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Concentrations of lead, cadmium and mercury in sardines, *Sardina pilchardus* (Walbaum, 1792) from the Algerian coast and health risks for consumers

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ABSTRACT

In order to assess the health risks associated with the consumption of *Sardina pilchardus* (Walbaum, 1792) and to study the spatiotemporal dynamics of three potentially toxic metals, lead (Pb), cadmium (Cd) and mercury (Hg) concentrations were measured in the muscle and the liver of 872 specimens of sardine sampled between October 2017 and September 2018 along the Algerian coast at 3 sites (Algiers, Dellys and Bejaia) subject to high pollution pressure. The concentrations of the three elements were higher in the liver than in the muscle of *S. pilchardus*. In Algiers, the average Pb concentrations in the liver were high (0.41 ± 0.17 μg/g wet weight) and similar to those noted in specimens from Bejaia. The highest average Cd liver concentrations were also recorded in Algiers specimens (1.53 ± 2.86 μg/g ww), as well as the highest average muscle concentrations of Pb and Cd (0.25 ± 0.29 μg/g ww and 0.31 ± 0.29 μg/g ww respectively). For Hg, the specimens from Bejaia (0.50 ± 0.0001 μg/g ww) and Dellys (0.20 ± 0.07 μg/g ww) presented the highest concentrations. The smaller specimens (total length (Lt) < 12 cm) and the larger specimens (Lt > 15 cm) often showed higher accumulation for the three contaminants analyzed, compared to the medium ones. No correlation was observed between contaminant concentrations and the biological indices measured in fish (Fulton's K and the hepato-somatic indices). The concentrations in the muscle were below the maximum levels set by the European Commission for the three elements (0.3 μg/g ww for Pb, 0.25 μg/g ww for Cd and 0.5 μg/g ww for Hg). The calculated target hazard quotients (THQs) were < 1.0 and the estimated weekly intake (EWI) were below the provisional tolerable weekly intake (PTWI), indicating that the consumption of *S. pilchardus* from Algerian coast was not likely to have adverse effect on human health.

1. Introduction

Metallic elements are present in all compartments of the environment and many have increased concentrations, as a result of anthropogenic activities. Gathering high human densities and being exposed to the effluents of catchment areas, the coastal ecosystems are often subject to the adverse effects of industrial discharges. Among the diffuse pollutants, trace metallic elements can be highly toxic to marine fauna and flora. Their bioavailability and their toxicity depend on their speciation. The soluble metal species are more mobile and bioavailable and are generally the most toxic metal fraction (de Paiva Magalhaes et al., 2015).

In seawater, these potentially toxic elements move up the food chain via zooplankton (Cole et al., 2013; Boucher et al., 2016), fish larvae and adults (Browne et al., 2013; Lusher et al., 2013; Rochman et al., 2013b), reaching humans through consumption of contaminated seafood (Couture, 2017). Thus, long term intake of metals above the unsafe levels through foods may result in disruption of many biological and biochemical processes in humans (Javed and Usmani, 2011; Balkhair and Ashraf, 2016).

Metallic elements such as lead (Pb), cadmium (Cd) and mercury (Hg) have been classified as toxic to humans by international organizations namely the World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA). These organizations have recommended tolerable intake levels, oral reference doses and health risk factors applicable to a large number of pollutants in order to assess

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the human health risk through seafood consumption (FAO/WHO 2011; USEPA Environmental Protection Agency, 2000). These three metallic elements are known to potentially affect marine ecosystems, given their ubiquity in the environment, their persistence, their bioaccumulative properties and their potential for toxicity (Ribeiro et al., 2005; Diop et al., 2016).

Regarding fish, such metals as zinc are known to cause cellular damages in the kidney (Gupta and Srivastava, 2006) and can even lead to fish mortality (Gebre-Mariam and Desta, 2002). Embryonic and larval stages are generally considered to be the most sensitive stage to metals during the entire fish life cycle (Osman et al., 2007; Zhang et al., 2012).

In Algeria, few data are available on the evaluation of discharges into the environment. Three thousand uncontrolled waste dumps are mostly located along the wadis that discharge into the sea. Seventeen urban wastewater treatment plants have been built along the Algerian coastline but only 5 of them are operating normally which represents about 25% of the total treatment capacity (A.E.E, 2006). Belkacem and Aurora (2018) reported that pollution in the Bay of Algiers was at the origin of the degradation of aquatic ecosystems and the reduction of fish resources.

To complete the assessment of the environmental and health risks of these metals in this region, this study focused on three sites along the Algerian coastline selected for their high pollution pressure (Fig. 1). The first site, the Bay of Algiers, is subject to strong anthropogenic pressures including a large chain of industrial activity bordering the bay in addition to the city of Algiers generating significant urban discharges. According to Rebzani-Zahaf (1992), the Algerian coast is certainly one of the zones where the alteration of the quality of coastal marine waters is most perceptible. The second site corresponds to the Gulf of Bejaia, an area also subject to relatively high anthropogenic pressure with a highly developed urban fabric, an industrial chain (manufacture of electrical equipment, plastic processing, oil and a commercial port) and agricultural and urban waste drained by the Soummam wadi (Mouni et al., 2009). The third site is the Bay of Boumerdes (Dellys), subject to less anthropogenic pressure than the first two sites, not far from the mouth of the Sebaou wadi, which drains discharges from various industries (Tireche, 2006; Ghobrini et al., 2017).

In these coastal areas, the sardine, *Sardina pilchardus* (Walbaum, 1792) occupies a very important place in Algerian fisheries and, with the sardinella, *Sardinella aurita* (Valenciennes, 1847), are of great economic value. Few studies dealing with this species on Algerian coasts (Ouabdesselam et al., 2017; Benguendouz, 2018; Hamida et al., 2018) reported low concentrations of Pb, Cd and Hg that did not exceed the limits set by regulations. The objective of the present work was to assess the spatial and temporal variations, as well as the organotropism (in muscle and liver) of Pb, Cd and Hg in *S. pilchardus* along the central-eastern coast of Algeria. Comparisons between sites, seasons and also according to the biological parameters of the species were carried out. Finally, the human health risks associated with the consumption of this species were assessed.

2. Materials and Methods

2.1. Sampling, biometrics and calculation of biological indices

872 specimens of *Sardina pilchardus* were sampled in the three targeted sites (the bays of Algiers, Dellys and the Gulf of Bejaia), during four seasons (autumn, winter, spring and summer) between October 2017 and September 2018. The fish were randomly sampled from the landings (fish caught by boats using seine nets for sardine fishing) (Table 1). The samples were transported to the laboratory in a 4 °C cooler. Upon arrival at the laboratory, the fish were identified at the species level and placed on a glass plate cleaned with doubly distilled water. The total length (Lt) in centimeter, the total weight (Pt) in gram and the eviscerated weight (Pe) in gram were measured on each fish specimen. The Fulton condition factor K was calculated according to the Eq. (1):

