



Baseline

Assessment of elemental composition in commercial fish of the Bay of Cádiz, Southern Iberian Peninsula

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ABSTRACT

The assessment of trace metal content in our fish diet is important due to the adverse effect on human health. Despite the increasing interest about the fish quality, little information is available for Southern Spain, a region characterized by high seafood intake. Nine species from the Bay of Cádiz with high commercial value were selected. Similar values were measured in the nine studied species for most of the elements, except for the macroelements Ca and S, and the microelements Fe, Mn and As, which showed significant differences among species. Metal Pollution Index (MPI) did not differ among species, and it was similar to those obtained for other Atlantic and Mediterranean locations. The values measured for the nine species were below the health limits provided by World, European and Spanish legislations, indicating that, in general terms, consumption of these species is safe in the study area.

There is an increasing interest about the food quality (Abadi et al., 2015). Indeed, the study of essential and toxic elements in foodstuffs has received a lot of attention due to the growing consideration about the health benefits and risks of food consumption (Guérin et al., 2011; Iamiceli et al., 2015; Korkmaz et al., 2017; Micheline et al., 2019). The concern of the trace metal content of our diet and its adverse effect on human health is particularly important for fish according to the dietary advice to be consumed 2–3 times a week (Kandyliari et al., 2021).

Fish is an excellent source of quality proteins, carbohydrates, vitamins, important micro and macro elements, and essential fatty acids such as omega 3 (Kalantzi et al., 2016; Kandyliari et al., 2021). Therefore, fish consumption has beneficial health effects, such as reduction in the incidence of diabetes and cardiovascular diseases, normal neurodevelopment, adequate enzyme reactions in metabolism and increase of antioxidant activity (Mendil et al., 2010; Olmedo et al., 2013; Merciai et al., 2018; Lounas et al., 2021). On the other hand, fish can also accumulate toxic substances such as non-essential toxic metals, which can involve health risks to consumers even at small concentrations (Bat

et al., 2022). These elements can naturally enter the marine environment mainly by geological weathering, erosion, forest fires and volcanic activities, but their distribution is mostly influenced by anthropogenic activities such as agricultural, untreated sewage and industrial discharges (Ghosh et al., 2020; Yilmaz, 2020; Kontas et al., 2022). The essential metals can also produce toxic effects when their intake is excessive (Celik and Oehlenschlager, 2007).

Fish is an essential ingredient in the Mediterranean feeding habits being a traditional component of Mediterranean diet (Merciai et al., 2018; Hidalgo-Mora et al., 2020; Lounas et al., 2021). Although many studies have measured element concentrations in fish and shellfish products throughout the world, there is a lack of efforts focused on the determination of a wide range of elements in species consumed in Andalusia (Southern Spain), a region characterized by high seafood intake (Olmedo et al., 2013). The Bay of Cádiz (Fig. 1) is located in the Gulf of Cádiz, along the Atlantic coast of Southern Spain, where the fishery has an important economic and employment role (Miró et al., 2020). This Bay comprises one of the largest areas of salt marshes on the

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Spanish South Atlantic coast, and it is a natural nursery habitat for several fish species of commercial interest (Sánchez-Lamadri, 2004; Yúfera and Arias, 2010). In fact, this area is very important for the professional fishing of these species (Muñoz-Pérez and Sánchez-Lamadri, 1994). In spite of the fishing interest of the Gulf of Cádiz, the potential risk of metal pollution in the area is high. It receives draining of the central and eastern part of the Iberian Pyrite Belt, rich in As, Pb, Cu and Zn, which deposits large amount of heavy metal-laden sediments and contaminated water via the Tinto and Odiel rivers into the Gulf of Cádiz (Periañez, 2009; Vicente-Martorell et al., 2009; González-Ortegón et al., 2019; Laiz et al., 2020; Martín-Vélez et al., 2021). Furthermore, the Huelva estuary, in the northwest part of the Gulf of Cádiz, is also a heavily industrialized area which discharges high concentration of heavy metals into the Atlantic Ocean (Pérez-López et al., 2011). Although the Bay of Cádiz is ca. 100 km away from Huelva estuary, the large amount of toxic metals generated in this area produces a plume of contaminants into the whole Gulf of Cádiz which even reaches the Mediterranean Sea through the Strait of Gibraltar (Martín-Vélez et al., 2021). Thence, a global assessment of the fish quality regarding micro and macroelemental composition (essential and nonessential) is necessary in this area.

Therefore, the main objectives of the present study are (i) to determine essential and non-essential elements in wild fish from Bay of Cádiz, Southern Spain, selecting a variety of species with high commercial value along the Mediterranean and nearby Atlantic, (ii) to compare element concentrations, and the Metal Pollution Index (MPI), among the species through univariate and multivariate approach, (iii) to compare the baseline data obtained in the present study with a compilation of the available information of these species in the literature for other Mediterranean and Atlantic areas, and (iv) to compare trace metal concentrations measured in the present study with standard health limits for human consumption recommended by different national and international regulations, including European Commission (EC), World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) among others.

Nine species with high commercial value were selected for the

present study: common two-banded seabream *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), white seabream *Diplodus sargus* (Linnaeus, 1758), red mullet *Mullus barbatus* Linnaeus, 1758, striped red mullet *Mullus surmuletus* Linnaeus, 1758, axillary seabream *Pagellus acarne* (Risso, 1826), common pandora *Pagellus erythrinus* Linnaeus, 1758, red-banded seabream *Pagrus auriga* (Valenciennes, 1843), common seabream *Pagrus pagrus* Linnaeus, 1758 and gilt-head seabream *Sparus aurata* Linnaeus, 1758. Fish (ten specimens per species) were collected by local fishermen using gill net fishing from the coast of the outer Bay of Cádiz and nearby areas (from Rota to Conil) (Fig. 1, Table 1). Specimens were collected from 10 February 2015 to 1 February 2016, at a depth ranging from 5 to 40 m along muddy, sandy and rocky bottoms (Table 1). The anonymity of the exact locations where the fish was collected is preserved, according to the wishes of the local fishermen.

Immediately after purchasing, all fish specimens were placed into separate polyethylene bags, individually labeled, and brought to the laboratory in ice-box. Since live animal samples were not included in the research, the study did not require ethical approval.

After arriving to the laboratory, length and wet weight of each fish were recorded. Fish were washed with sterile distilled water and immediately dissected with aseptic plastic forceps and knife. Muscle samples (edible part) were frozen at -80°C . Previously to analyses of macro- and microelements, samples were freeze-dried for 48 h into a iShin Biobase Europe lyophilizer model FD8512 to constant weight. Muscle samples of 10 specimens of each species were used for analysis of essential macroelements (Ca, K, Mg, Na, P and S), essential microelements (Co, Cr, Cu, Fe, Mn, Ni and Zn) and non-essential microelements (Al, As, Ba, Cd, Pb and Sr). Freeze-dried samples (approximately 500 mg) were mineralized in Teflon digestion vessels, in a closed microwave digestion using 0.5 ml of nitric acid HNO_3 and 1.5 ml of hydrogen peroxide H_2O_2 as reagents (Suprapur® grade, Merck, Darmstadt, Germany). Quantification of elements in the extracts was achieved using a VARIAN ICP 720-ES (simultaneous ICP-OES with axially viewed plasma), equipped with ultrasonic nebulizer CETAC U5000AT+ (Guerra-García et al., 2011). Standard solutions for the devices' calibration were used. For preparation of standards we used $<18\text{ M}\Omega/\text{cm}$

