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Potentially toxic elements in commonly consumed fish species from the western Mediterranean Sea (Almería Bay): Bioaccumulation in liver and muscle tissues in relation to biometric parameters

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ABSTRACT

Marine pollution is one of today's most relevant problems. Public awareness has been raised about the harmful potential of heavy metals (HMs) accumulating in edible fish and possibly ending up in human diet through the food chain. This study aimed to characterize and evaluate As, Cd, Cr, Cu, Ni and Pb contents in four edible fish species from the western Mediterranean Sea. Liver and muscle toxic elements were determined by GF-AAS in *Mullus surmuletus*, *Merluccius merluccius*, *Auxis rochei* and *Scomber japonicus* from Almería Bay (Spain). Muscular composition, biometrics and trophic levels were also determined. The mean PTE concentration levels (mg kg^{-1}, DW) in fish muscle tissue were: As (2.90–53.74), Cd (0.01–0.18), Cr (0.53–2.01), Cu (0.78–6.93), Ni (0.06–0.24), Pb (0.0–0.32). These concentrations did not exceed the maximum limits set by European legislation (Commission Regulation (EC) No. 1881/2006) for the intake of these marine species. Accumulation of toxic elements tends to be seen in the liver (As (7.31–26.77), Cd (0.11–8.59), Cr (0.21–2.94), Cu (2.64–16.90), Ni (0.16–1.03), Pb (0.0–0.99)). As was the element at highest risk in this Mediterranean region, especially due to red mullet values in muscle. The high As contents with living habits as benthic species that feed near the coast. HMs, especially muscle Cd contents, were associated with higher contents of lipids and organic matter, and bigger specimen size (length and weight), while As was linked to higher fish protein content. However, these relationships between potentially toxic elements (PTE) and biometric indices and body composition parameters depend on species. Finally, the THQ indices indicated that eating fish from Almería Bay poses no human health risk despite pollution from the Almería coastline.

1. Introduction

For a considerable time, oceans and seas have been considered a means with a practically unlimited capacity to accumulate pollutants, with no immediate harmful effects for the marine environment or human health. However, we now realize that this is not true. The general public is aware of the harmful potential of some trace elements, commonly known as heavy metals (HMs) and metalloids (Alahabadi and Malvandi, 2018). These potentially toxic elements (PTE) may enter the environment through human activities, like mining and smelting processes (Gutiérrez et al., 2016; Kaitantzian et al., 2013; Odumo et al., 2014), or from fossil combustion (Kelepertzis and Argyraki, 2015; Rodríguez Martín et al., 2013a, 2014). In any case, human activities have substantially raised HMs concentration levels in recent decades (Rodríguez Martín et al., 2015). The pollutants are able to be bioconcentrated in the fish, even at very low concentrations, and most specifically in the liver. In fact the liver has been proposed as a biomarker of HM pollution (Ardeshir et al., 2017). Moreover, muscle is an edible part of fish and the part to which legislation refers. To evaluate the risk posed by metal bioaccumulation and biomagnification, more than monitoring only PTE levels in sediment or water samples must be accomplished to generate sufficient information (Jiang et al., 2018). Hence testing aquatic organisms like fish when monitoring marine pollution is very useful as they accumulate contaminants by direct absorption from water, and indirectly through food chains (Łuczyńska et al., 2018). In addition, given the long life span of some fish species, detecting the long-term effects of PTE on aquatic ecosystems is possible (Jiang et al., 2018). PTE can bioaccumulate in vital organs of fish, such as liver, kidneys and gills, several hundred times higher than can be detected in the surrounding environment (Chua et al., 2018). Bioaccumulation of PTE in fish tissues can be affected by factors like feeding habits, age, gender, genetic tendencies, and different swimming behaviors of fish species

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(Anandkumar et al., 2018). The consideration of Mediterranean Sea pollution is associated with semi-enclosed sea intensification of contamination effects (Aston and Fowler, 1985). The origin of PTE on the Almería coastline is related with various sources of pollution, of which the following stand out: atmospheric deposition associated with emissions released to the atmosphere, like Cu related with the local cement-making industry, As, Cd and Ni deriving from energy production, or Pb and Zn related with the area's metal industry. Spillages of mainly Pb and As in Almería Bay waters is also a major source of pollution, and are associated with abusive fertilizer use in the greenhouse crops located all along the Almería coastline (Kelepertzis, 2014; Rodríguez Martín et al., 2013b). There are also spillages of wastewater into the bay, which considerably contribute Cd and Cu (Casadevall et al., 2016). Coastal ecosystems are also conditioned by a growing anthropogenic impact.