$$K = \left(\frac{Pt}{Lt^3}\right) \times 100, \quad (1)$$

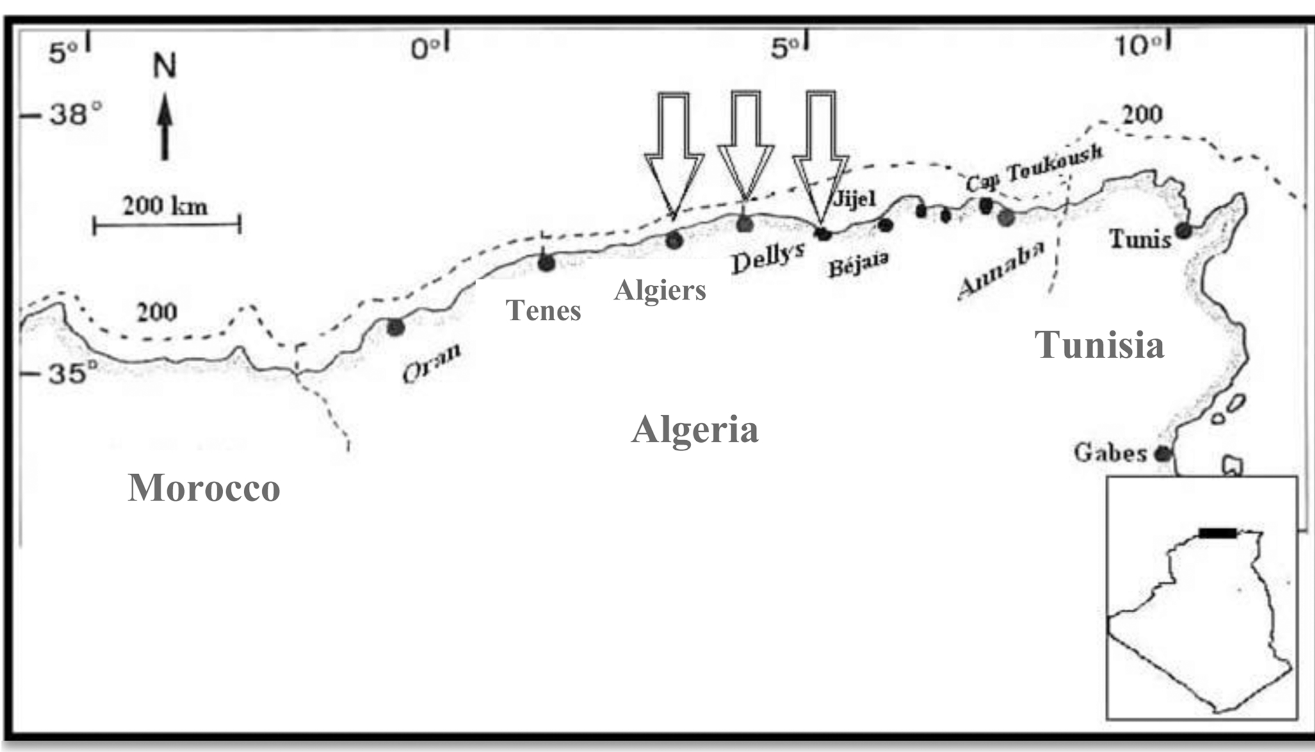

Fig. 1. Location of the study stations on the Algerian coastline, shown by the arrows (Map modified from Hamida, 2005).

Table 1
Characteristics of the examined biological material.

Sardina pilchardus (Walbaum, 1792) (N = 872)							
Study site/class/number		**Small specimens class**		**Medium specimens class**		**Large specimens class**	
Algiers	**Size class**	Lt < 12 cm		12 < Lt < 15 cm		Lt > 15 cm	
	Number/size class	n = 253		n = 222		n = 20	
	Number/Season	Autumn (n = 51)	Winter (n = 153)		Spring (n = 121)		Summer (n = 170)
Total		**N = 495**					
Bejaia	**Number/size class**	n = 148		n = 81		n = 09	
	Number/Season	Autumn (n = 73)	Winter (n = 69)		Spring (n = 36)		Summer (n = 60)
Total		**N = 238**					
Dellys	**Number/size class**	n = 52		n = 68		n = 19	
	Number/Season	Autumn (n = 39)	Winter (n = /)		Spring (n = 37)		Summer (n = 63)
Total		**N = 139**					

Fulton's condition factor is widely used in fisheries and general fish biology studies. This factor is calculated from the relationship between the weight of a fish and its length and aims to describe, the "condition" of that individual (Nash et al. 2006).

The hepato-somatic index (HSI) was calculated according to the Eq. (2):

$$HSI = \left(\frac{Pf}{Pe}\right) \times 100, \quad (2)$$

With Pf: the liver weight (g).

The hepato-somatic index (HSI) is expressed as the ratio of the liver weight to the total weight, and it provides information about the health status of the fish and the quality of water (Dane and Şişman 2020).

2.2. Sample preparation and analysis

The individuals were classified into three size ranges (small: Lt < 12 cm; medium: 12 < Lt < 15 and large: Lt > 15 cm) (Table 1). Organs and tissues for analysis (liver and muscle) were removed and weighed, placed in plastic pill boxes (sterile, labelled and coded) and then freeze-dried in a freeze-dryer (CHRIST Betta 1–8/ Laboratory freeze drying systems/Germany) in order to stop any chemical or biological transformation. The lyophilisates were then ground and mineralised in Teflon reactors: 500 mg of matrix (muscle or liver) were taken from each size class batch (500 mg × 3 size classes/muscle and 500 mg × 3 size classes/liver), a total of 120 samples/3 study sites and 30 samples/season were analysed.

Each 500 mg sample was mixed with 8 mL HNO_3 and 2 mL H_2O_2 and submitted to the fishmeal digestion program (200 °C, 40 min) in the microwave (SPEEDWAVE TWO V.2.0/ BERGHOF/Germany). The mineralized samples were transferred into tubes and supplemented up to 50 mL with ultrapure water. The tubes were stored at low temperature (−18 °C) pending analysis. Cd and Pb determinations were performed by ICP/MS Agilent 7700 Series Inductively Coupled Plasma Mass Spectrometry (Agilent/United States). Hg determination was carried out by NIC "Mercury Analyzer" (Nippon Instruments Corporation/Japan). In order to ensure reliability of the results, several internal quality controls (IQC) were used. The data were considered valid when all acceptance criteria were satisfied. IQCs included calibration, blanks and Limit of Quantification (LOQ) checking, internal standards, spikes, Certified Reference Material's (CRM) and duplicates results were acceptable if the relative standard deviation (RSD) ≤ 20%, when mean value ≥ 5 × LOQ or RSD ≤ 40%, when mean value ≥ LOQ and < 5 × LOQ. The accuracy of the analytical results was also verified through two inter-calibration exercises carried out by the Association Générale Laboratoires Analyse Environnement (AGLAE, 2018a for Pb, Cd and 2018b for Hg) on the basis of statistical exploitation of the results. The analyses were carried out at the geochemistry laboratory of the SONATRACH research and development centre (CRD).

The concentrations of each metal were expressed in μg/g of wet weight tissue or organ (μg/g ww). The calculation of the values assigned to the material (mean m) and the standard deviation for the assessment of suitability (standard deviation used for the calculation of the z-score) were evaluated with an improved version of the algorithm A of ISO 13528.

2.3. Statistical study

The results of this work were presented in tables in the form of "mean ± standard deviation for all the variables analysed. To test the hypothesis of the difference of the means between the modalities of the explanatory factors, an analysis of variance (ANOVA) was used after a verification of the normal distribution of the variables (concentrations of Pb, Cd and Hg) via Shapiro and Wilk's normality test. Following a significant ANOVA ($p \leq 0.05$), a post-hoc comparison per mean pair was performed using the Bonferroni correction for a significance level $p \leq 0.05$. These statistical treatments were carried out using IBM SPSS 24 software.