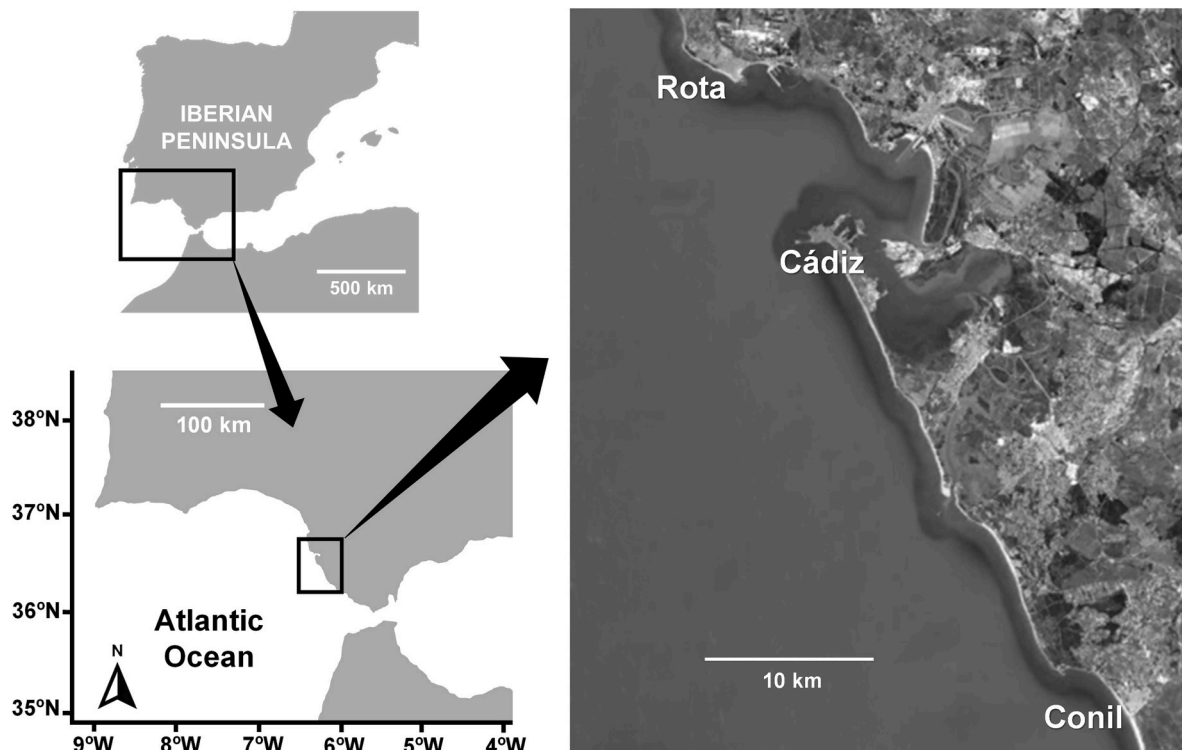


Fig. 1. Study area showing the location of the Bay of Cádiz in the Gulf of Cádiz, Southern Spain. Fish collection were conducted from Rota to Conil.

Table 1Characteristics of fish specimens used in this study. SE: Standard error of the mean ($n = 10$). M = muddy, S = sandy, R = rocky.

	Bottom		Collection date	Length (cm)		Weight (g)	
	Bottom	Depth (m)		Range	Mean \pm SE	Range	Mean \pm SE
Fish species							
<i>Diplodus vulgaris</i> (1)	M, S, R	20–30	9 Jun-3 Sep 2015	18.5–26.7	24.2 \pm 0.8	226–331	250 \pm 14
<i>Diplodus sargus</i> (2)	R	5	10 Feb-13 Mar 2015	19.0–20.0	19.6 \pm 0.1	102–132	122 \pm 3
<i>Mullus barbatus</i> (3)	M	10–15	10 Feb-23 Jul 2015	17.2–25.6	19.2 \pm 0.8	63–186	87 \pm 12
<i>Mullus surmuletus</i> (4)	R	15–40	6 May-30 Jun 2015	17.3–21.7	19.8 \pm 0.4	70–138	106 \pm 7
<i>Pagellus acarne</i> (5)	S, R	25–40	3 Jul-9 Sep 2015	18.8–24.8	21.2 \pm 0.7	147–235	192 \pm 11
<i>Pagellus erythrinus</i> (6)	M, S, R	14–31	20 May 2015–1 Feb 2016	18.4–27.0	23.6 \pm 0.9	96–277	198 \pm 20
<i>Pagrus auriga</i> (7)	M, S, R	15–20	13 Mar-26 May 2015	21.4–26.8	24.6 \pm 0.5	191–360	272 \pm 17
<i>Pagrus pagrus</i> (8)	M	15–40	28 Apr-22 Jun 2015	26.1–28.4	27.0 \pm 0.2	287–420	327 \pm 13
<i>Sparus aurata</i> (9)	S	9–11	2 Sep 2015–1 Feb 2016	21.0–25.0	22.0 \pm 0.4	232–362	280 \pm 13
One-way ANOVA:							
F-statistic				20.34***		42.49***	
SNK				(2 = 3 = 4 = 5) \neq (1 = 6 = 7 = 9) \neq 8		(2 = 3 = 4) \neq (5 = 6) \neq (1 = 7 = 9) \neq 8	

*** $p < 0.001$.

ultrapure water supplied from a Milli-Q Millipore system (Bedford, MA, USA) and Tracepure™ HNO₃ from Merck (Darmstadt, Germany). Calibration and Quality Control (QC) solutions were prepared from an ICP multi-element standard solution IV Certipur obtained from Merck and Spectrascan certified reference solution from LGC Standards GmbH (Wesel, Germany). To prevent contamination of the samples with any traces of metal, all material used for sample storing and treatments and all labware equipment was soaked in 2% v/v HNO₃ solution followed by two washes with Milli-Q water. The calibration blank was prepared with 2% v/v HNO₃. Analytical blanks and standard reference materials were run in the same way as samples. The accuracy of the analytical methods was assessed through reference sample (TORT-2: Lobster Hepatopancreas Reference Material for Trace Metals). The differences in concentrations between analyzed and certified values were < 10%.

To compare the total essential and non-essential microelement content at the different species, the metal pollution index (MPI) was used. This was obtained with the following equation: $MPI = (C_1 \times C_2 \dots \times C_n)^{1/n}$; where C_i is the concentration of metal i in the sample (essential and non-essential microelements were considered separately) and n is the number of total microelements (Usero et al., 1996, 1997, 2005; Marengo et al., 2018; Arechavala-Lopez et al., 2019).

To compare size and weight among the specimens of the nine species, one-way ANOVA was conducted. Prior to statistical analysis, the homogeneity of variances was tested with Cochran's C-test (Underwood, 1997). Where ANOVA indicated a significant difference for a given factor, the source of difference was identified using Student-Newman-Keul (SNK) tests. Concentrations of essential and non-essential elements, and MPI values, were also compared among species. Non-compliance with parametric ANOVA assumptions led to use of the Kruskal-Wallis non-parametric tests to evaluate the existing differences in element concentration and MPI values among species. When significant differences were found, Bonferroni post-hoc testing was used to determine the differences among species (Arechavala-Lopez et al., 2019). Results were expressed as mean \pm SE and $p < 0.05$ was considered statistically significant. Additionally, principal component analyses (PCAs) were carried out to show the relationship among fish species according to element composition. Data of elements were transformed with $\log(x + 1)$. Differences among species based on the element composition were also tested by the use of a permutational multivariate analysis of variance (PERMANOVA). Analysis was based on Euclidean distance. Significant P values were obtained by computing 9999 permutations under a model of unrestricted permutation of raw data, which is recommended when there is only a single factor (Anderson, 2005). Pairwise comparisons were then used. Univariate analyses were conducted with GMAV5 (Underwood et al., 2002) and multivariate analyses were carried out using the PRIMER v.6 plus PERMANOVA package (Clarke and Gorley, 2001).

Although the specimens studied had a length and weight range of the

same magnitude order (always lower than 30 cm and 500 g respectively), some significant differences were found among species, being *D. sargus*, *M. barbatus* and *M. surmuletus* smaller than the other species (Table 1).

