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Concentrations of lead, cadmium, and mercury in *Mullus barbatus barbatus* (L.) from the Algerian coast and health risks associated to its consumption

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ABSTRACT

The Algerian coast receives significant inputs of metallic pollutants. To assess the accumulation of potentially toxic elements (Pb, Cd, Hg) by benthic fish and the consequent health risks for consumers, red mullet specimens (*Mullus barbatus barbatus*, Linnaeus, 1758) were sampled (n=424) in 3 sites along the Algerian coast (Algiers, Bejaia and Dellys) and analysed for Pb, Cd and Hg in muscles and liver. Spatio-temporal and biological variations were highlighted. The highest concentrations were observed in the liver for the three elements. Mean Pb and Cd concentrations are higher in Algiers specimens. However, the specimens from Dellys are more contaminated by Hg in the liver (0.35±0.0001µg/g). Medium and large specimens show higher concentrations of the three metals. There was no clear seasonal pattern in concentration of Pb, while Hg concentrations are higher in autumn, and Cd in summer, spring and winter. No impact of the three metal elements on Fulton's K and the hepato-somatic indexes was observed. Despite the average concentrations of Pb and Cd exceeded the recommended regulatory values, few risk related to the consumption of contaminated *M. barbatus barbatus* inhabiting the Algerian coasts were demonstrated.

1. Introduction

The coastal ecosystem is often subject to the adverse effects of industrial discharges (toxic to marine life). The Mediterranean Sea is an enclosed and polluted sea. Pollutants enter the food chain and could be transferred to humans through the consumption of seafood (Couture, 2017).

The Algerian coast receives significant inputs of metallic pollutants. Indeed, several studies have highlighted the fact that the Algerian coast is subject to significant anthropogenic pressure (Tireche, 2006; Guendouzi, 2015; DIPI, 2015; Ghobrini et al., 2016). According to Cossa and Fileman (1989), Algeria is ranked among the top mercury producing countries in the world. About 65% of the population is concentrated in the north of the Algeria enhancing therefore pressures on the coastal environment. Thereby, 3000 uncontrolled waste dumps are mostly located along the wadis that discharge into the sea, 17 urban wastewater treatment plants have been built along the Algerian coastline but unfortunately only 5 of them are operating normally which represents about 25% of the total treatment capacity (A.E.E, 2006). Belkacem and Aurora (2018) report clearly that pollution is the cause of the degradation of aquatic ecosystems, and the reduction of fisheries resources in the Bay of Algiers.

The studied species, *Mullus barbatus barbatus* (Linnaeus, 1758) is regularly used as sentinel species for pollution assessment along the Mediterranean coast (e.g. MedPOL programme; FAO-CGPM., 2002). Meinesz, 2011 considers that *M. barbatus barbatus*, as it incorporates in its tissues old contamination stored in the sediments, could constitute a potential indicator of the historical contamination of the environment and play an important role in a monitoring network for the Mediterranean coasts, due to its benthic compartment and sedentary lifestyle.

In Algeria, this bentho-demersal species is a species of interest to fishermen and highly appreciated by consumers. Despite its economic importance, few studies have been carried out on the health risks that could result for the consumption of contaminated specimens. Also studies on the assessment of the quality of coastal ecosystems using this species as bio-indicator are still

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rare. The only recorded works are: Bachouche et al. (2017) reported that the muscle tissue of *M. barbatus* from Algiers Bay contained metal elements (Cd and Hg) exceeding recommended standards; Atoui et al. (2019) reported that Cd and Pb concentrations measured in the flesh of *M. barbatus* present higher levels than regulatory values; Bentata-Keddar (2015) showed that Cd concentrations in the liver and muscle of *M. barbatus* are lower than the FAO recommendations.