2.4. Evaluation of health risks linked to the consumption of Sardina pilchardus

2.4.1. Target hazard quotient (THQ)

THQ is the ratio of an exposure level of a single substance over a specified period of time (e.g. subchronic) to a reference dose (RfD) for that substance derived from a similar exposure period (USEPA Environmental Protection Agency, 2000). THQ was calculated according to the Eq. (3):

$$THQ = \left[\frac{(EFr \times EDtot \times Wfood \times Ci)}{(RfDo \times Bw \times ATn)}\right] \times 10^{-3}, \quad (3)$$

THQ is the risk associated with a single element,

EFr is the frequency of exposure (set at 365 days/year),

EDtot is the duration of exposure (76 years) equivalent to life expectancy at birth,

Wfood is the fish intake rate in Algeria (12 g/person/day) (MADRP 2016),

Ci is the concentration of the metal element in the sample (μg/g ww),

RfDo is the reference oraldose: Cd $= 1 \times 10^{-3}$ μg/g/day, Hg $= 1.6 \times 10^{-4}$ μg/g/day (USEPA Environmental Protection Agency, 2018), Pb $= 4 \times 10^{-3}$ μg/g/day) (USEPA Environmental Protection Agency, 2000),

Bw is the average body weight (75 kg for adults) (Abbes, 2017),

ATn is the average exposure time for non-carcinogens (365 days / year × 76 years),

Once this ratio has been calculated, several scenarios may arise:

-THQ < 1.0 indicates that daily exposure does not cause adverse effects on human health over a lifetime,

-THQ ≥ 1.0 indicates possible side effects.

To assess the risk of the three metals together, the total THQ (TTHQ) was calculated by adding the THQ for each element measured according to the formula (Eq. 4):

$$\text{TTHQ} = \text{THQ}(\text{toxicant1}) + (\text{toxicant2}) + \ldots\ldots(\text{toxicant n}), \quad (4)$$

2.4.2. Estimated weekly intake

Consumer exposure to Cd, Pb and Hg was also determined by calculating the estimated weekly intake according to the Eq. (5) of Pastorelli et al. (2012):

$$\text{EWI} = \frac{(\text{C} \times \text{Ci})}{\text{Bw}} \quad (5)$$

EWI is the estimated weekly intake, (μg/kg/week).

C is the weekly fish consumption rate (84 g/week) (MADRP, 2016),

Ci is the level of contaminant in the food (μg/g ww); Bw is the average body weight (75 kg for adults) (Abbes, 2017),

THQ provided an indication of the level of risk due to Pb, Cd and Hg in the *S. pilchardus* muscle but did not serve as a quantitative criterion. It is rather the estimation of the probability of exposure of a population experiencing an inverse health effect (Storelli, 2008).

The assessment of the exposure and the sanitary risks was carried out comparing the EWI certain provisions applicable to EFSA (European Food Safety Security) and particularly.

The PTWI "Provisional tolerable weekly intake". The FAO/WHO Expert Commission on Contaminants in Foods (JECFA) has established the PTWI for Pb at 25 μg/week/kg b.w, for Cd at 7 μg/week/kg b.w and for Hg at 4 μg/week/kg b.w (JECFA, 2000).

3. Results and discussions

3.1. Levels of contamination in the liver and the muscle of S. pilchardus

The average concentrations of the three contaminants (Pb, Cd and Hg) measured in the liver and muscle of *S. pilchardus* are presented in the Table 2. For all elements, the levels in the liver were higher than those recorded in the muscle regardless of the site and the fish size. Chahid (2016) confirmed that the liver is a good indicator of chronic exposure to metals and plays an important role in their storage and inactivation. This author noted that the liver of *S. pilchardus* has higher concentrations than the other organs and is an accumulating and detoxifying organ for Pb and Cd. Ennouri et al., (2017) reported that in *Liza ramada* (Risso 1810), the liver is the preferential accumulation organ for Hg. According to El Morhit et al. (2012), the fish muscle presents a low metal content due to its low metabolic activity.

On the other hand, metallothionein (MT), a low molecular weight protein rich in cysteine, concentrates mainly in the liver tissue of fish (Scudiero et al., 2005) and therefore plays a role comparable to a trap in relation to various metals, particularly with mercury (IFREMER Scientific and Technical Reports 1990). Metallothioneins protect cells against metal ion aggression (detoxification role by capturing excess metals of exogenous origin) (Bensakhria, 2018).

Regarding cadmium, this element has chemical characteristics close to those of calcium, in particular ionic radiation, thus facilitating its penetration into organisms (Borchardt, 1985).

Statistically significant differences ($p < 0.05$) were observed between Hg concentrations of the three *S. pilchardus* size classes from the three sites. The highest concentrations of Hg were mostly found in the medium and large size specimens (Table 2). The Table 3 present the relationships between heavy metal concentrations and the total fish lengths of *Sardina pilchardus*, whatever the site.

Highly significant relationships were recorded between the size of *S. pilchardus* and the Hg content in liver and muscle ($p < 0.001$) confirming that mercury contamination increase in the medium and large specimens of this species.

Previous evidence suggested that nearly all of the mercury (> 85%) in the muscle tissue of fish occurs as MeHg (Hight and Cheng, 2006; Krystek and Ritsema, 2005).

MeHg is better retained by higher-level organisms compared to other Hg species and is the predominant form of mercury that biomagnifies in the aquatic food chain (Renner, 2004).

Regarding Pd and Cd, their concentrations in two of the studied sites

Table 2
Variations of annual mean concentrations of Pb, Cd and Hg (in μg/g wet weight) in the liver and the muscle, as well as the biological parameters of *Sardina pilchardus*, sampling at the three sites.

Site	Size	Lt (cm)	K (%)	HSI (%)	Pb *(μg/g ww)*		Cd *(μg/g ww)*		Hg *(μg/g ww)*	
					liver	muscle	liver	muscle	liver	muscle
Algiers	**Small (n = 253)**	11.1_a	0.78_a	0.93_a	0.41_a	0.25_a	1.53_a	0.04_a	0.23_a	0.11_a
(n = 495)	**(Lt < 12 cm)**	(0.65)	(0.10)	(0.40)	(0.17)	(0.29)	(2.86)	(0.06)	(0.14)	(0.04)
	Medium (n = 220) (12 cm < Lt <15 cm)	13.1_b	0.76_a	1.15_b	0.29_b	0.21_a	0.95_a	0.06_a	0.29_a	0.10_a
		(0.92)	(0.08)	(0.51)	(0.16)	(0.23)	(0.54)	(0.08)	(0.24)	(0.04)
	Large (n = 20)	15.8_c	0.79_a	1.49_c	.[1]	.[1]	1.04_a	0.31_b	0.18_a	0.09_a
	(Lt > 15 cm)	(0.97)	(0.03)	(0.42)			(0.08)	(0.14	(0.03)	(0.03)
	Meaning of Statistical Test (P)	***< 0.001***	***0.161***	***< 0.001***	***0.001***	***0.545***	***0.155***	***< 0.001***	***0.043***	***0.084***
Bejaia	**Small (n = 148)**	11.05_a	0.70_a	0.70_a	0.41_a	0.09_a	0.73_a	0.08_a	0.14_a	0.08_a
(n = 238)	**(Lt < 12 cm)**	(0,88)	(0,07)	(0,40)	(0,33)	(0,02)	(0.42)	(0.06)	(0.08)	(0.03)
	Medium (n = 81)	12.87_b	0.75_b	$0.96_{a,b}$	0.35_a	0.09_a	0.57_a	0.01_b	0.16_a	0.10_a
	(12 cm< Lt < 15 cm)	(1.17)	(0.07)	(0.42)	(0.31)	(0.0001)	(0.25)	(0.0001)	(0.01)	(0.02)
	Large (n = 09)	16.06_c	$0.72_{a,b}$	1.23_b	.[1]	.[1]	0.73_a	$0.04_{a,b}$	0.32_b	0.50_b
	(Lt > 15 cm)	(0,97)	(0,04)	(0,40)			(0.0001)	(0.0001)	(0.0001)	(0.0001)
	Meaning of Statistical Test (P)	***< 0.001***	***0.018***	***0.005***	***0.607***	***0.843***	***0.251***	***< 0.001***	***< 0.001***	***< 0.001***
Dellys (n = 139)	**Small (n = 52)**	11.51_a	0.77_a	0.73_a	0.05_a	0.06_a	$0.81_{a,b}$	0.02_a	0.05_a	0.06_a
	(Lt <12 cm)	(0.52)	(0.07)	(0.37)	(0.0001)	(0.0001)	(0.55)	(0.0001)	(0.01)	(0.01)
	Medium (n = 68)	14.1_b	0.75_b	$0,96_{a,b}$	0.16_b	0.03_b	0.62_a	0.01_b	0.14_b	0.13_b
	(12 cm < Lt < 15 cm)	(1.14)	(0.07)	(0.42)	(0.06)	(0.01)	(0.36	(0.01)	(0.09)	(0.08)
	Large (n = 19)	17.55_c	0.99_b	1.63_c	0.49_c	$0,01_c$	1.05_b	.[1]	0.46_c(0.10)	0.20_c
	(Lt > 15 cm)	(1.42)	(0.19)	(0.22)	(0.0001)	(0.0001)	(0.58)			(0.07)
	Meaning of Statistical Test (P)	***< 0.001***	***0.002***	***< 0.001***	***< 0.001***	***< 0.001***	***0.044***	***0.032***	***< 0.001***	***< 0.001***