Regarding elemental composition, similar values were measured in the nine studied species for most of the elements, except for the macroelements Ca and S, and the microelements Fe, Mn and As, which showed significant differences among species (Table 2). Ca concentration was significantly high in *D. sargus*, *P. auriga* and *P. pagrus*. S was lower in *P. acarne* and *P. erythrinus*. Fe showed significantly higher values in *M. barbatus*, *P. erythrinus* and *P. pagrus*. *P. erythrinus* showed significant lower concentration of Mn than the remaining species. As levels were significant higher in *M. barbatus*, *M. surmuletus*, *P. pagrus* and *S. aurata*. In PCA analyses, As, Fe and Ca were also the elements that better explained the differences among species, especially As which separated the different species along axis 2 of the PCA based on non-essential microelements (Fig. 2). These elements showed the highest significant correlations ($p < 0.001$) with the ordination axes (As: $r = 0.99$, axis 2; Fe: $r = -0.96$, axis 1; Ca: $r = -0.76$, axis 1). Multivariate differences among species according to the elemental composition were also supported by PERMANOVA results (Table 3). Türkmen et al. (2009) also found significant differences in some metal values (Cd, Co, Cr, Cu, Zn, Mn, Fe, Ni and Pb) among fish species collected from the Aegean Sea and Mediterranean Sea and suggested that this could indirectly indicate the different environmental contamination along the coastal areas which species were inhabiting. Çelik and Oehlenschläger (2005) also reported differences in the Zn and Cu contents in fish collected from the eastern Mediterranean. In fact, they found that *M. barbatus* presented the highest Cu concentration of demersal fish from Mersin Bay. During the present study we also found higher concentrations of some elements, i.e. S, Fe, Mn and As, in this species. Feeding habits of *M. barbatus* are associated to muddy bottoms, which are usually more polluted areas, and the small invertebrates ingested could contribute to some metal accumulation. Heavy metals potentially show an affinity for mud particles and accumulate where fine-grained sediments are present (George et al., 2007). Kontas et al. (2022) pointed out that the importance of bivalves and gastropods in the diet of *M. barbatus* could be the main reason to explain higher heavy metal levels since these molluscs are especially prone to accumulate metals. Indeed, element uptake by fish may be either waterborne (directly from water to organism through respiratory surface) or dietborne (with food and consequent absorption in the digestive tract) (Rozon-Ramilo et al., 2011). Many authors have pointed out that bioaccumulation largely depends on the fish species, but also on size and age, sex, feeding behaviour, physiological conditions, spawning status or migration, parasites and environmental factors such as salinity, pH and temperature (Žvad Rožič et al., 2014; Merciai et al., 2018).

In spite of the differences measured for some elements, MPI values

Table 2

Elemental concentrations (mg/kg dw) in the muscle of the different species measured in the present study. Values are mean \pm standard error, n = 10 for each species. n.s.: not significant. Dissimilar letters (a, b, c) denote significant differences between groups according to post-hoc Bonferroni test. MPI: Metal pollution index.

	<i>D. vulgaris</i>	<i>D.sargus</i>	<i>M.barbatus</i>	<i>M.surmuletus</i>	<i>P. acarne</i>	<i>P. erythrinus</i>	<i>P.auriga</i>	<i>P. pagrus</i>	<i>S. aurata</i>	KRUSKAL-WALLIS p-value
Essential macroelements										
Ca	787.49 \pm 116.34 ^a	1074.83 \pm 378.37 ^b	839.58 \pm 139.39 ^a	642.36 \pm 34.83 ^c	628.35 \pm 36.56 ^c	508.57 \pm 29.03 ^c	1176.20 \pm 281.37 ^b	1231.62 \pm 486.41 ^b	530.66 \pm 20.42 ^c	0.005**
K	22,236.22 \pm 1795.78	26,499.09 \pm 1968.96	20,332.26 \pm 1293.15	21,975.22 \pm 1769.00	20,938.61 \pm 1433.46	20,955.68 \pm 1573.68	25,184.48 \pm 1778.66	22,656.27 \pm 1783.32	22,042.38 \pm 1581.34	0.256 n.s.
Mg	1666.99 \pm 59.84	1744.30 \pm 145.05	1688.80 \pm 113.98	1560.63 \pm 54.60	1569.51 \pm 61.35	1485.35 \pm 38.70	1636.47 \pm 55.49	1626.14 \pm 65.47	1607.76 \pm 43.30	0.587 n.s.
Na	2606.36 \pm 312.66	2668.22 \pm 306.85	3432.12 \pm 186.61	2626.35 \pm 243.70	3073.51 \pm 326.84	3194.16 \pm 443.47	2511.56 \pm 226.37	2405.82 \pm 285.09	3443.70 \pm 417.76	0.123 n.s.
P	1349.52 \pm 94.03	1484.57 \pm 120.14	1271.19 \pm 93.75	1306.87 \pm 89.08	1310.36 \pm 88.62	1308.24 \pm 78.05	1417.65 \pm 82.82	1442.89 \pm 93.64	1329.77 \pm 78.90	0.786 n.s.
S	1360.61 \pm 89.92 ^a	1775.94 \pm 173.98 ^a	1450.59 \pm 107.27 ^a	1420.54 \pm 91.19 ^a	1176.40 \pm 81.72 ^b	1109.91 \pm 99.51 ^b	1578.96 \pm 104.77 ^a	1335.45 \pm 105.17 ^a	1398.22 \pm 89.81 ^a	0.004**
Essential microelements										
Co	0.03 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.04 \pm 0.01	0.04 \pm 0.02	0.03 \pm 0.01	0.01 \pm 0.01	0.02 \pm 0.01	0.514 n.s.
Cr	0.29 \pm 0.04	0.46 \pm 0.13	0.31 \pm 0.05	0.26 \pm 0.03	0.27 \pm 0.04	0.30 \pm 0.04	0.37 \pm 0.04	0.43 \pm 0.09	0.28 \pm 0.02	0.181 n.s.
Cu	0.74 \pm 0.43	1.53 \pm 1.01	1.34 \pm 0.36	0.51 \pm 0.19	1.00 \pm 0.17	0.68 \pm 0.32	0.58 \pm 0.16	0.69 \pm 0.42	0.66 \pm 0.22	0.118 n.s.
Fe	6.19 \pm 0.49 ^a	27.88 \pm 19.99 ^b	53.27 \pm 35.07 ^c	9.67 \pm 0.93 ^a	16.80 \pm 3.78 ^b	56.01 \pm 35.01 ^c	19.99 \pm 6.48 ^b	45.35 \pm 21.28 ^c	5.93 \pm 0.60 ^a	0.000***
Mn	0.30 \pm 0.04 ^a	0.32 \pm 0.14 ^a	0.47 \pm 0.06 ^a	0.48 \pm 0.06 ^a	0.41 \pm 0.07 ^a	0.18 \pm 0.05 ^b	0.56 \pm 0.10 ^a	0.39 \pm 0.10 ^a	0.36 \pm 0.05 ^a	0.015*
Ni	0.76 \pm 0.16	1.96 \pm 0.67	1.49 \pm 0.70	0.98 \pm 0.20	1.07 \pm 0.27	0.92 \pm 0.32	1.81 \pm 0.64	1.23 \pm 0.45	1.61 \pm 0.90	0.547 n.s.
Zn	27.82 \pm 2.34	46.11 \pm 9.66	32.07 \pm 5.88	26.85 \pm 3.50	32.91 \pm 3.61	26.72 \pm 3.51	39.24 \pm 4.14	31.22 \pm 3.95	32.50 \pm 3.70	0.211 n.s.
MPI	0.54 \pm 0.09	0.83 \pm 0.37	0.95 \pm 0.12	0.55 \pm 0.07	0.84 \pm 0.09	0.66 \pm 0.16	0.82 \pm 0.07	0.54 \pm 0.05	0.55 \pm 0.08	20.693 n.s.
Non-essential microelements										
Al	5.93 \pm 2.95	9.41 \pm 3.92	8.02 \pm 2.16	9.05 \pm 2.92	15.30 \pm 7.37	14.78 \pm 6.41	12.69 \pm 6.40	7.36 \pm 2.76	5.86 \pm 2.39	0.969 n.s.
As	23.49 \pm 2.86 ^a	19.76 \pm 3.14 ^a	59.05 \pm 10.48 ^b	42.52 \pm 4.03 ^b	9.79 \pm 1.36 ^a	20.56 \pm 2.56 ^a	19.80 \pm 2.27 ^a	48.48 \pm 8.40 ^b	54.86 \pm 6.83 ^b	0.000***
Ba	0.65 \pm 0.33	14.52 \pm 12.95	44.95 \pm 29.88	0.47 \pm 0.23	5.92 \pm 4.67	17.74 \pm 13.14	2.77 \pm 1.35	34.91 \pm 29.26	0.66 \pm 0.28	0.584 n.s.
Cd	0.04 \pm 0.01	0.11 \pm 0.03	0.06 \pm 0.02	0.07 \pm 0.02	0.13 \pm 0.04	0.07 \pm 0.03	0.17 \pm 0.08	0.09 \pm 0.02	0.07 \pm 0.02	0.240 n.s.
Pb	0.71 \pm 0.16	1.99 \pm 0.57	1.56 \pm 0.56	0.97 \pm 0.24	1.31 \pm 0.26	1.12 \pm 0.37	1.26 \pm 0.19	1.35 \pm 0.43	1.24 \pm 0.29	0.478 n.s.
Sr	2.76 \pm 0.72	3.22 \pm 1.52	3.08 \pm 0.96	1.86 \pm 0.21	2.33 \pm 0.32	1.81 \pm 0.18	4.05 \pm 1.28	4.74 \pm 2.00	1.70 \pm 0.22	0.832 n.s.
MPI	0.85 \pm 0.22	2.00 \pm 0.90	2.63 \pm 1.19	1.22 \pm 0.29	1.57 \pm 0.45	1.77 \pm 0.91	1.94 \pm 0.41	2.43 \pm 1.09	1.26 \pm 0.35	9.998 n.s.