Eating fish is a recognized healthy habit that forms part of the Mediterranean diet, but can also be the main way by which PTE enter humans (WHO, 2017). The health benefits that fish intake can imply for people lie in the proteins, minerals and vitamins they contain, as well as their unsaturated essential fatty acid (UFAs) contents, such as omega-3 UFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The Mediterranean Sea has been exploited since ancient times, and currently supplies fish to >480 million people (EEA, 2015). Spain is the third ranked country in the world for eating fish. Fish accumulate PTE from the sea, which depends not only on fish species, but also on environmental factors, and on the concentration levels and chemical forms in which elements are present (Perugini et al., 2014). Red mullet (*Mullus surmuletus*), European hake (*Merluccius merluccius*), bullet tuna (*Auxis rochei*) and Pacific Chub Mackerel (*Scomber japonicus*) are well-known and widely exploited species in the Mediterranean basin, and feature among the highly commercialized species on the Almería Fish Market, where *Scomber japonicus* is the second most caught species (2015–2017, IDAPES). Monitoring metal concentrations in different commercial fish is, therefore, important to ensure compliance with food safety regulations and consequent consumer protection (Bosch et al., 2016). Recent studies have shown that the concentration levels of HMs in fish in the Mediterranean Sea not only vary according to species, but are also strongly influenced by the area where fish are caught (Copat et al., 2018; Cresson et al., 2015; Ouali et al., 2018).

This study aimed to explore the potential toxicity risk of PTE in the four most consumed species on the Andalusian coast (S Spain) to: 1) determine the concentration levels of As, Cd, Cr, Cu, Ni and Pb contents in liver and muscle tissues; 2) study the relationship linking trace element contents and fish biometric composition; 3) evaluate the risk for the local population of developing health problems and diseases from eating these fish through Target Hazard Quotient (THQ) indices.

2. Material and methods

2.1. Fish sampling and biometric parameters

The coast of Almeria is an important tourist area of southern Spain. It has a subtropical arid climate with scarce, but heavy, rains capable of mobilizing sediments. Almería has very few rivers that are reduced to “ramblas”, where the water flow usually disappears underground for most of the year to appear in heavy rain episodes. The urban pressure on the coast, as well as intensive agriculture, is a source of impact on the coast. In the East of the province there are a power plant, two coal groups, a desalination plant, a biodiesel factory; a cement plant and a sewage treatment plant release considerable atmospheric emissions and discharges to sea. The mining activity of recent centuries is also a source of pollution in Almeria, specifically, gold mining in Rodalquilar, and iron and lead mining in Las Menas de Seron area. Surface currents are predominantly from west to east, but bottom currents come from the east, from the Levante, as does the prevailing wind. Fig. 1 illustrates the areas where the four studied fish species (Red mullet, European hake, Bullet tuna and The Pacific Chub Mackerel) are caught. The morphological characteristics in re-

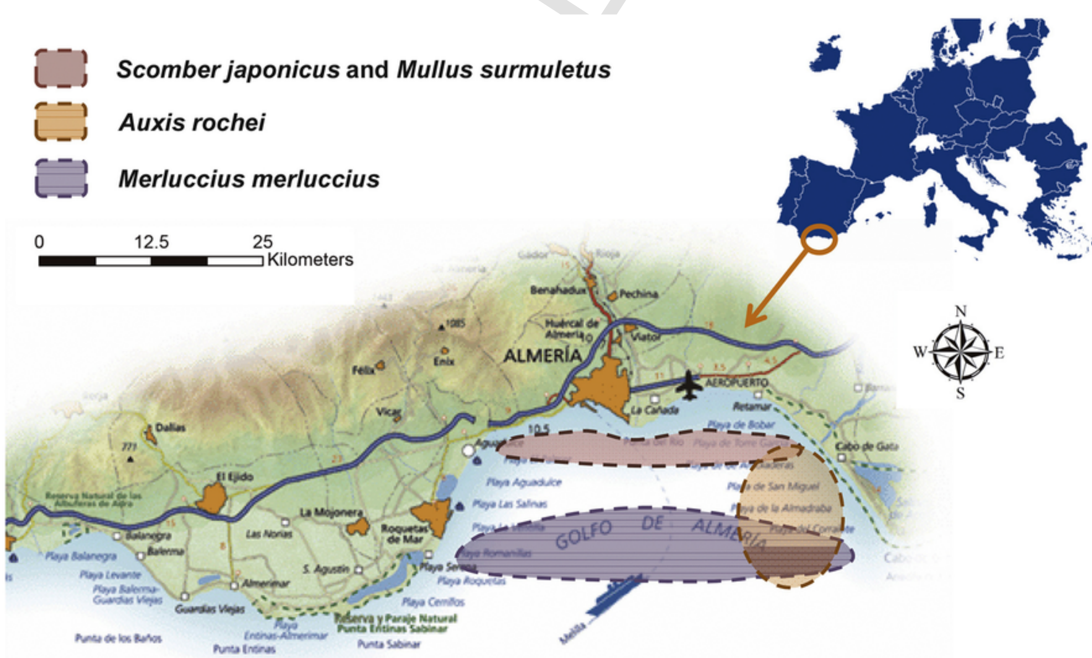

Fig. 1. Fishing areas for four species in Almeria Bay, the Western Mediterranean Sea.