ww wet weight
Lt total length
Pt total weight
K Fulton Condition Coefficient
HSI Hepato-somatic index
Note: The values in brackets represent the standard deviation
The letters a, b and c indicate significant differences between the different class size regarding the biological parameters or the metal concentrations in each matrix.
[1] not detected

Table 3
The relationships between heavy metal concentrations and total fish lengths in the tissues of *Sardina pilchardus*.

Tissue	Data	Pb	Cd	Hg
Liver	DF	292	292	292
	Equation	Y= 0031–0,01(x)	Y= 1504–0037(x)	Y= 0006 + 0017(x)
	R value	-0006	-0047	0199
	P value	NS	NS	* **
Muscle	DF	292	292	292
	Equation	Y= 0482–0,0255(x)	Y= −0176 + 0019(x)	Y= −0086 + 0016(x)
	R value	-0209	0361	0390
	P value	* *	* **	* **

DF is degree of liberty. In the equations, Y is metal concentration (μg /g w.w.) and X is total fish length (cm). Asterisks indicate significant results. NS, not significant, P > 0.05. * *P < 0.01. * **P < 0.001.

(Algiers and Dellys), were significantly higher in the small size class of *S. pilchardus* (Table 2).

Regarding all sites, non-significant relationships were found between the size of *S. pilchardus* and the levels of Pb and Cd in liver. In muscle, however, an inverse relationship was observed for Pb ($p < 0.01$) and a positive one for Cd ($p < 0.001$) (Table 3).

This observation corroborates some previous results on metal accumulation, which has been shown to be higher in young (small specimens) than in old fish (Nussey et al., 2000; Widianarko et al., 2000). Hoang et al. (2004) noticed that higher metal concentrations in young or juvenile fish may be related to the early life stages which generally show a higher sensitivity to metals. Many authors have also suggested that negative correlations observed between size and metal concentrations within the same species result from higher metabolic rates of younger individuals compared to the older ones (Canli and Atli, 2003; Bosch et al., 2016; Vieira et al., 2011; Galitsopoulou et al., 2012; Farkas et al. 2003; Yi and Zhang, 2012; Tang et al. 2017; Guo et al. 2016). Metal bioaccumulation is likely to reach a steady state after a certain age (Canli and Atli, 2003; Yi and Zhang, 2012; Galitsopoulou et al., 2012; Guo et al., 2016).

Our results corroborate those of Canli and Atli (2003) who reported that Pb concentrations in *S. pilchardus* are negatively related to the length of the fish. Farkas et al. (2003) reported negative correlations between Pb and Zn bioaccumulation and fish *Abramis brama* L. age and size and suggested that dilution takes place in older individuals due to their higher lipid content. No significant correlation was found between Pb levels in muscle tissue and the length of *Merluccius merluccius* (Linnaeus, 1758) and *Mullus barbatus barbatus* (Linnaeus, 1758) (Gašpić et al., 2002).

Correlations between the body size of sardine and anchovy from Greek coast to the metal load or bioaccumulation have been reported mostly for Cu, Fe, Hg, Cd, Ni, As, Pb, Zn, Se, Li, Rb, Ba, Tl, V and Cs (Sofoulaki et al., 2018). Most references reported negative correlations suggesting that smaller fish size can be associated with higher levels of metals and elements (Sofoulaki et al., 2018).

The correlations found in literature were usually associated with growth efficiency. If organisms grow faster than they accumulate metals, then metal levels should decrease with increasing body size (Tang et al. 2017). Gašpić et al. (2002) showed that cadmium and lead concentrations in the liver decreased with increasing length of fish.

However, results are often controversial since positive or non-significant correlations may also appear (Sarkar et al., 2008; Bosch et al., 2016; Canli and Atli, 2003; Joiris and Holsbeek, 1999; Vieira et al., 2011; Yi and Zhang, 2012; Galitsopoulou et al., 2012; Farkas et al., 2003; Hayase et al., 2009; Dang and Wang, 2012; Guo et al., 2016; Tang et al., 2017). For exemple,

Salam et al. (2019) observed a positive correlation of Pb concentration with fish size of four commonly consumed fish (*Euthynnus affinis*, *Pampus argenteus*, *Descapterus macrosoma*, *Leiognathus daura*) and a negative correlation of Pb content in fish muscle and Pb content in other organs of fish.

This trend towards a decrease in the concentration of potentially toxic elements in larger fish was also highlighted in the study of Pourang et al. (2005) who explained this result by a dilution effect with growth and ion exchange in the fish environment. In *Atherinella brasiliensis* muscle, Pb concentrations tend to decrease with length, being weakly correlated (Vieira et al., 2020).

If the growth of the organism is faster than the accumulation of contaminants, the concentration of trace metals observed can decrease with age and weight, even though the total level of contaminants may increase. In this sense, Kayalto et al. (2014) reported that the concentration of Cr decreased in the liver, the flesh and the bone with increasing age.

Possible overshadowing of size effect on metal bioaccumulation due to environmental factors has also been reported by Hayase et al. (2009) and Guo et al. (2016). Size specific bioaccumulation has been reported to be affected, apart from the site, by the species and the metal/element studied (Guo et al., 2016), by the ontogenetic stage (e.g. juveniles) (Guo et al., 2016) and finally by different exposure ro of marine organisms to metals (e.g. diet, water, sediment) (Tang et al., 2017).

Regarding biological indices, the Fulton condition factor K showed statistically significant differences between sardines from the three size classes at Bejaia and Dellys (Table 2). In small specimens from Algiers, despite the highest concentrations of Pb, Cd and Hg, high values of Fulton's K ($K = 0.78$) and hepato-somatic index ($HSI = 0.93$) were recorded (Table 2). The same observation was made for medium-sized specimens (from Bejaia and Algiers). The maximum value for hepato-somatic index ($HSI = 1.15$) was in Algiers, the most exposed site to pollution (Table 2). Regarding the large specimens, the highest values of K and HSI were also noted in Dellys and Algiers sites (Table 2). However, the analyses of correlations between the 3 metallic elements (Pb, Cd and Hg) concentrations and these two biological indices (K and HSI) revealed very low correlation coefficients (< 0.20) in liver and muscle except for a negative linear relationship between Pb concentrations and HSI in *S. pilchardus* muscle ($r = -0.277$) (see Fig. 3A–C for K, and Fig. 4A–C for HSI). The Fulton's condition factor K tends to increase slightly (Fig. 3C) or remained stable (Fig. 3A and B) with the increase of the 3 metallic elements. For HSI, it declined or increased slightly with increasing concentrations of the metallic elements in the liver or the muscle (Fig. 4A–C).

