

In this study, heavy metals concentrations were found to vary between species. Overall, sharks had the highest concentrations of cadmium (Cd) and copper (Cu), while chimaeras had the highest concentration of lead (Pb). Although rays had the lowest concentrations of heavy metals. At the species level, *Etmopterus spinax* had the highest Cd and Cu concentrations, and *Chimaera monstrosa* of Pb. It should be noted that concentration threshold delimitated by the EU Commission Regulation 2023/915 for Cd was exceeded by *E. spinax*, in the same way that certain specimens of *C. monstrosa* exceeded the concentration limit established for Pb.

Significant negative correlations linked heavy metal concentrations to species size and trophic level, with positive correlations related to depth. This suggests that heavy metal concentrations may depend not only on diet but also on habitat. In terms of diet, the species that mainly fed on invertebrates had high Cu concentrations, while those primarily consuming Reptantia crustaceans had the most Pb.

When considering the Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) of heavy metals, the consumption of the analysed species would not pose a risk to adults. However, *C. monstrosa* may pose a risk to children due to their lower body weight. Thus, the consumption of *C. monstrosa*, as well as other chondrichthyan species, may not be completely recommended for children and pregnant people. Therefore, further research is essential to fully understand the potential effects of pollutants on Mediterranean chondrichthyans and the risks derived from their consumption.

CRediT authorship contribution statement

Pol Carrasco-Puig: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **Ana I. Colmenero:** Project administration, Resources, Supervision, Validation, Writing – review & editing. **David Ruiz-García:** Methodology, Resources, Supervision, Validation, Writing – review & editing. **Alejandro J. Molera-Arribas:** Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **Ana M. Hernández-Martínez:** Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Juan A. Raga:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Claudio Barría:** Conceptualization, Project administration, Resources, Supervision, Validation, Writing – review & editing, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft.

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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