lation to the biometric indices and body composition are provided in Table 1. A description of the areas where they are caught and the methodology followed to estimate these parameters are all provided in detail in Sánchez-Muros et al. (2018). It is noteworthy that these four species are widely distributed in the Mediterranean Sea and are very important for the commercial fishing sector in Almería (Sánchez-Muros et al., 2018). Red mullet (*M. surmuletus*) is a teleost fish of the Order Perciformes with a wide distribution. It can be found in the Mediterranean and the NE Atlantic, chiefly in very shallow continental shelf areas (0–30 m), among sand, mud and rocks Despite it having a vast coastal distribution, it inhabits depths up to 400 m, where other red mullets (*M. barbatus*) do not inhabit (Lombarte et al., 2000). This benthic carnivore feeds largely on small invertebrates like the polychaetes, mollusks and crustaceans that live inside or on the marine substrate. European hake (*M. merluccius*) belongs to the order Gadiformes and is a demersal teleost species. Its distribution throughout the Mediterranean and in the Atlantic NE is wide. It inhabits depths between 50 m and 750 m, but its most widespread is deep in the continental shelf zone (150–300 m). The opportunistic *M. merluccius* carnivore feeds on adult stages of teleost fish, euphausiidae and large decapods, including myctophids, and feeds after daily vertical migrations (Cartes et al., 2004). The small Bullet tuna (*A. rochei*) of the Order Perciformes is distributed worldwide in tropical and temperate waters, including the Mediterranean. *A. rochei* feeds largely on zooplankton organisms. Its main preference is planktonic crustaceans like eufasiaceans and amphipods, and fish larvae, small cephalopods, and adult fish of larger bullet tuna size (>35 cm) (Mostarda et al., 2007). The Pacific Chub Mackerel (*S. japonicus*), a small pelagic, is of the Order Perciformes. This neritic pelagic species is distributed chiefly at depths of 50–200 m in tropical and temperate waters in the southern and northern hemispheres, including the Mediterranean. *S. japonicus* feeds mainly on different fish stages, including annelids, decapods and cannibalism (Castro, 1993).

2.2. Potentially toxic elements (PTE) and analytical methods

According to The World Health Organization (WHO), PTE has attracted much attention for years given the persistence and toxicity of these chemical species since they can pose very health problems in many parts of the world. Hg, As, Pb and Cd are included as the 10 chemicals of major public health concern (WHO, 2019. www.who.int). Mercury is toxic to human health that bioaccumulates in fish and shellfish. The mercury contents in fish from Almería bay has been studied recently by Sánchez-Muros et al. (2018). Some metals, as copper, are an essential nutrient for animal and human, but the most heavy metals have deleterious effect on the human health. The Arsenic is a metalloid element, which forms a number of poisonous compounds and it is prominently toxic and carcinogenic. The lead affects multiple body systems, including the neurologic, hematologic, gastrointestinal, cardiovascular, and renal systems. Cadmium is classified as a human carcinogen. The chrome has toxic and carcinogenic effects and the chronic Cu toxicity can result in liver disease and severe neurological defects (WHO, 2019. www.who.int).

The fish samples (liver and muscle tissues) were digested by aqua regia technique using microwave acid digestion (ETHOS SEL Model Milestone, Monroe, CT, USA). The concentrations of PTE (As, Cd, Cr, Cu, Ni and Pb) in tissue extracts were determined by Atomic Absorption Spectrometry (PerkinElmer, Shelton, CT, USA 06484-4794) using Graphite Furnace Atomic Absorption Spectrophotometry (GF-AAS) equipment. The limits of detection (LoDs) of GF-AAS are 0.05 mg kg^{-1} for As, 0.002 mg kg^{-1} for Cd, 0.2 mg kg^{-1} for Cr, 0.014 mg kg^{-1} for Cu, 0.07 mg kg^{-1} for Ni, and 0.05 mg kg^{-1} for Pb. To check the accuracy and precision of the measurements and to validate the applied methods for the PTE analysis were performed using two certified reference materials, tuna fish (CRM 463) and ERMI-CE278 (muscle tissue) from European Reference Materials. Recoveries for fish samples were good with an average of 98.6% for Cd, 101% for Cr, 95.7%, 94.4% for Cu, 95.7% for Ni, 98.24% for Pb, 103.6% for As. Two replicates were analyzed per sample and the concentrations were present as mg kg^{-1} dry matter (DM).

2.3. Target Hazard Quotient

Target Hazard Quotient (THQ) is frequently used to calculate the risk of exposure to toxins *via* food in humans (Copat et al., 2013, 2018; Heshmati et al., 2017; Yabanli and Alparslan, 2015). THQ represents the ratio between exposure to a toxin and the reference dose. Even though this index does not provide a quantitative estimation of adverse effects on health (Yi et al., 2011), it provides an estimate of the risk of non carcinogenic effects on the health of the population exposed to a given pollutant.