* $p < 0.05$.** $p < 0.01$.*** $p < 0.001$.

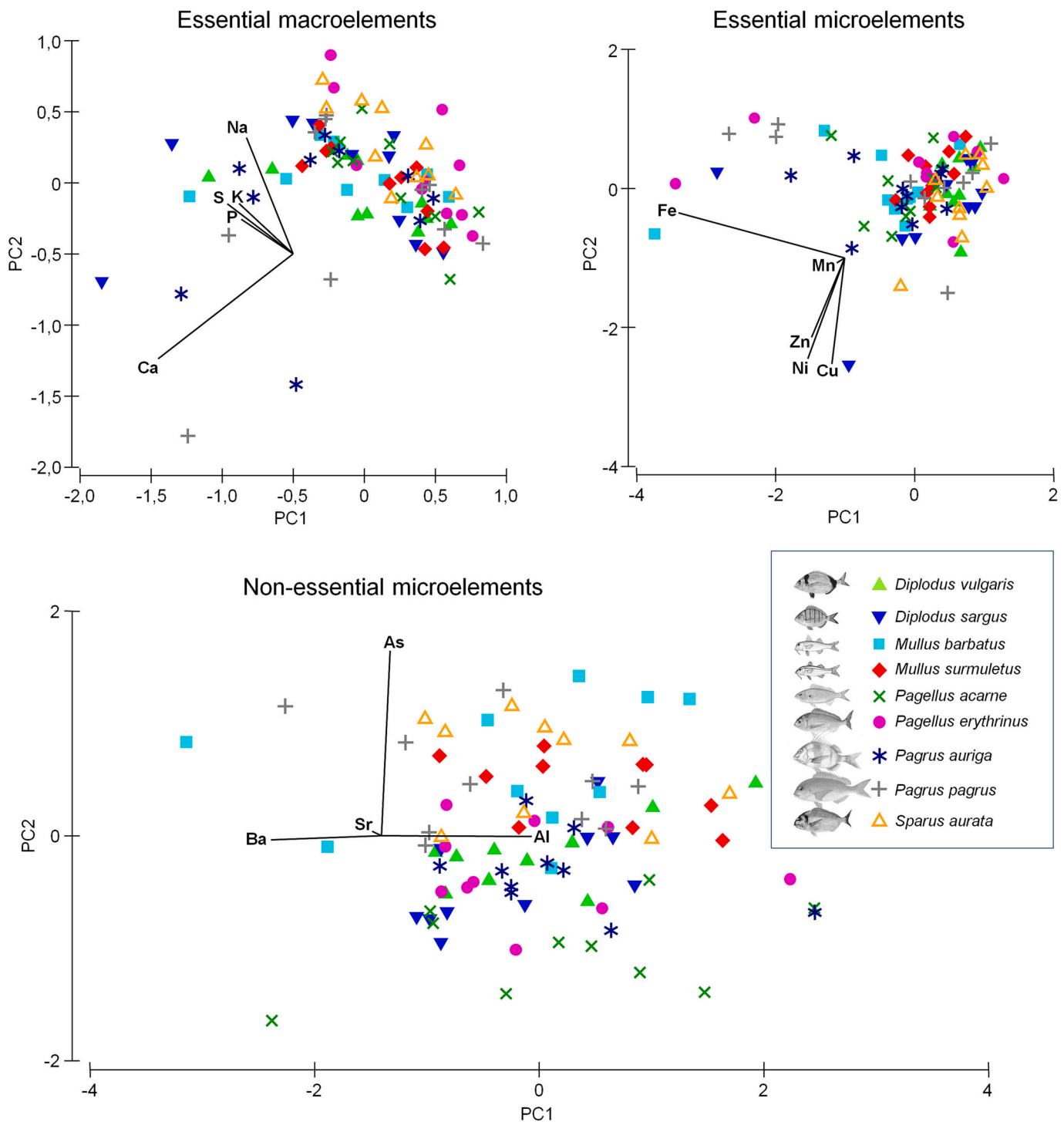


Fig. 2. Principal component analysis (PCA) plots based on the concentrations of essential macroelements and essential and non-essential microelements in muscle tissues of the nine species considered in the present study. Only those elements which significantly correlated with the axes PC1 and PC2 were graphically represented.

did not show significant differences among species for essential and non-essential microelements (Table 2). MPI allows the evaluation of trace element variation among species and their origins; the higher the index, the greater the contamination (Usero et al., 1996; Marengo et al., 2018). Values obtained in the present study (ranging 0.54–0.95 for essential and 0.85–2.63 for non-essential microelements, Table 2) are of the same magnitude order or lower than those obtained for other Mediterranean areas (e.g. Kalantzi et al., 2016; Marengo et al., 2018; Merciai et al., 2018; Kontas et al., 2022). Interestingly, although differences were not significant, the highest MPI values were measured in those species

collected exclusively from muddy bottoms, *M. barbatus* and *P. pagrus* (see Table 1). MPI values are, indeed, usually related with enrichment of metal levels in sediments (Kontas et al., 2022).

Element concentrations measured in the present study are, in most cases, similar (same order of magnitude) to those from other Mediterranean and Atlantic locations (see Table S1, supplementary material). Most studies have been focused in *Sparus aurata*, *Mullus barbatus* and *Diplodus* spp., and very scarce information is available for the species *P. acarne*, *P. auriga* and *P. pagrus*. More efforts should be conducted to properly assess the elemental composition of these species, provided

Table 3

Summary of the one-way PERMANOVA results based on data of essential macroelements, essential microelements and non-essential microelements. Star symbols indicates significant differences, * $P < 0.05$. MS = Mean Square. Only significant differences in pairwise are included. 1: *D. vulgaris*, 2: *D. sargus*, 3: *M. barbatus*, 4: *M. surmuletus*, 5: *P. acarne*, 6: *P. erythrinus*, 7: *P. auriga*, 8: *P. pagrus*, 9: *S. aurata*.