The purpose of the present study is to investigate the levels of Pb, Cd and Hg (potentially toxic elements) concentrations in the muscle and liver of *M. barbatus* from the central-eastern Algerian coast, in order to evaluate the dynamics of these three contaminants according to spatiotemporal parameters and biological parameters, to assess possible health risks associated with the consumption of this species, and finally, to investigate the quality of the living habitat of this fish. Recommendations will be communicated to deciders for best management of both fish resource and environmental quality.

2. Materials and methods

2.1. Study site

The sampling took place in the Bay of Algiers and the Gulf of Bejaia (Fig. 1), two sites subject to strong anthropic pressure, and the Bay of Dellys less polluted site regarding the two first sites (Fig. 1) (Tireche, 2006; Ghobrini et al., 2016).

2.2. Sampling, biometry and calculation of biological indices

424 specimens of *M. barbatus* (Linnaeus, 1758) were sampled in the three targeted sites during four seasons (autumn, winter, spring and summer) from October 2017 to September 2018 (Table 1). In the laboratory, sampled specimens were identified and cleaned with distilled water. The total length (Lt) in centimetres (cm), total weight (Pt) in grams (g) and eviscerated weight (Pe) in (g) were measured on each fish specimen. The specimens were classified into 3 size classes (small: Lt < 13 cm; medium: 13 < Lt < 16; large: Lt > 16 cm). Liver and muscle were collected and weighed, then placed in plastic pillboxes (sterile, labelled and coded) and freeze-dried in a freeze-dryer (CHRIST Betta 1–8) to stop any chemical or biological transformation.

The Fulton condition factor K was calculated according to the formula:

$$K = (Pt/Lt^3) \times 100.$$

The hepato-somatic index (HSI) was calculated according to the formula:

$$HSI = Pf/Pe \times 100$$ with Pf: liver weight (g).

2.3. Determination of potentially toxic elements

The freeze-dried materials were ground and mineralised in Teflon reactors in the microwave (SPEEDWAVE TWO V. 2 .0) as follow: 500 mg of sample (liver or muscle), 8 mL of HNO_3 and 2 mL of H_2O_2 mineralised at 200 °C during 40 min. The mineralised samples were transferred to tubes and made up to 50 mL with ultrapure water. The tubes were kept at low temperature (4 °C) pending analysis. The determination of Cd and Pb was carried out by Inductively Coupled Plasma Mass Spectrometry (ICP/MS) Agilent Series 7700 at the SONATRACH research and development centre (CRD). Standards of different concentrations (5 ppb, 10 ppb, 20 ppb, 50 ppb ...) are prepared by diluting an intermediate solution using two multi-element standards and a single element mercury (Hg) standard supplied by Agilent. The determination of Hg was carried out by Mercury Analyzer (NIC) in the same centre. The accuracy of the analytical results was verified through two interlaboratory proficiency tests "Metals on clean waters" carried out by the General Association Laboratories Analysis Environment (AGLAE, 2018a for Pb, Cd and 2018b for Hg) on the basis of statistical exploitation of the results.

The calculation of the assigned material values (mean m) and the standard deviation for suitability assessment (standard deviation used for the calculation of the z-score) were evaluated with an improved version of Algorithm A of ISO 13528.

2.4. Statistical study

The 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.

2.5. Evaluation of health risks linked to the consumption of M. barbatus barbatus

(a) 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, 2000). THQ is calculated for a single element, as follows:

$$THQ = [(EFr \times EDtot \times Wfood \times Ci)/(RfDo \times Bw \times ATn)] \times 10^{-3}$$

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., 2018),

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

RfDo is the reference oraldose (Cd = 1×10^{-3} μg/g/day, Hg = 1.6×10^{-4} μg/g/day, Pb = 4×10^{-3} μg/g/day) (USEPA, 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 indicates that daily exposure does not cause adverse effects on human health over a lifetime,
- THQ $\geq$ 1 indicates possible side effects.