THQ is calculated for each element considered to be a pollutant by bearing in mind the adults who include these species in their diet and eat them. This calculation is done using the equation below:

$$THQ = \frac{(EF \times ED \times IR \times \mathrm{C})}{(RfD \times BW \times AT)} \tag{1}$$

where EF represents exposure frequency; ED is exposure duration; IR is the ingestion rate that indicates the amount of fish eaten by the Andalusian population which, in this case, we consider to be a mean intake of 9.4 kg yr^{-1} (25.8 g day^{-1}) (MAGRAMA, 2017; Martín Cerdeño, 2017); C is the concentration of the polluting element in the tissue of the studied fish species, expressed as wet weight (mg kg^{-1} wet weight); RfD is the oral reference dose (μg g^{-1} day^{-1}), set by the Environmental Protection Agency of the USA (US-EPA) (USEPA,

Table 1
The capture area and biometric fish parameter for each fish species.

	A. rochei	*M. merluccius*	*S. japonicus*	*M. surmuletus*
Capture area	El Cantillo	Medio Canto	La Terralia	La Terralia
Wt (g)	955.80 ± 0.66	199.20 ± 15.97	183.00 ± 0.75	47.60 ± 4.43
Lt (cm)	37.42 ± 0.66	31.88 ± 1.04	24.88 ± 0.75	15.98 ± 0.31
Age/T. level	2.5/4.3	1/3.44	2/3.4	1/2.44
Moisture (%)	65.03 ± 2.16	80.86 ± 1.07	72.00 ± 1.79	[a]
lipids (%DM)	23.70 ± 4.90	5.41 ± 2.20	11.96 ± 6.45	[a]
Protein (%DM)	66.32 ± 5.34	84.21 ± 2.45	73.88 ± 9.49	[a]
O.M. (%DM)	95.36 ± 0.55	94.68 ± 0.67	95.53 ± 0.27	[a]
NFE (% DM)	5.34 ± 3.79	5.05 ± 3.14	9.68 ± 6.95	[a]

[a] For *M. surmuletus* the data were not determined due to lack of sample quantity (Sánchez-Muros et al., 2018).

2000); BW is the mean body weight of adults (in kg); AT represents the averaging time, which equals (EF × LT), where LT represents the individuals' mean lifetime. The THQ value for the As was calculated assuming that the inorganic As represents 5% of the total concentration (Copat et al., 2018). Following the recommendations of Copat et al. (2018), we took an EF of 365 days yr^{-1}, an ED of 26 yr and a mean BW of 70 kg in adults. The arithmetic sum of the different THQs provides the ΣTHQ by considering the additive effects of exposure to several contaminating elements (Chien et al., 2002). If the obtained ΣTHQ value equals or exceeds 1, no risk is posed for consumer health. If the THQ value exceeds 1, the intake of certain species should be restricted.

2.4. Statistical analysis

Classic statistics (mean, median, coefficient of variation, standard deviation, *etc.*) was carried out to evaluate the contents of PTE (As, Cd, Cr, Cu, Ni and Pb) in both muscle and the liver. ANOVA was used to explore the effects of fish species and tissues in the evaluated PTE contents. The multi-way analysis of variance model included the main effects (tissues and species) and the interactions between the main effects (tissue × species). However, these classical statistical approaches ignore relationships between groups of variables. One approach to study the relationship between the two sets of variables is to use the canonical correlation analysis (CCorA), which describes the relationship between PTE in muscle and liver tissues and the fish biometrics (size and analytical body composition). The CCorA is a multivariate analysis of correlation where one set of variables is not necessarily independent and the other is dependent, although that may potentially be the approach. This method is used considerably in ecology and, unlike redundancy analysis (RDA), this method is symmetrical. Let Y1 and Y2 (PTE contents in muscle and liver tissues, and response variables (Y2) based on the fish biometric indices and body composition parameters), with variables p and q, respectively, we obtain:

$$\rho(\mathrm{i}) = cor(\mathrm{Y1a(i)}, \mathrm{Y2b(i)}) = \frac{\mathrm{cov(Y1a(i), Y2b(i))}}{\mathrm{var(Y1a(i)) \cdot var(Y2b(i))}} \tag{2}$$

The CCorA provide two vectors, a(i) and b(i), which are maximized. Constraints must be introduced so that the solution for a(i) and b(i) is unique as the ultimate intention is to maximize the covariance between Y1a(i) and Y2b(i) and to minimize their respective variance (Jobson, 1992; Takoutsing et al., 2018). The CCorA results are presented as graphical bi-plot scaling to evaluate the relationship between biological variability and sensitivity to chemical disturbance (Campos-Herrera et al., 2016; Losi et al., 2013; Takoutsing et al., 2017). All the statistical analyses were carried out by the XLSTAT (Addinsoft Version, 2012.2.02) package for Windows.

3. Results and discussion

The summaries of the descriptive statistics of the biometric indices and body composition are presented in Table 1. The weight and total length of the fish species are associated with the age and trophic levels of specimens. The muscular composition study (lipids, protein, nitrogen-free extracts, *etc.*) was related with the interspecies differences between species, and fell within the range described for these species (FAO, 2017). These fish parameters are described in detail in the study of Sánchez-Muros et al. (2018).