Source of variation	df	MS	Pseudo-F	P	Unique permutations
Essential macroelements					
Fish species	8	0.979	1.923	0.012*	9899
Residual	81	0.509			
Total	89				
Pair-wise tests	1 ≠ 6, 2 ≠ 6, 2 ≠ 9, 3 ≠ 6, 5 ≠ 7, 6 ≠ 7, 6 ≠ 8, 7 ≠ 9, 8 ≠ 9				
Essential microelements					
Fish species	8	2.475	1.678	0.041*	9909
Residual	81	1.475			
Total	89				
Pair-wise tests	1 ≠ 3, 1 ≠ 5, 1 ≠ 7, 3 ≠ 4, 3 ≠ 9, 4 ≠ 5, 4 ≠ 7, 5 ≠ 9, 7 ≠ 9				
Non-essential microelements					
Fish species	8	6.288	1.592	0.048*	9896
Residual	81	3.948			
Total	89				
Pair-wise tests	1 ≠ 3, 3 ≠ 5, 4 ≠ 5, 5 ≠ 8, 5 ≠ 9, 7 ≠ 9				

their high commercial values. Global comparisons can be difficult because some studies express elemental concentrations per fish dry weight (e.g. Carvalho et al., 2005; Vasconcelos et al., 2011; Žvad Rožič et al., 2014; Bouchoucha et al., 2018; Yigit et al., 2018; Kontas et al., 2022; present study) while others refer them to fish wet weight (e.g. Afonso et al., 2018; Korkmaz et al., 2019; Renieri et al., 2019; Lounas et al., 2021). Humidity percentage can differ among species and environmental conditions, and it is expected that dry weight should be more adequate for comparisons. However standard limits in national and international regulations are all traditionally provided per wet weight (see Table 4) so this factor probably make many authors to give their results by wet weight. Several studies use theoretical conversion factors to compare concentrations expressed to wet or dry mass (e.g. 4.31 in Minganti et al., 2010; 5 in Cresson et al., 2017), but some studies show large discrepancy driven by biological variables (e.g. values can be affected by the muscle lipid content), varying from 3 to 6 (Cresson et al., 2017). For these reasons it would be advisable to reach a consensus among members of the scientific community and authorities to use the same measure (wet or dry mass) to facilitate comparison. In the present study we have used concentrations per dry mass and have used 4.31 as multiplicative factor when comparison with wet mass was required according to Minganti et al. (2010).

The availability of baseline databases of element composition is mandatory to a proper assessment of the fish quality regarding accumulation of toxic elements. Different approaches provide tools to evaluate the adequacy for human consumption, such as estimation of daily (EDI) or weekly (EWI) intakes of elements and comparison with recommended safety guidelines, use of bioaccumulation index (IMBI) or metal pollution index (MPI), calculation of the hazard index (HI), target hazard quotient (THQ) and target cancer risk (TR) among others (e.g. Olmedo et al., 2013; Abadi et al., 2015; Marengo et al., 2018; Korkmaz et al., 2019; Renieri et al., 2019; Kumar et al., 2021; Lounas et al., 2021; Sadeghi et al., 2021; Shalini et al., 2021; Kontas et al., 2022). Indeed, International authorities (e.g. World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), US Environment Protection Agency and European Commission (EC)) have published threshold levels for potentially hazardous chemicals to prevent health problems (Kosker, 2020).

Besides calculation of MPI for fish from the Bay of Cádiz, which turned out to be similar or lower than those of other Mediterranean areas, we conducted a compilation of the maximum acceptable limits for trace elements in fish for human consumption recommended by different national and international regulations. These maximum limits are given in Table 4 together with the mean levels measured in the present study. Legal thresholds are not available for essential elements in Europe since the European Community has proposed limits only for the nonessential metals Cd, Pb and Hg. Some national and international regulations also provide values for Cr, Cu, Zn and As. Indeed, lead, cadmium and arsenic are considered to be the most toxic metals and have been associated with serious adverse health effects (Dural et al., 2007). Lead exposure may result in physical and mental growth retardation in infants, learning deficiency in children, and kidney problems, hypertension, anemia and muscle paralysis (Yabanli and Alparslan, 2015). Cadmium exposure can cause kidney and skeletal damages, neurological disorders and endocrine disruption as well as cardiovascular dysfunction and carcinogenic effects (Renieri et al., 2019). Arsenic is a known carcinogen in humans according to the International Agency for Research on Cancer (IARC), causing lung, liver, skin and bladder cancer (Aydin and Tokaloğlu, 2015).

The values measured in the present study for the nine species are below the limit values provided by these World, European and Spanish legislations for all the elements except for As (Table 4). However, it is well known that As is present in fish under various chemical forms with the most abundant being the non-toxic organic arsenic (arsenobetaine) (Abadi et al., 2015; Micheline et al., 2019). Arsenic shows its toxicity mainly via inorganic species such as As (III) and As (V). Therefore, the current guidelines for As exposure are provided only for inorganic As (Table 4), since the organic forms of arsenic has no toxicological concern (Micheline et al., 2019). Consequently, the relatively high values obtained for As in the present study do not reflect the health hazard because the toxic fraction (inorganic As) in fish ranges between 0.02 and 11 % of the total As (Muñoz et al., 2000). In this sense, some authors (e.g. Kalantzi et al., 2016) estimate human risk due to As based on inorganic fraction calculated as 10 % of total As. According to this, values of inorganic As in the present study would range 0.02–0.12 mg/kg, which are below the limits proposed by some national and international regulations (Table 4). For a more accurate assessment of As health effects in future studies, speciation analysis of As, as those carried out by Bouchoucha et al. (2019), should be carried out (Micheline et al., 2019).

Although European regulations have not established limits of As for fish and other foodstuff in human consumption yet (see Table 4), Regulation No 1006/2015 (EC, 2015b) provided limits of 0.1–0.30 of inorganic As (sum of As (III) and As (V)) in rice and derivatives. Rice is an important ingredient in a broad variety of food for children and the occurrence data demonstrate that rice waffles, wafers, crackers and cakes can contain high levels of inorganic arsenic (EC, 2015b). Taking this rice-case as example, further efforts should be conducted by European commission and other institutions to establish health limits not only for As in foodstuffs but also to other nonessential and essential elements. Erecting complete baseline data of element concentrations in different regions is the first step to integrate all available information (ecotoxicological tests, health effects measured) to establish coherent and useful hazard limits by national and international organizations.

Although further studies regarding speciation of some metals, and based on a larger number of fish specimens are needed, this baseline research based on element concentrations in edible parts of nine examined fish species indicated that, in general terms, consumption of these species is safe in the study area.

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Table 4

Limits (in mg/kg ww) for some trace elements in fish for human consumption recommended by different national and international regulations. Average values (mg/kg ww) obtained in the present study for the nine species are included for comparison (Mean dry weight values of the nine species were converted in wet weight values dividing by 4.31 for comparison, according to [Minganti et al., 2010](#)). Reference limits for As are provided for inorganic As ([Micheline et al., 2019](#)), while values in the present study refers to the total As. Limits for Hg were not included since Hg was not measured in the present study.

	As	Cd	Cr	Cu	Pb	Zn	Reference
National and International Regulations:							
Spanish legislation		1		20	2		BOE (Boletín Oficial del Estado), 1991, 2006
Croatian legislation	2	0.05–0.3			0.3		Ordinance, 2008; Žvad Rožič et al., 2014
Turkish legislation		0.1		20	1	50	Anonymous, 1996; Dural et al., 2007; Korkmaz et al., 2017
Ministry of Agriculture, Fisheries and Food, UK		0.2		20	2	50	MAFF, 2000; Korkmaz et al., 2017
European Commission		0.05–0.25			0.2–0.4		EC, 2001, 2006, 2008, 2014, 2015a, 2015b; Lozano et al., 2017
United States Environmental Protection Agency		2	8	120	4	120	USEPA, United States Environmental Protection Agency, 2011; Kumar et al., 2021
Government of Canada	3.5				0.5		Government of Canada, 2020
Australia New Zealand Food Authority	2				0.5		Australian Government, 2021
China National Standards	0.5	0.1	2	50	0.5		CNSMD (China National Standards Management Department), 2001
Brazilian authorities		1		30	2	50	Joyeux et al., 2004
Food Safety and Standards Authority of India		0.3			0.3		FSSAI, 2015; Kumar et al., 2021
Malaysian Food Regulation		1		30	2	100	MFR, 1985; Kumar et al., 2021
South African Department of Health	3	1			0.5		Bosch et al., 2016
World Health Organization		1	50	30	2	100	WHO, 1989; Kumar et al., 2021
Food and Agriculture Organization of the United Nations (FAO)	0.1–6	0.05–2	1	10–100	0.5–6	30–150	Nauen, 1983
Species from Bay of Cádiz (present study):							
<i>Diplodus vulgaris</i>	5.45	0.01	0.07	0.17	0.16	6.45	
<i>Diplodus sargus</i>	4.58	0.03	0.11	0.35	0.46	10.70	
<i>Mullus barbatus</i>	13.70	0.01	0.07	0.31	0.36	7.44	
<i>Mullus surmuletus</i>	9.86	0.02	0.06	0.12	0.22	6.23	
<i>Pagellus acarne</i>	2.27	0.03	0.06	0.23	0.30	7.64	
<i>Pagellus erythrinus</i>	4.77	0.02	0.07	0.16	0.26	6.20	
<i>Pagrus auriga</i>	4.59	0.04	0.09	0.13	0.29	7.64	
<i>Pagrus pagrus</i>	11.24	0.02	0.10	0.16	0.31	7.24	
<i>Sparus aurata</i>	12.72	0.02	0.06	0.15	0.29	7.54	