Louiz et al. (2018) reported an increase in hepato-somatic ratios in *Zosterisessor ophiocephalus* (Pallas, 1814) living in an environment characterised by chronic chemical contamination (Bizerte lagoon). They reported low values of the condition indices (K) of this fish in a reference site with low contamination. Fluctuations in these both indices can be attributed to various factors such as reproductive status and food availability (Chellappa et al., 1995; Gilliers et al,. 2004). Several studies have also mentioned the value of using these parameters in assessing the effects of environmental stressors on a target organ or a whole organism (Lloret and Planes, 2003; Amara et al., 2007). In the present study, our results showed that the potential effects of Pb, Cd and Hg on the health status of *Sardina pilchardus* could not be observed using the Fulton's condition coefficient or the HSI.

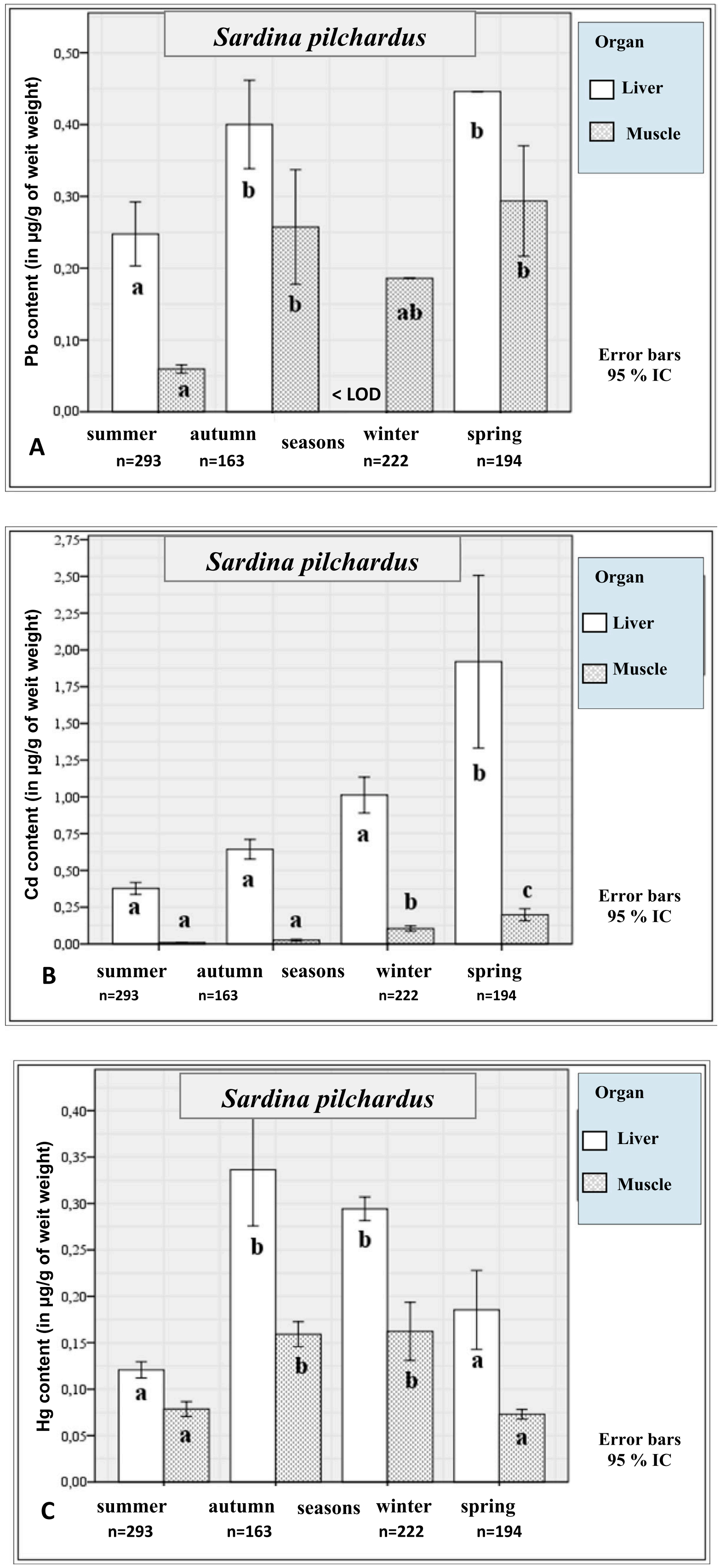

Fig. 2. Variation in the average concentrations (µg/g ww) of Pb (A), Cd (B), Hg (C) in the liver and in the muscle of *Sardina pilchardus* according to the seasons (The letters a, b, c indicate significant seasonal differences for the same matrix). (< LOD: below the Limit of Detection).

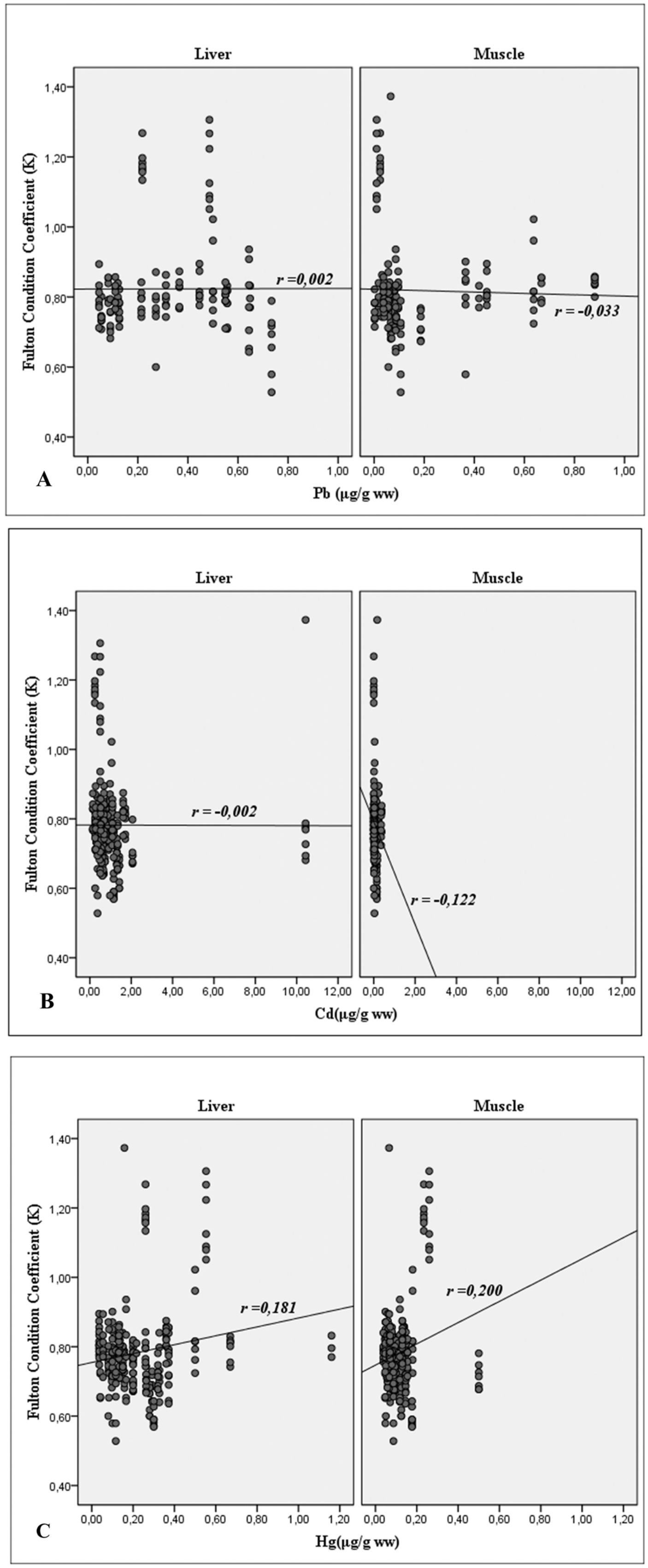

Fig. 3. Variation in the average concentrations (µg/g ww) of Pb (A), Cd (B), Hg (C) in the liver and in the muscle of *Sardina pilchardus* according to Fulton Condition Coefficient (K).