3.1. Concentration levels of PTE in fish

S. japonicas (chuck mackerel) is one of the most important fishing resources in the world. According to our study, this species had the highest Cu concentration levels in both muscle (6.93 mg kg^{-1} DM) and the liver (16.9 mg kg^{-1} DM), and also of Ni and Pb in the liver (1.03 mg kg^{-1} DM, 0.99 mg kg^{-1} DM, respectively) (Table 2). Nonetheless, the concentration of these PTE in chuck mackerel from Almería Bay was below that observed in other Mediterranean regions, like the Aegean Sea (Türkmen et al., 2009) or the Croatian coast (Bilandžić et al., 2018) where, for instance, the concentration of As or Cu was roughly 2- or 3-fold higher than those obtained in the present study. *A. rochei* (bullet tuna) was the species with the highest Cd contents in both muscle (0.18 mg kg^{-1} DM) and the liver (8.59 mg kg^{-1} DM) (Table 2), which contrasts with the concentrations found for this species in other Mediterranean regions (southern Adriatic, southern Tyrrhenian or the Ionian Sea), where the Cd concentration in the muscle of this species was below the LoD (0.01 mg kg^{-1}) (Iamiceli et al., 2015). *M. merluccius* (European hake) is one of the most important species found in the Mediterranean Sea (Chapela et al., 2007). The HMs contents obtained herein (see Table 2) fell within the ranges observed in other Mediterranean regions like the Croatian Sea (Bilandžić et al., 2018) or the Italian coast (Pastorelli et al., 2012). Finally, *M. surmuletus* (red mullet) is one of the species to be most widely distributed around the Mediterranean coast. It presented considerably higher As levels in muscle (53.74 mg kg-1 DM), which were even higher than those found in the liver (26.77 mg kg^{-1} DM in the liver) (Table 2). Unlike the other studied species, Red mullet tends to accumulate As in muscle tissue (Dorta et al., 2015; Falcó et al., 2006) and a high As concentration in muscle appears to be a typical characteristic of this species. Copat et al. (2013) also found that the As concentration in Red mullet muscle was significantly higher than in the other species they investigated in the eastern Mediterranean. These authors concluded that benthonic species from sandy seabeds accumulate large quantities of As. In the Black Sea (Durmuş et al., 2018), *M. surmuletus* has also been found to present high As levels, and to show Cd and Pb pollution. Nevertheless, the fish that feed close to the coastline or on the seabed tend to accumulate more HMs (Casadevall et al., 2016).

Fig. 2 clearly shows (demonstrates) differential performance for accumulating toxic elements in fish tissues. Despite all the fish species being caught in the same fishing area and at the same time of year, the accumulation of PTE in fish is different for elements as well as fish species studied. Significant statistical differences were found by species for As, Cu, Ni and Pb contents (Table 3). Generally speaking, the PTE content was higher in the liver, and concentrations were sometimes around 50-fold higher to those in muscle tissue; *e.g.*, Cd accumulation in *A. rochei* (Fig. 2). Most marine species tend to accumulate PTE in their livers (Bachouche et al., 2017; Debipersadh et al., 2018; Ersoy and Çelik, 2010), particularly Cd (Castro-González and Méndez-Armenta, 2008). As previously mentioned however, the As and Cr contents in *M. surmuletus* were concentrated more in muscle, with statistically significant differences (Table 3). As is one of the most widespread PTE present in aquatic ecosystems (Kumari et al., 2017). Nonetheless, the As encountered in our fish species investigated as an organic form and does not pose a severe health risk (Taylor et al., 2017), which has been verified with different teleost fish in the East Alborán Sea (the same area as in this study) (Casadevall et al., 2016).

The pollution in the area where the fish species are caught is one of the main factors that most conditions PTE concentration levels in

Table 2
PTE concentrations in the muscle and liver of four fish species. Mean values ± STD in mg kg^{-1} (DM).

Species	Trace elements in muscle						Trace elements in liver					
	As	Cd	Cr	Cu	Ni	Pb	As	Cd	Cr	Cu	Ni	Pb
S. japonicus	2.90 ± 0.75	0.01 ± 0.01	0.53 ± 0.65	6.93 ± 6.58	0.24 ± 0.26	0.20 ± 0.05	16.81 ± 4.95	7.86 ± 3.29	0.59 ± 0.40	16.90 ± 3.21	1.03 ± 0.48	0.99 ± 0.34
A. rochei	2.78 ± 1.24	0.18 ± 0.22	2.01 ± 2.07	5.24 ± 1.32	0.32 ± 0.19	0.45 ± 0.69	7.31 ± 2.69	8.59 ± 12.67	0.89 ± 0.60	14.43 ± 3.62	0.49 ± 0.23	<LOD
M. merluccius	9.60 ± 1.33	0.01 ± 0.01	1.98 ± 0.89	2.30 ± 0.79	0.15 ± 0.09	<LOD	7.80 ± 9.62	0.11 ± 0.10	2.94 ± 2.05	7.41 ± 1.95	0.16 ± 0.19	0.11 ± 0.07
M. surmuletus	53.74 ± 18.61	0.02 ± 0.02	3.44 ± 1.54	0.78 ± 0.45	0.06 ± 0.04	<LOD	26.77 ± 11.49	0.35 ± 0.10	0.21 ± 0.09	2.64 ± 1.85	0.46 ± 0.24	<LOD

<LOD below detection limit.