CRedit authorship contribution statement

José M. Guerra-García: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft. **Sandra Calero-Cano:** Investigation, Data curation, Methodology, Writing – review & editing. **Íñigo Donázar-Aramendía:** Investigation, Methodology, Writing – review & editing. **Inmaculada Giráldez:** Investigation, Methodology, Writing – review & editing. **Emilio Morales:** Investigation, Methodology, Writing – review & editing. **Pablo Arechavala-Lopez:** Investigation, Methodology, Writing – review & editing. **J. Lucas Cervera-Currado:** Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

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Data availability

Data will be made available on request.

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References

- Abadi, D.R.V., Dobaradaran, S., Nabipour, I., Lamani, X., Ravanipour, M., Tahmasebi, R., Nazmara, S., 2015. Comparative investigation of heavy metal, trace, and macro element contents in commercially valuable fish species harvested off from the Persian Gulf. *Environ. Sci. Pollut. Res.* 22 (9), 6670–6678.
- Afonso, A., Gutiérrez, Á.J., Lozano, G., González-Weller, D., Lozano-Bilbao, E., Rubio, C., Caballero, J.M., Revert, C., Hardisson, A., 2018. Metals in *Diplodus sargus cadenati* and *Sparisoma cretense*—a risk assessment for consumers. *Environ. Sci. Pollut. Res.* 25 (3), 2630–2642.
- Anderson, M.J., 2005. PERMANOVA: A FORTRAN Computer Program for Permutational Multivariate Analyses of Variance. Department of Statistics, University of Auckland, New Zealand.
- Anonymous, 1996. Handbook of Quality Control on Fish Products. Ministry of Agriculture and Rural Affairs, Ankara.
- Arechavala-Lopez, P., Capó, X., Oliver-Codorniu, M., Sillero-Rios, J., Busquets-Cortés, C., Sanchez-Jerez, P., Sureda, A., 2019. Fatty acids and elemental composition as biomarkers of *Octopus vulgaris* populations: does origin matter? *Mar. Pollut. Bull.* 139, 299–310.
- Australian Government, 2021. Australia New Zealand Food Standards Code—Schedule 19—Maximum levels of contaminants and natural toxicants. <https://www.legislation.gov.au/Details/F2017C00333>. (Accessed 23 February 2022).
- Aydin, D., Tokaloğlu, S., 2015. Trace metals in tissues of the six most common fish species in the Black Sea, Turkey. *Food Addit. Contam. Part B Surveill.* 8, 25–31. <https://doi.org/10.1080/19393210.2014.949873>.
- Bat, L., Şahin, F., Öztekin, A., Arici, E., 2022. Toxic metals in seven commercial fish from the southern Black Sea: toxic risk assessment of eleven-year data between 2009 and 2019. *Biol. Trace Elem. Res.* 200, 832–843.
- BOE (Boletín Oficial del Estado), 1991. In: Normas microbiológicas, los límites de contenido en metales pesados y los métodos analíticos para la determinación de metales pesados para los productos de la pesca y la acuicultura. (Microbiological Standards, Limits on Heavy Metal Content, and Analytical Methods for Determining the Heavy Metal Content of Fishery and Agricultural Products). BOE No. 195, 15 August 1991, pp. 27153–27155.
- BOE (Boletín Oficial del Estado), 2006. In: Orden SCO/3517/2006, de 13 de noviembre, por la que se modifica la Orden de 2 de agosto de 1991, por la que se aprueban las normas microbiológicas, los límites de contenido en metales pesados y los métodos analíticos para la determinación de metales pesados para los productos de la pesca y de la acuicultura. BOE No. 275, 17 November 2006, pp. 40287–40288.
- Bosch, A.C., O'Neill, B., Sigge, G.O., Kerwath, S.E., Hoffman, L.C., 2016. Heavy metals in marine fish meat and consumer health: a review. *J. Sci. Food Agric.* 96 (1), 32–48.
- Bouchoucha, M., Brach-Papa, C., Gonzalez, J.L., Lenfant, P., Darnaude, A.M., 2018. Growth, condition and metal concentration in juveniles of two *diplodus* species in ports. *Mar. Pollut. Bull.* 126, 31–42.
- Bouchoucha, M., Chekri, R., Leufroy, A., Jitaru, P., Millour, S., Marchond, N., Chafey, C., Testu, C., Zinck, J., Cresson, P., Mirallès, F., Mahe, A., Arnich, N., Sanaa, M., Bemrah, N., Guérin, T., 2019. Trace element contamination in fish impacted by bauxite red mud disposal in the cassidaigne canyon (NW french Mediterranean). *Sci. Total Environ.* 690, 1626. <https://doi.org/10.1016/j.scitotenv.2019.06.474>.
- Carvalho, M.L., Santiago, S., Nunes, M.L., 2005. Assessment of the essential element and heavy metal content of edible fish muscle. *Anal. Bioanal. Chem.* 382 (2), 426–432.
- Çelik, U., Oehlenschläger, J., 2005. Zinc and copper content in marine fish samples collected from the eastern Mediterranean Sea. *Eur. Food Res. Technol.* 220 (1), 37–41.
- Çelik, U., Oehlenschläger, J., 2007. High contents of cadmium, lead, zinc and copper in popular fishery products sold in Turkish supermarkets. *Food Control* 18, 258–261.
- Clarke, K.R., Gorley, R.N., 2001. PRIMER v5: User Manual/Tutorial. PRIMER-E, Plymouth, UK.
- CNSMD (China National Standards Management Department), 2001. Safety Qualification for Agricultural Product for Non-environmental Pollution Aquatic Products. GB 18406.4-2001. Beijing, China.
- FSSAI Safety, Food, 2015. Proposes Standard Limits of Metal Contaminants in New Food Articles. <https://doi.org/10.1016/B978-0-12-374845-4.00002-3>. ISSN 0250-563X.
- MAFF Fisheries and Food, Ministry of Agriculture, 2000. Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea. <https://www.cefas.co.uk/publications/aquatic/emr51.pdf>. (Accessed 23 February 2022).
- Cresson, P., Travers-Trolet, M., Rouquette, M., Timmerman, C.A., Giraldo, C., Lefebvre, S., Ernande, B., 2017. Underestimation of chemical contamination in marine fish muscle tissue can be reduced by considering variable wet: dry weight ratios. *Mar. Pollut. Bull.* 123 (1–2), 279–285. <https://doi.org/10.1016/j.marpollbul.2017.08.046>.
- Dural, M., Göksu, M.Z.L., Özak, A.A., 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. *Food Chem.* 102 (1), 415–421. <https://doi.org/10.1016/j.foodchem.2006.03.001>.
- European Commission Regulation (EC), 2001. EC No 466/2001 setting maximum levels for certain contaminants in foodstuffs. <https://eur-lex.europa.eu/legal-content/ES/ALL/?uri=celex%3A32001R0466>. (Accessed 17 February 2022).
- European Commission Regulation (EC), 2006. EC No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32006R1881>. (Accessed 17 February 2022).
- European Commission Regulation (EC), 2008. EC No 629/2008 amending Regulation (EC) No 629/2008 as regards maximum levels of lead in foodstuffs. <https://eur-lex.europa.eu/legal-content/ES/ALL/?uri=CELEX%3A32008R0629>. (Accessed 17 February 2022).
- European Commission Regulation (EC), 2014. EC No 488/2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in foodstuffs. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32014R0488>. (Accessed 17 February 2022).
- European Commission Regulation (EC), 2015a. EC No 1005/2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in foodstuffs. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2015.161.01.0014.01. ENG. (Accessed 17 February 2022).
- European Commission Regulation (EC), 2015. EC No 1006/2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs. <https://eur-lex.europa.eu/legal-content/ES/ALL/?uri=CELEX:32015R1005>. (Accessed 17 February 2022).
- George, D.A., Hill, P.S., Milligan, T.G., 2007. Flocculation, heavy metals (Cu, Pb, Zn) and the sand–mud transition on the Adriatic continental shelf, Italy. *Cont. Shelf Res.* 27 (3–4), 475–488.
- Ghosh, M., Mahfouz, C., Chekri, R., Khalaf, G., Guérin, T., Jitaru, P., Amara, R., 2020. Seasonal and spatial variability of trace metals in livers and muscles of three fish species from the eastern Mediterranean. *Environ. Sci. Pollut. Res.* 27, 12428–12438.
- González-Ortegón, E., Laiz, I., Sánchez-Quiles, D., Cabello-García, A., Tovar-Sánchez, A., 2019. Trace metal characterization and fluxes from the Guadiana, tinto-odiell and Guadalquivir estuaries to the Gulf of Cadiz. *Sci. Total Environ.* 650, 2454–2466. <https://doi.org/10.1016/j.scitotenv.2018.09.290>.
- Government of Canada, 2020. List of contaminants and other adulterating substances in food. Part 2. <https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/chemical-contaminants/contaminants-adulterating-substances-foods.html>. (Accessed 23 February 2022).
- Guérin, T., Chekri, R., Vastel, C., Sirot, V., Volatier, J.L., Leblanc, J.C., Noël, L., 2011. Determination of 20 trace elements in fish and other seafood from the french market. *Food Chem.* 127 (3), 934–942. <https://doi.org/10.1016/j.foodchem.2011.01.061>.
- Guerra-García, J.M., Navarro-Barranco, C., Martínez-Laiz, G., Moreira, J., Giráldez, I., Morales, E., Fernández-Romero, A., Florido, M., Ros, M., 2011. Assessing environmental pollution levels in marinas. *Sci. Total Environ.* 762, 144169.
- Hidalgo-Mora, J.J., García-Vigara, A., Sánchez-Sánchez, M.L., García-Pérez, M.A., Tarín, J., Cano, A., 2020. The Mediterranean diet: a historical perspective on food for health. *Maturitas* 132, 65–69.
- Iamiceli, A.L., Ubaldi, A., Lucchetti, D., Brambilla, G., Abate, V., de Felip, E., de Filippis, S.P., Dellatte, E., de Luca, S., Ferri, F., Fochi, I., Fulgenzi, A.R., Iacovella, N., Moret, I., Piazza, R., Roncarati, A., Melotti, P., Fanelli, R., Fattore, E., di Domenico, A., Miniero, R., 2015. Metals in Mediterranean aquatic species. *Mar. Pollut. Bull.* 94, 278–283. <https://doi.org/10.1016/j.marpollbul.2015.02.034>.
- Joyeux, J.C., Campanha Filho, E.A., Jesus, H.C.D., 2004. Trace metal contamination in estuarine fishes from Vitória Bay, ES, Brazil. *Braz. Arch. Biol. Technol.* 47 (5), 765–774.
- Kalantzi, I., Pergantis, S.A., Black, K.D., Shimmield, T.M., Papageorgiou, N., Tsapakis, M., Karakassis, I., 2016. Metals in tissues of seabass and seabream reared in