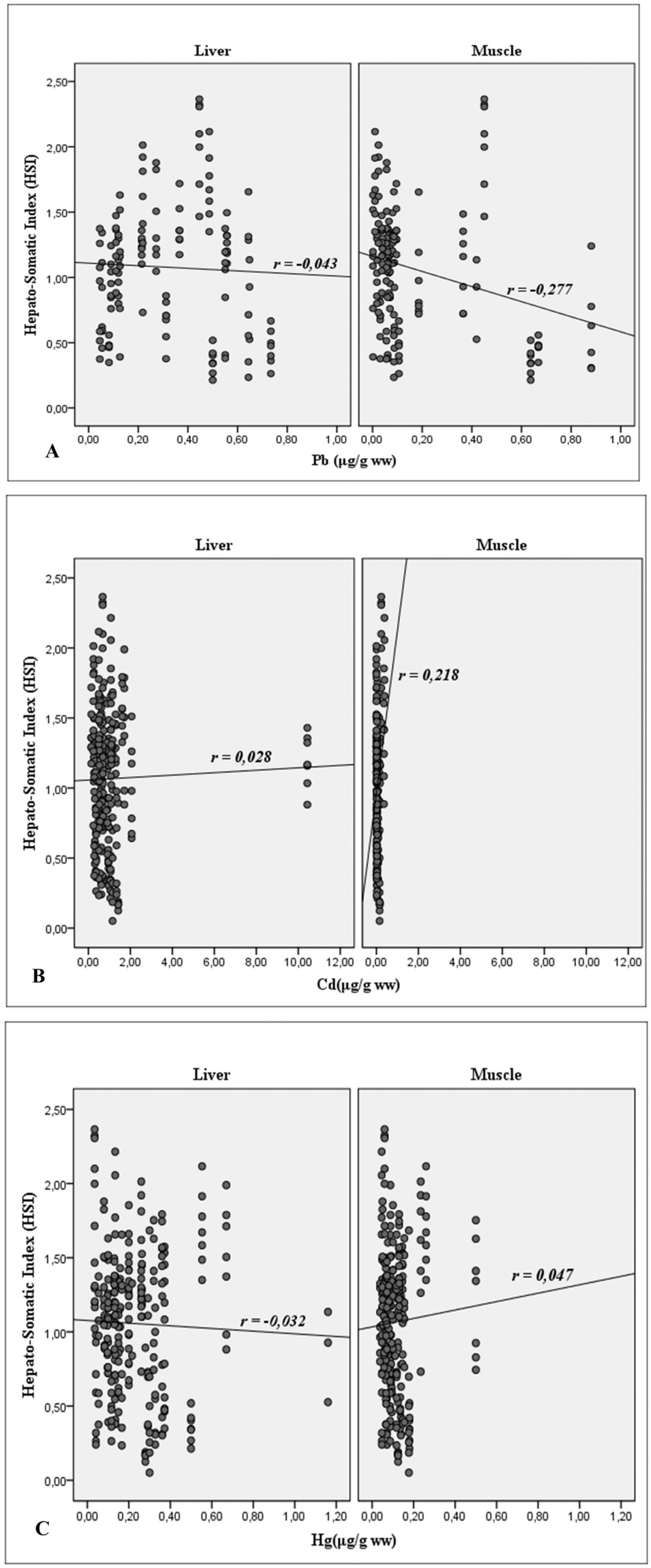

Fig. 4. Variation in the average concentrations (µg/g ww) of Pb (A), Cd (B), Hg (C) in the liver and in the muscle of *Sardina pilchardus* according to Hepato-Somatic Index (HSI).

3.2. Seasonal and spatial variations

The seasonal variation was evaluated only in the two sites most subject to the pollution pressure (Algiers and Bejaia).

The three metals showed higher concentrations in the liver than in the muscle for all four seasons. Despite fluctuating environmental parameters, the liver of *S. pilchardus* represents the storage and the detoxification site for these three elements. The highest concentrations of these three metals were observed during the autumn (Hg) and spring season for Pb and Cd (Figs. 2A–C).

The highest percentage of stomachs fullness of *S. pilchardus* was observed during autumn and winter indicating that this species feeds intensively during these periods (Kaidi et al., 2019), thus favouring the bioaccumulation of toxic contaminants. On the Portuguese coasts, Garrido et al. (2007) have clearly shown that spring and winter are the seasons when *S. pilchardus* has an intense diet feeding exclusively on zooplankton.

The highest average Hg concentrations in *S. pilchardus* were recorded in large individuals from Dellys (0.46 μg/gww in liver) and Bejaia (0.50 μg/gww in muscle) bays (Table 2). According to Tireche (2006), mercury is found in discharges from the pharmaceutical and chemical industries and in effluents from textile factories in the Boumerdes region (Dellys). Existing cement factories in the Boumerdes region (Dellys) could also be a source of Hg. Furthermore, according to Aranguren (2008), the mining activity recorded in the Boumerdes region (Dellys site) could therefore generate higher concentrations in soils, sediments and water than the geochemical background, existing prior to mining in the region. The explosives factory in Boumerdes (Dellys site) is also a proven source of mercury. In the Bejaia region, the effluents of the textile and electrolysis plants of the Kherrata region located upstream of the Elthnine Souk wadi could be a Hg source, as well as the oil industry near the port which may cause Hg leaks.

The highest average Pb concentrations were recorded in the liver of *S. pilchardus* (0.49 μg/g ww) sampled in Dellys (Table 2). On the other hand, the highest average concentration in the muscle (0.25 μg/g ww) were recorded in Algiers (Table 2). At the Dellys site, these high Pb concentrations could be mainly related to human activity linked to domestic, industrial (pigments and batteries) and agricultural uses. These contaminants are often transported via the Sebaou wadi and discharged into the site (Ghobrini et al., 2017). According to the same author, lead emissions have long been dominated by car transport due to the presence of lead in petrol (Tireche, 2006). The port activity through its wide use of lead in Algiers, in fishermen's nets, the high fuel consumption by motor boats as well as the nature of the hull paintings of the different boats could be at the origin of high lead concentrations in this area (Chouba and Mzoughi, 2005).

The highest average Cd concentrations in liver and muscle of *S. pilchardus* were recorded in Algiers, respectively 1.53 μg/g ww and 0.31 μg/g ww (Table 2). In the Bay of Algiers, the presence of the thermal power station at the port of Algiers may explain these high levels of Cd (e.g. fly ash loaded with cadmium). The values found in the analysis of fly ash samples by Moufti and Mountadar (2004) are higher than the international standards for some metals (Cd, Al and Ti). Two wadis flow into the Bay of Algiers, the El Hamiz wadi and the El Harrach wadi. The latter drains domestic and industrial waste water, especially from the city of Algiers, which is only 8% treated and is discharged directly into the bay (PAC, 2005). Following Algiers, the highest average concentrations of Cd was attributed to the Dellys site (1.05 μg/g ww in the liver of large individuals), a site characterised by agricultural activity nearby and which receives discharges from a fertiliser industry. Cadmium is a minor constituent of fertilisers used in agriculture.