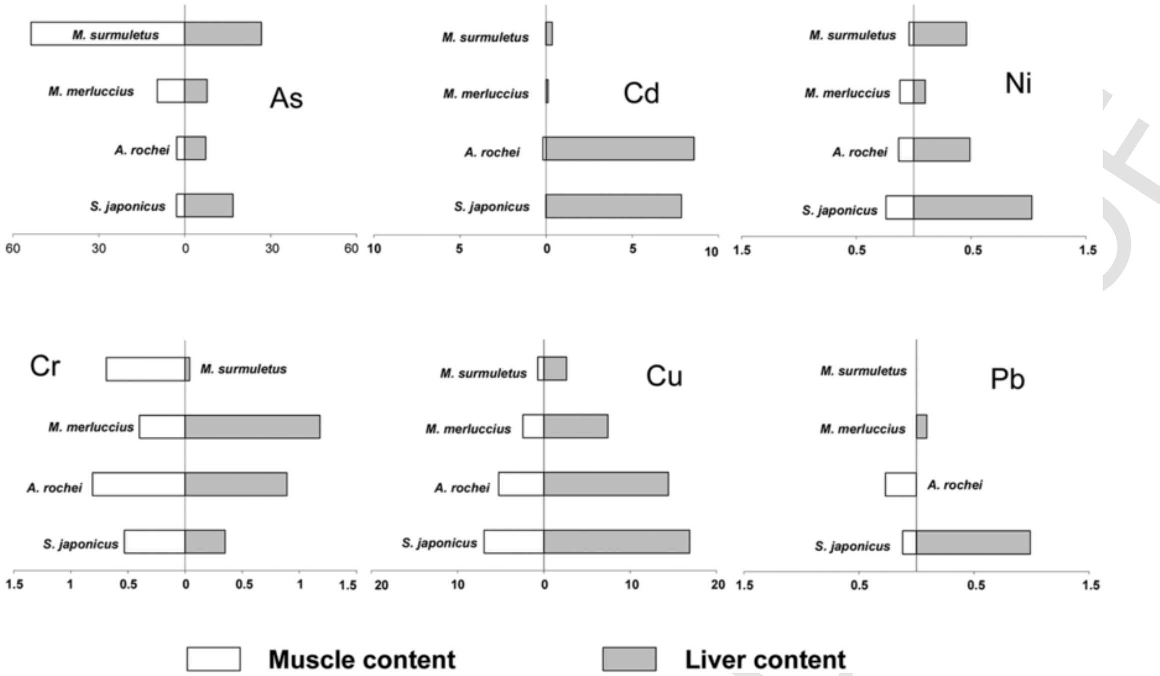

Fig. 2. Trace element concentrations in muscle *versus* liver in the four fish species.

Table 3
Statistic report on the multi-way analysis of variance of the fish species specimens and the sampling tissues of fish (main effects) with the interaction of the main effects.

Source	Df	As	Cd	Cr	Cu	Ni	Pb
Fish species	3	*P* < 0.01	n.s	n.s	*P* < 0.01	P < 0.01	P < 0.01
Tissue	1	n.s	P < 0.01	n.s	P < 0.01	P < 0.01	*p* < 0.05
Interaction tissue × Species	3	P < 0.01	n.s	n.s	n.s	p < 0.05	P < 0.01

Significant at 95% ($p < 0.05$) and 99% ($p < 0.01$). n.s: not significantly different.

fish (Bae and Lim, 2012; Debipersadh et al., 2018; Türkmen et al., 2009). All the fish in our study were caught in the same area (Fig. 1) and variation could be attributed only to species. Nevertheless, accumulating pollutants differ according to distinct tissues. Hence this variability is related mainly with the different ways by which pollutants accumulate in each species (Canli and Atli, 2003). This aspect corroborated significantly with the tissue/species interaction for As, Ni and Pb (Table 3), which indicates the distinct performance attributed to species. For example, *M. surmuletus* presented higher As concentrations than pelagic fish (Iamiceli et al., 2015). These high As levels in both muscle and liver may be related to the fact that As is present naturally in rocks and would, therefore, affect mainly coastal ecosystems rather than pelagic ones. Indeed other studies have also demonstrated high As concentrations in this part of the Mediterranean Sea (Casadevall et al., 2016; Núñez et al., 2016). As synthesis, it was noteworthy that neither the Pb levels or Cd exceeded the corresponding maximum levels in wet weight set out by Commission Regulation (EC) No. 1881/2006 that setting maximum levels only for certain contaminants in foodstuffs (for Pb 0.3 $mg\,kg^{-1}$ and for Cadmium 0.05 $mg\,kg^{-1}$ in general muscle meat of fish (0.1 $mg\,kg^{-1}$ in *Scomber* sp. and 0.15 $mg\,kg^{-1}$ in *Auxis* sp.)).