- sites with oxic and anoxic substrata and risk assessment for consumers. *Food Chem.* 194, 659–670. <https://doi.org/10.1016/j.foodchem.2015.08.072>.
- Kandyliari, A., Karavoltos, S., Sakellari, A., Anastasiadis, P., Asderis, M., Papandroulakis, N., Kapsofetalou, M., 2021. Trace metals in six fish by-products of two farmed fishes, the gilthead sea bream (*Sparus aurata*) and the meager (*Argyrosomus regius*): interactions with the environment and feed. *Hum. Ecol. Risk Assess.* Int. J. 27 (4), 1126–1146.
- Kontas, A., Alyuruk, H., Bilgin, M., Uluturhan, E., Ünlüoğlu, A., Darılmaz, E., Altay, O., 2022. Metal bioaccumulation and potential health risk assessment in different tissues of three commercial fish species (*Merluccius merluccius*, *Mullus barbatus*, and *Pagellus erythrinus*) from Edremit Bay (Aegean Sea), Turkey. *Biol. Trace Elem. Res.* 200 (2), 868–880.
- Korkmaz, C., Ay, Ö., Çolakfakioğlu, C., Ciciik, B., Erdem, C., 2017. Heavy metal levels in muscle tissues of *Solea solea*, *Mullus barbatus*, and *Sardina pilchardus* marketed for consumption in Mersin, Turkey. *Water Air Soil Pollut.* 228 (8), 1–10.
- Korkmaz, C., Ay, Ö., Ersoyal, Y., Köroğlu, M.A., Erdem, C., 2019. Heavy metal levels in muscle tissues of some fish species caught from north-East Mediterranean: evaluation of their effects on human health. *J. Food Compos. Anal.* 81, 1–9.
- Kosker, A.R., 2020. Metal and fatty acid levels of some commercially important marine species from the northeastern Mediterranean: benefits and health risk estimation. *Environ. Monit. Assess.* 192, 358.
- Kumar, P., Sivaperumal, P., Manigandan, V., Rajaram, R., Hussain, M., 2021. Assessment of potential human health risk due to heavy metal contamination in edible finfish and shellfish collected around Ennore coast, India. *Environ. Sci. Pollut. Res.* 28 (7), 8151–8167.
- Laiz, I., Plecha, S., Teles-Machado, A., González-Ortega, E., Sánchez-Quiles, D., Cobelo-García, A., Roque, D., Peliz, A., Sánchez-Leal, R.F., Tovar-Sánchez, A., 2020. The role of the Gulf of Cadiz circulation in the redistribution of trace metals between the Atlantic Ocean and the Mediterranean Sea. *Sci. Total Environ.* 719, 134964.
- Lounas, R., Kasmi, H., Chermali, S., Amarni, N., Ghebriout, L., Hamdi, B., 2021. Heavy metal concentrations in wild and farmed gilthead sea bream from southern Mediterranean Sea—human health risk assessment. *Environ. Sci. Pollut. Res.* 28, 30732–30742. <https://doi.org/10.1007/s11356-021-12864-3>.
- Lozano, G., Lozano, E., Gutiérrez, A.J., Hardisson, A., Melón, E., 2017. Legislación de la Unión europea sobre metales pesados en alimentos marinos. *Vierae* 45, 381–392.
- Marengo, M., Durieux, E.D., Ternengo, S., Lejeune, P., Degrange, E., Pasqualini, V., Gobert, S., 2018. Comparison of elemental composition in two wild and cultured marine fish and potential risks to human health. *Ecotoxicol. Environ. Saf.* 158, 204–212. <https://doi.org/10.1016/j.ecoenv.2018.04.034>.
- Martín-Vélez, V., Hortas, F., Taggart, M.A., Green, A.J., Óhanlon, N.J., Sánchez, M.I., 2021. Spatial variation and biovectoring of metals in gull faeces. *Ecol. Indic.* 125, 107534.
- Mendil, D., Demirci, Z., Tuzen, M., Soyлак, M., 2010. Seasonal investigation of trace element contents in commercially valuable fish species from the Black sea, Turkey. *Food Chem. Toxicol.* 48 (3), 865–870.
- Merciai, R., Rodríguez-Prieto, C., Torres, J., Casadevall, M., 2018. Bioaccumulation of mercury and other trace elements in bottom-dwelling omnivorous fishes: the case of *Diplodus sargus* (L.) (Osteichthyes: Sparidae). *Mar. Pollut. Bull.* 136, 10–21.
- MFR, 1985. Malaysian Law on Food and Drugs. Malaysia. https://doi.org/10.4194/1303-2712-v13_2_21 (accessed 23 February 2022).
- Micheline, G., Rachida, C., Céline, M., Gaby, K., Rachid, A., Petru, J., 2019. Levels of pb, cd, hg and as in fishery products from the eastern Mediterranean and human health risk assessment due to their consumption. *Int. J. Environ. Res.* 13 (3), 443–455.
- Minganti, V., Drava, G., Pellegrini, R.D., Siccardi, C., 2010. Trace elements in farmed and wild gilthead seabream, *Sparus aurata*. *Mar. Pollut. Bull.* 60, 2022–2025. <https://doi.org/10.1016/j.marpolbul.2010.07.023>.
- Miró, J.M., Megina, C., Donázar-Aramendía, I., Reyes-Martínez, M.J., Sánchez-Moyano, J.E., García-Gómez, J.C., 2020. Environmental factors affecting the nursery function for fish in the main estuaries of the Gulf of Cadiz (south-West Iberian Peninsula). *Sci. Total Environ.* 737, 139614 <https://doi.org/10.1016/j.scitotenv.2020.139614>.
- Muñoz, O., Devesa, V., Suñer, M.A., Vélez, D., Montoro, R., Urieta, I., Jalón, M., 2000. Total and inorganic arsenic in fresh and processed fish products. *J. Agric. Food Chem.* 48, 4369–4376.
- Muñoz-Pérez, J.L., Sánchez-Lamadrid, A., 1994. El medio físico y Biológico en la Bahía de Cádiz: Saco Interior. *Consejería de Agricultura y Pesca, Junta de Andalucía, Sevilla*, 151 pp.
- Nauen, C.E., 1983. Compilation of legal limits for hazardous substances in fish and fishery products. In: *FAO Fisheries Circular (FAO)*. No. 764, 102 pp.
- Olmedo, P., Hernández, A.F., Pla, A., Femia, P., Navas-Acien, A., Gil, F., 2013. Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples. Risk and nutritional assessment and mercury-selenium balance. *Food Chem. Toxicol.* 62, 299–307. <https://doi.org/10.1016/j.fct.2013.08.076>.