3.3. Health risk assessment

3.3.1. Mercury (Hg)

In this study, Hg concentrations in the muscle and liver of *S. pilchardus* did not exceed the regulatory threshold (0.5 μg/g ww) (CE, 2008) whatever the study site (Table 2).

The highest values in *S. pilchardus* were recorded from the western Mediterranean basin in Italy (0.31 ± 0.26 μg/g ww) by Copat et al. (2012) from the eastern Mediterranean basin in Egypt (0.72 ± 0.01 μg/g ww) by Ali Shokr et al. (2019) (Table 4). The rest of the studies carried out in the other localities of the Mediterranean basin showed Hg values lower than 0.287 ± 0.910 μg/g ww.

In Algeria, our results at the Algiers site (0.10 ± 0.03 μg/g ww) were similar to those found by Benguendouz (2018) (0.091 μg/g ww) at the same site.

The studies carried out along the Algerian coastline in the muscle of three species of pelagic fish (Table 5) revelated that *S. pilchardus*, is the species for which the highest Hg values were recorded (results of the present study) in the Bay of Bejaia and the Bay of Boumerdes (Table 2).

3.3.2. Lead (Pb)

The comparison of the recorded Pb concentrations with European standards showed that the Pb concentrations in *S. pilchardus* muscle did not exceed the regulatory threshold value (0.3 μg/g ww) (CE, 2015). This is not the case for the liver where maximum Pb concentrations were slightly higher in small specimens in Algiers and Bejaia (0.41 μg/g ww in both sites) and in large specimens in Dellys (0.49 μg/g ww) (Table 2), which represents 51%, 62% and 13% of contaminated fish respectively. The results of Pb concentrations in *S. pilchardus* muscle obtained in Boumerdes (from 0.01 to 0.06 μg/g ww) are similar to the work of Hamida et al. (2018) who reported low Pb concentrations in *S. pilchardus* muscle from the Bay of Boumerdes (from 0.021 to 0.055 μg/g ww), not exceeding regulatory values. Our results corroborate the work of Benguendouz (2018) who reported that the Pb values measured in the flesh of *S. pilchardus* from the Bay of Algiers did not exceed the regulatory standard. The highest mean Pb concentrations in *S. pilchardus* were reported by Ouabdesselam et al. (2017) in Boumerdes (0.99 ± 0.11 μg/g ww) and El Morhit et al. (2012) in Morocco (0.55 ± 0.03 μg/g ww), for the North African Mediterranean basin and by Korkmaz et al. (2017) in Turkey (0.91 ± 0.08 μg/g ww) for the Eastern Mediterranean basin. For the Western Mediterranean basin, Pb concentrations remained low and did not exceed 0.05 μg/g ww (Table 4). Scientific work carried out in the muscle of three pelagic fish species along the Algerian coast (Table 5) revealed that *S. pilchardus* was the species with the highest Pb values (in Boumerdes, Ouabdesselam et al., 2017 and Algiers, present study) compared to *Sardinella aurita* (Valenciennes, 1847) and *Trachurus trachurus* (Linnaeus, 1758).

3.3.3. Cadmium (Cd)

The maximum concentrations of Cd in the muscle of *S. pilchardus* (0.31 μg/g ww in Algiers) slightly exceed the regulatory threshold (0.25 μg/g ww) (CE, 2014). The maximum concentrations recorded in the liver, on the other hand, were well above the norms in specimens of the three size classes in Algiers, Bejaia and Dellys corresponding to 100% contaminated fish (with a maximum of 6 times higher in small specimens in Algiers, almost 3 times higher in small and large specimens in Bejaia and 4 times higher in large specimens in Dellys) (Table 2). These high concentrations could reflect the poor state of water quality in these coastal ecosystems. The highest Cd values were recorded respectively in the Eastern Mediterranean basin in Turkey (0.46 ± 0.56 μg/g ww) by Yabanli (2013) and in *S. pilchardus* from the North African Mediterranean basin in Oran (10.99 ± 3.93 μg/g ww) by Merbouh (1998) and in Bejaia (present study) (Table 4). Studies carried out along the Algerian coastline on three species of pelagic fish (Table 5) revelated that *S. pilchardus*, is the species presenting the highest Cd values in muscle (Oran, Merbouh, 1998 and Algiers, the present study) compared to *Sardinella aurita* (Valenciennes, 1847) and *Trachurus trachurus* (Linnaeus, 1758).

The THQPb, THQCd and THQHg calculated for Pb, Cd and Hg in the three study sites were < 1.0 indicating that the concentrations of these

Table 4
Comparison of the concentrations of the three metals (in µg/g wet weight) in *Sardina pilchardus* muscle according to the different regions of the Mediterranean basin.

Study site		Pb (µg/gww) Mean ± sd (min-max)	Cd (µg/gww) Mean ± sd (min-max)	Hg (µg/gww) Mean ± sd (min-max)	Reference
Mediterranean Basin occidental	Spain (Catalonia)	0.01 – 0.08	0.002 – 0.01	0.07 – 0.09	Falco et al. (2006)
	Spain (Valenvia)	0.05 (0.02–0.36)	0.01 (0.003–0.021)	0.04 (0.02–0.3)	Yusa et al. (2008)
	France	0.04	–	–	Guerin et al. (2011)
	Italy (Sicily)	< 0.06	0.045 ± 0.020	0.31 ± 0.26	Copat et al. (2012)
	Italy (Sicily)	< LD	0.087 ± 0.031	0.132 ± 0.108	Naccari et al. (2015)
Mediterranean Basin Oriental	Egypt (Suez Canal in Port- Said Harbour)	–	0.028 ± 0.012 0.02–0.04	0.287 ± 0.910 0.20–0.52	Soliman (2006)
	Egypt (Kafr El–sheikh Governorate)	0.33 ± 0.01 0.10–0.62	–	0.72 ± 0.01 0.17–1.19	Ali Shokr et al. (2019)
	Turkey (Izmir)	0.14 ± 0.17 LD-0.38	0.46 ± 0.56 LD-1.25	0.03 ± 0.02 0.01–0.05	Yabanli (2013)
	Turkey (Mersin)	0.91 ± 0.08 0.57–1.37	< 0.0004	–	Korkmaz et al. (2017)
Mediterranean Basin North Africa	Morocco (estuaire du bas loukkos)	0.55 ± 0.03 0,51–0,59	0.08 ± 0,15 0.009–0310	–	El Morhit et al. (2012)
	Morocco (Laayoune)	0.002 ± 0.001 0.001–0.008	0.09 ± 0.06 0.01–0.23	–	El Morhit et al. (2013)
	Oran	0.02 ± 0.01	10.99 ± 3.93	–	Merbouh (1998)
	Ghazouet (Tlemcen)	0.013	–	0.077	Benguendouz (2018)
	Beni saf (Ain T é mouchent)	0.018	–	0.101	
	Mostaganem	0.024	–	0.117	
	Jijel	0.017	–	0.129	
	Algiers	0.016	–	0.091	
	Boumerdes	0.99 ± 0.11	00	–	Ouabdesselam et al. (2017)
	Boumerdes	0.055 ± 0.021	0.031 ± 0.017	–	Hamida et al. (2018)
	Algiers	0.23 ± 0.26 (0.21–0.25)	0.13 ± 0.09 (0.04–0.31)	0.1 ± 0.03 (0.09–0.11)	**Present study**
	Bejaia	0.09 ± 0.01 (0.09–0.09)	0.04 ± 0.02 (0.01–0.08)	0.22 ± 0.01 (0.08–0.50)	**Present study**
	Dellys	0.03 ± 0.003 (0.01–0.06)	0.015 ± 0.005 (0.01–0.02)	0.13 ± 0.05 (0.06–0.20)	**Present study**

< LD = Detection limit of the device
Mean ± sd (min-max) = mean ± standard deviation (minimum-maximum)
ww = wet weight

Table 5
Concentrations of Pb, Cd and Hg (in µg/g wet weight) in the muscle of pelagic fish species caught along the Algerian coast.