3.2. Assessing links between trace elements and fish characteristics

The tendency of certain marine species to accumulate PTE is often associated with either the species biometric parameters or its nutritional levels, and reflects its feeding habits (Sofoulaki et al., 2018). From this point of view, and regardless of the species being studied, we analyzed the PTE relation in muscle and liver tissues *versus* the fish biometric indices and body composition parameters. Canonical correlation analyses (CCorA) were used to study these relationships. The CCorA results were plotted separately for both tissues: muscle (Fig. 3a) and the liver (Fig. 3b). CCorA evidenced an association between the lipids content of the studied specimens and their length (Lpa) and total weight (tW). As expected, a bigger sized fish is associated with a higher content of fats. However, a higher content of proteins is related with a higher trophic level and negatively related with fish size (Fig. 3). In general terms, those fish at a higher level in the food chain tend to accumulate more pollutants (Debipersadh et al., 2018).

The first analysis (Fig. 3a) to relate the PTE concentration levels in muscle accounted for 69% of total variance. What we firstly observed was that HMs (Cd, Cr, Cu, Pb) obtained negative values in F1, while the As ones were isolated in the figure and took a high positive value on this axis. Mainly the contents of Cd, but also of the other PTE in muscle, were associated with higher contents of lipids and organic matter and a bigger specimen size, whereas the As that we found in muscle was associated with a higher fish proteins content. The distinct performance observed for As compared to the other assessed PTE was due to the high concentration levels found in *M. surmuletus* muscle. In relation to As, this performance has also been observed in scorpaenid fish in Marseille Bay (Ourgaud et al., 2018).

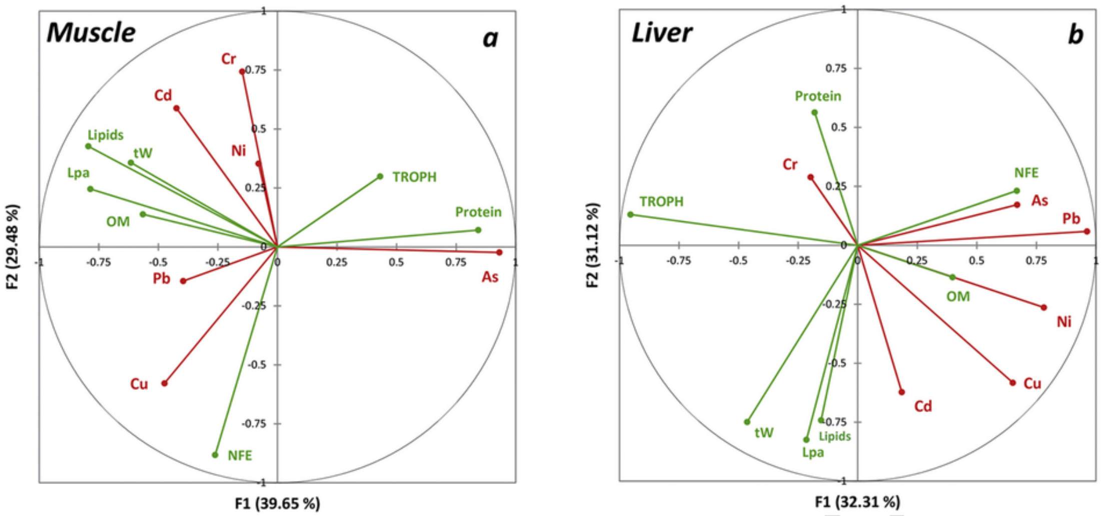

Fig. 3. Ordination diagram based on the CCorA of PTE in muscle and liver tissues *versus* fish biometric indices and body composition parameters.

As mentioned in the previous section, the distribution of PTEs contents in various fish tissues is associated mainly with the species under study. For sardine and anchovy, a higher PTE concentration in muscle tissue is associated with lower lipids contents (Sofoulaki et al., 2018). In sea bass and sea bream, a higher PTE content is associated with a higher proteins content (Kalantzi et al., 2016).

The association of elements in the liver is provided in Fig. 3b. Unlike muscle, As was found among the other PTE (chiefly Pb, Ni and Cu), which demonstrates common performance for the set of the evaluated pollutants in liver. Generally speaking, the liver is the organ in which aquatic species accumulate HMs (Endo et al., 2002), so all these elements are expected to be related to one another. Moreover, we did not find any relation between fish size (length and weight) and greater accumulation or presence of toxic elements in the liver as only Cd was apparently related with specimen size (Fig. 3b). Nonetheless, what this analysis evidenced was that the liver HMs contents were inversely related with the species' trophic level, and the only nutritional parameter that seemed to be positively related with HMs contents was NFE (nitrogen-free extracts). In short, the distribution of PTE in the liver and muscle differs. This is not only due to the biometric parameters, but also to the species' typical characteristics. This fact could be related with the species' different diets (Signa et al., 2017).