- Ordinance, 2008. Ordinance setting maximum levels for certain contaminants in foodstuffs. *Official Gazette* 154, 4198.
- Pérez-López, R., Nieto, J.M., López-Cascajosa, M.J., Díaz-Blanco, M.J., Sarmiento, A.M., Oliveira, V., Sánchez-Rodas, D., 2011. Evaluation of heavy metals and arsenic speciation discharged by the industrial activity on the Tinto-Odiel estuary, SW Spain. *Mar. Pollut. Bull.* 62 (2), 405–411.
- Periañez, R., 2009. Environmental modelling in the Gulf of Cadiz: heavy metal distributions in water and sediments. *Sci. Total Environ.* 407 (10), 3392–3406.
- Renieri, E.A., Safenkova, I.V., Alegakis, A.K., Slutskaya, E.S., Kokaraki, V., Kentouri, M., Dzantiev, B.B., Tsatsakis, A.M., 2019. Cadmium, lead and mercury in muscle tissue of gilthead seabream and seabass: risk evaluation for consumers. *Food Chem. Toxicol.* 124, 439–449. <https://doi.org/10.1016/j.fct.2018.12.020>.
- Rozon-Ramilo, L.D., Dubé, M.G., Squires, A.J., Niyogi, S., 2011. Examining waterborne and dietborne routes of exposure and their contribution to biological response patterns in fathead minnow (*Pimephales promelas*). *Aquat. Toxicol.* 105, 466–481. <https://doi.org/10.1016/j.aquatox.2011.07.006>.
- Sadeghi, P., Loghmani, M., Yousuf, D.J., Abadi, Z.T.R., 2021. Ecological and human health risk assessment of trace element pollution in sediments and five important commercial fishes of the Oman Sea. *Mar. Pollut. Bull.* 173, 112962.
- Sánchez-Lamadrid, A., 2004. Effectiveness of releasing gilthead sea bream (*Sparus aurata*, L.) for stock enhancement in the bay of Cádiz. *Aquaculture* 231 (1–4), 135–148. <https://doi.org/10.1016/j.aquaculture.2003.08.015>.
- Shalini, R., Jeyasekaran, G., Shakila, R.J., Sundhar, S., Arisekar, U., Jawahar, P., Surya, T., 2021. Dietary intake of trace elements from commercially important fish and shellfish of Thoothukudi along the southeast coast of India and implications for human health risk assessment. *Mar. Pollut. Bull.* 173, 113020.
- Türkmen, M., Türkmen, A., Tepe, Y., Töre, Y., Ateş, A., 2009. Determination of metals in fish species from Aegean and Mediterranean seas. *Food Chem.* 113 (1), 233–237.
- Underwood, A.J., 1997. *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, Cambridge.
- Underwood, A.J., Chapman, M.G., Richards, S.A., 2002. *GMAV-5 for Windows. An Analysis of Variance Programme*. Centre for Research on Ecological Impacts of Coastal Cities. Marine Ecology Laboratories, University of Sydney, Australia.
- USEPA, United States Environmental Protection Agency, 2011. *USEPA Regional Screening Level (RSL) summary table: November 2011*. <http://www.epa.gov/regshw/md/risk/human/Index.htm>. (Accessed 23 February 2022).
- Usero, J., González-Regalado, E., Gracia, I., 1996. Trace metals in the bivalve mollusc *Chamelea gallina* from the Atlantic coast of southern Spain. *Mar. Pollut. Bull.* 32, 305–310.
- Usero, J., González-Regalado, E., Gracia, I., 1997. Trace metals in the bivalve molluscs *Ruditapes decussatus* and *Ruditapes philippinarum* from the Atlantic Coast of southern Spain. *Environ. Int.* 23 (3), 291–298.
- Usero, J., Morillo, J., Gracia, I., 2005. Heavy metal concentrations in molluscs from the Atlantic coast of southern Spain. *Chemosphere* 59 (8), 1175–1181. <https://doi.org/10.1016/j.chemosphere.2004.11.089>.
- Vasconcelos, R.P., Reis-Santos, P., Maia, A., Ruano, M., Costa, M.J., Cabral, H.N., 2011. Trace metals (Cu, Zn, Cd and Pb) in juvenile fish from estuarine nurseries along the portuguese coast. *Sci. Mar.* 75 (1), 155–162.
- Vicente-Martorell, J.J., Galindo-Riáño, M.D., García-Vargas, M., Granado-Castro, M.D., 2009. Bioavailability of heavy metals monitoring water, sediments and fish species from a polluted estuary. *J. Hazard. Mater.* 162, 823–836. <https://doi.org/10.1016/j.jhazmat.2008.05.106>.
- WHO, 1989. *Heavy metals environmental aspects*. In: *Environmental Health Criteria*. World Health Organization, Geneva, Switzerland. ISSN 0250-563X.
- Yabanlı, M., Alparlan, Y., 2015. Potential health hazard assessment in terms of some heavy metals determined in demersal fishes caught in eastern Aegean Sea. *Bull. Environ. Contam. Toxicol.* 95 (4), 494–498.
- Yigit, M., Dwyer, R., Celikkol, B., Yilmaz, S., Bulut, M., Buyukates, Y., Kesbic, O.S., Acar, Ü., Ozalp, Maita M., Ergün, S., 2018. Human exposure to trace elements via farmed and cage aggregated wild axillary seabream (*Pagellus acarne*) in a copper alloy cage site in the northern Aegean Sea. *J. Trace Elem. Med. Biol.* 50, 356–361.
- Yilmaz, M., 2020. Effect of cage culture environment on farmed fish in terms of metal accumulation. *Aquac. Res.* 51 (7), 3025–3036.
- Yúfera, M., Arias, A.M., 2010. Traditional polyculture in “Esteros” in the Bay of Cádiz (Spain). Hopes and expectatives for the prevalence of a unique activity in Europe. *Aquac. Europe* 3, 22–25.
- Žvad Rožič, P.Ž., Dolenc, T., Baždarić, B., Karamarko, V., Kniewald, G., Dolenc, M., 2014. Element levels in cultured and wild sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) from the Adriatic Sea and potential risk assessment. *Environ. Geochem. Health* 36 (1), 19–39. <https://doi.org/10.1007/s10653-013-9516-0>.