Metals / Fish species	Site	Pb (µg/g ww) Mean ± sd (min-max)	Cd (µg/g ww) Mean ± sd (min-max)	Hg (µg/g ww) Mean ± sd (min-max)	Reference
Sardina pilcardus	Oran	0.02 ± 0.01	10.99 ± 3.93	–	Merbouh (1998)
	Boumerdes	0.99 ± 0.11	0	–	Ouabdesselam et al. (2017)
	Ghazaouet/Tlemcen	0.013	–	0.077	Benguendouz (2018)
	Benisaf	0.018	–	0.101	
	Mostaganem	0.024	–	0.117	
	Algiers	0.016	–	0.091	
	Jijel	0.017	–	0.129	
	Boumerdes	0.055 ± 0.021	0.031 ± 0.017	–	Hamida et al. (2018)
	Algiers	0.23 ± 0.26 (0.21–0.25)	0.13 ± 0.09 (0.04–0.31)	0.10 ± 0.03 (0.09–0.11)	**Present study**
	Bejaia	0.09 ± 0.01 (0.09–0.09)	0.04 ± 0.02 (0.01–0.08)	0.22 ± 0.01 (0.08–0.50)	
	Dellys	0.03 ± 0.003 (0.01–0.06)	0.015 ± 0.005 (0.01–0.02)	0.13 ± 0.05 (0.06–0.20)	
Sardinell aaurita	Oran	0.024	3.193	–	Benamar (2006)
Trachurus trachurus	Oran	0.02 ± 0.002	1.85 ± 0.76	–	Benadda (2009)

Mean ± sd (min-max) = mean ± standard deviation (minimum-maximum)

metals in the muscle of *S. pilchardus* presented no adverse health risks for the consumers (Table 6). The total THQ (TTHQ) was also calculated to include the three metals together (Table 6). The results showed that the sum did not exceed 1.0, and therefore, no health risks can be noticed.

The estimated weekly intake (EWI) calculated for the 3 metals (Hg, Pb and Cd) in the 3 study sites did not exceed the threshold of the provisional weekly intake (PTWI) set by the EFSA (Table 6). The largest contributions of Pb (EWI = 0.257 µg/kg ww) and Cd (EWI = 0.152 µg/kg ww) were recorded in the most polluted site (Algiers). The largest contribution of Hg (EWI = 0.253 µg/kg ww) was recorded in the second most polluted site (Bejaia). Dellys recorded the lowest contributions of EWI.

The average intake of Pb through the consumption of *S. pilchardus* muscle represents 1%, 0.4% and 0.14% of the PTWI in Algiers, Bejaia and Dellys respectively. For Cd, the average intake represents respectively 2.17%, 0.68% and 0.22% of the PTWI in Algiers, Bejaia and Dellys. For Hg, it represents respectively 2.8%, 6.32% and 3.62% of the PTWI in Algiers, Bejaia and Dellys. These results showed that no risk was highlighted for the health of the consumer.

Considering others studies and the different regions of the Mediterranean basin, the THQs calculated for Pb, Cd and Hg were well below the value of 1.0, indicating that the concentrations of these metals in

Table 6
Target Hazard Quotient (THQ) and Estimated Weekly Intake (EWI; µg/µg ww) related to the consumption of Pb, Cd and Hg in *S. pilchardus* muscle from different regions of the Mediterranean basin.

Study sites		THQ			EWI			References
		Pb	**Cd**	**Hg**	**Pb**	**Cd**	**Hg**	
Western Mediterranean Basin	**Spain**	0.01	0.02	0.11	0.25	0.13	0.38	Trabalón et al. (2015)
	Italy (Sicily-Catania)	13×10^{-6}	64×10^{-6}	51×10^{-6}	0.037	–	0.037	Copat el al. (2012)
	Italy (Sicily-Catania)	–	–	0.19	–	–	0.13	Traina et al. (2018)
Eastern Mediterranean Basin	**Turquie**	0.03	0.01	0.79	0.73	0.06	0.56	Özdenand Erkan (2015)
North African Mediterranean Basin	**Morocco**	–	0.04	0.12	–	–	–	(Chahid, 2016)
	Algeria (Algiers-Bejaia-Oran)	8.08×10^{-6}	1.61×10^{-6}	–	0.112	0.0112	–	Mehouel et al. (2019)
	Algeria (Algiers)	0.003	0.008	0.037	0.257	0.152	0.112	**Present study**
	Algeria (Bejaia)	0.001	0.002	0.084	0.100	0.048	0.253	**Present study**
	Algeria (Dellys)	0.0004	0.0009	0.048	0.036	0.016	0.145	**Present study**

S. pilchardus do not present an adverse effect for the health of the consumer (Table 6). The Western Mediterranean basin has the lowest THQ values. In Italy the values increased between 2011 (THQHg = 51 $\times 10^{-6}$) and 2018 (THQHg = 0.19) according to the respective studies of Copat et al. (2012) and Traina et al. (2018) in the same site (Catania). The North African Mediterranean basin seems to be the less contaminated. The THQ values of the three metals calculated in Algeria were lower than those in Morocco, 0.008 for Pb as an example in the present study and 1.61 $\times 10^{-6}$ in the study of Mehouel et al. (2019), compared to 0.04 in Morocco (Chahid, 2016). The Eastern Mediterranean basin presents an intermediate position. In Turkey (Özden and Erkan, 2015), the THQs of Pb and Cd were low except for Hg (THQHg = 0.79 and TTHQ = 0.95). Regarding the estimated weekly intake (EWI), the values recorded in the different regions of the Mediterranean basin do not exceed the threshold of the provisional weekly intake (PTWI) set by the EFSA (Table 6). Large intakes of Pb (EWI = 0.73) and Hg (EWI = 0.56) have been recorded in Turkey (Özden and Erkan, 2015). The largest contribution for Cd (EWI = 0.15) was recorded in Algeria (Algiers) (this study).

4. Conclusion

The average concentrations of Pb, Cd and Hg measured in the liver of *S. pilchardus* were always higher than those recorded in the muscle regardless of the site and the size of the fish. Sardine specimens from the two bays, Bejaia and Dellys, showed high average Hg concentrations. The highest concentrations of Pb and Cd have been noticed in sardines from the bays of Dellys and Algiers. These results also showed that the intake of Pb, Cd and Hg contained in the *S. pilchardus* muscle did not exceed the safety level. The North African and Eastern basins record the highest concentrations of potentially toxic elements (Pb, Cd and Hg). On the other hand, the concentrations of these metals remain low in the Western basin (apart from Hg, which had a high value in Italy). The three contaminants measured in the muscle of *S. pilchardus* (edible part) sampling along the Algerian coast do not seem to present some health risk for consumers.

CRediT authorship contribution statement

Souad Aissioui: Conceptualization Ideas, Methodology Development, Validation Verification, Investigation Conducting a research and investigation process, Resources Provision of study materials, Writing, Writing – review & editing, Visualization Preparation, Supervision, Project administration, **Laurence Poirier:** Methodology Development, Formal analysis, Writing – review & editing, Visualization, Preparation, **Rachid Amara:** Methodology Development, Writing – review & editing, Visualization Preparation, **Zouhir Ramdane:** Conceptualization Ideas, Methodology Development, Validation Verification, Writing, Writing – review & editing, Visualization Preparation, Supervision, Project administration.

Declaration of Competing Interest

On behalf of all authors, I state that there is no conflict of interest.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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