3.3. Target Hazard Quotient

The benefits of eating fish in human diet have long since been stressed. In recent years however, given the social alert about our seas being polluted, the potential health risk owing to PTE content in our seas must be taken into account. From this viewpoint, THQ is an index that is normally employed to calculate the risk that foods pose (Copat et al., 2018). It makes estimates based on the concentrations found in muscle (the edible fish part). The THQ values of several elements and the ΣTHQ estimated for all the fish species under study are provided in Table 4. As had the highest THQ value for all the species (ranging from 0.13 in *M. surmuletus* to 0.011 in *S. japonicus*). Copat et al. (2018) also revealed a higher exposure risk for PTE. Other studies conducted with fish species caught in the north eastern Mediterranean Sea indicate THQ values up to 1.6 for As (Marengo et al., 2018), which exceeds this indicator's limit, and they consider the potential intake hazard. What this evidences is that As is the element that poses the highest health risk in the Mediterranean. In fact the WHO (World Health Organization) indicates that As is one of the most hazardous elements for human diet. One study conducted by the European Food Safety Agency (European-Food-Safety, 2014) about assessing As levels in foods and the extent to which the European population might be affected, stresses the importance of eating fish for As exposure, and it is a particular concern for children (Castro-González and Méndez-Armenta, 2008). Nevertheless, only 1–5% of total As is found in inorganic form, the most dangerous for health (Krishnakumar et al., 2016; Wang et al., 2014); normally arsenic is found in fish such as arsenobetaine, which it is non toxic (Juncos et al., 2019; Zhang et al., 2018). In addition, our results indicate that both each element's THQ value and the ΣTHQ value for the studied species were well below 1 (Table 4). The ΣTHQ values revealed that *M. surmuletus* was the species with the highest values (0.132), followed by *A. rochei* (0.035), *M. merluccius* (0.027), and finally by *S. japonicus* (0.020), which all indicate that eating these species caught in Almería Bay poses no health risk. Our results agree with those reported in other studies, which have evaluated the same species in other areas, such as chub mackerel in Atlantic coasts (Vieira et al., 2011) which, despite presenting higher THQ values than for our As, Cd and Pb results, did not exceed a THQ value of 1; or in Croatia where eating hake and chub mackerel did not pose any health risk (Bilandžić et al., 2018). However, it is necessary to consider that constant spillages of pollutants to the sea, particularly those from the intensive farming practiced in the area, along with growing local indus-

Table 4
The estimated target hazard (THQ) coefficients of the elements evaluated in the muscle of the fish species in Almería Bay. Inorganic As was assumed to be 3% of the total concentrations. Cr was assumed to be 100% of the total concentration (Copat et al., 2018).

THQ	*S. japonicus*	*A. rochei*	*M. merluccius*	*M. surmuletus*
As	11.00×10^{-3}	13.00×10^{-3}	25.00×10^{-3}	130.00×10^{-3}
Cd	0.56×10^{-3}	8.90×10^{-3}	0.24×10^{-3}	0.54×10^{-3}
Cr	0.014×10^{-3}	0.67×10^{-3}	0.35×10^{-3}	0.56×10^{-3}
Cu	6.60×10^{-3}	6.30×10^{-3}	1.50×10^{-3}	0.47×10^{-3}
Ni	0.047×10^{-3}	0.77×10^{-3}	0.20×10^{-3}	0.071×10^{-3}
Pb	1.90×10^{-3}	3.50×10^{-3}	0.33×10^{-3}	0.30×10^{-3}
ΣTHQ	0.020	0.033	0.028	0.132

Pb THQ for *M. merluccius* and *M. surmuletus* were estimated with the LOD value.

trial activity, might degrade the marine environment and end up affecting all pollutant levels in fish.

4. Conclusions

According to the present research results, the concentration levels of PTE examined in the muscle tissue of the four studied species evaluated in Almería Bay do not exceed the maximum limits set out by European legislation (Commission Regulation (EC) No. 1881/2006) on eating these marine species. Moreover, the low THQ indices that we obtained indicate that eating fish from this fish-catching area poses no health risk. Notwithstanding, As is the element with the highest possible risk in this Mediterranean Region, especially in relation to red mullet intake which, unlike the other assessed species, tends to accumulate As in muscle, which reflects its different lifestyle and feeding habits. The fish that feed close to the coast or on the seabed tend to accumulate more pollutants. The tendency displayed by the European hake, bullet tuna and Pacific Chub Mackerel is to accumulate PTE in the liver, which is related with higher contents of lipids and organic matter and a bigger specimen size. Although eating these species presently caught in the Gulf of Almería entails no risk, we cannot rule out that this may change in the future. Increased industrial activity on the Almería coast, abuse of fertilizers and phytosanitary products in greenhouse agriculture in the area, the gradual increase in tourism in this region, along with wastewater spillages in Almería Bay, may all increase the content of both As and HMs in the Almería Bay coastal waters, which could, in turn, degrade the marine habitat and affect these species' food.

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