(b) Estimated weekly intake

Consumer exposure to Cd, Pb, Hg was also estimated by calculating the weekly intake estimated as follows (Pastorelli et al., 2012):

$$EWI = (C \times Ci)/Bw$$

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

C is the weekly fish consumption rate (84 g/week) (MADRP., 2018),

Ci is the level of contaminant in the food (μg/g ww);

THQ and EWI of Pb, Cd and Hg in the *M. barbatus* muscle analysed in present study provide an indication of the level of risk due to these toxic metals but do 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).

Our assessment of the risk of potentially toxic elements associated with the consumption of *M. barbatus* muscle is based on certain provisions applicable to EFSA (European Food Safety

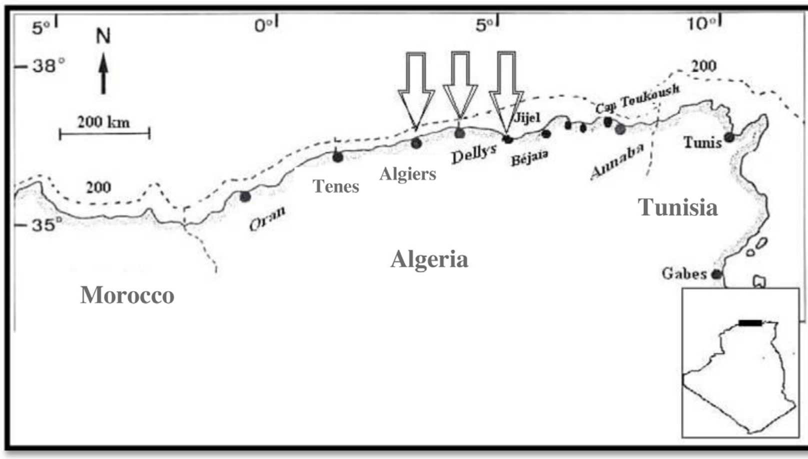

Fig. 1. Location of the sampling sites on the Algerian coastline, shown by the arrows (map modified from Hamida, 2005).

Table 1
Characteristics of the biological material examined.

Mullus barbatus barbatus (Linnaeus, 1758) (N = 425)							
Study site/class/number		Small specimens class		Medium specimens class		Large specimens class	
Algiers	Size class	Lt < 13 cm		13 < Lt < 16 cm		Lt > 16 cm	
	Number/size class	n = 49		n = 125		n = 49	
	Number/Season	**Autumn** (n = 42)	**Winter** (n = 41)		**Spring** (n = 77)		**Summer** (n = 63)
Total		N = 223					
Bejaia	Number/size class	n = 64		n = 54		n = 26	
	Number/Season	**Autumn** (n = 43)	**Winter** (n = 47)		**Spring** (n = 31)		**Summer** (n = 23)
Total		N = 144					
Dellys	Number/size class	n = 15		n = 29		n = 14	
	Number/Season	**Autumn** (n = 19)	**Winter** (n = /)[a]		**Spring** (n = 22)		**Summer** (n = 17)
Total		N = 58					

[a]No sample.

Security). The PTWI "Provisional tolerable weekly intake" is a reference value established by EFSA. The FAO/WHO Expert Commission on Contaminants in Foods (JECFA) has established the PTWI for Pb at 25 μg/kg, for Cd at 2.5 μg/kg and for Hg at 4 μg/kg (JECFA, 2000). The exposure assessment for potentially toxic elements was carried out using the EWI.

2.6. Results and discussion

2.6.1. Level of contamination of liver and muscle of M. barbatus barbatus

The Pb, Cd and Hg concentrations are summarised in Table 2. Whatever the site and the fish size classes, the average concentrations of Pb, Cd and Hg in the liver of *M. barbatus* are higher than those recorded in the muscle (7 times higher than those recorded in the muscle for Pb and Cd and 2 times higher than those recorded in the muscle for Hg) . The liver is the assimilating organ of these 3 metals. Chahid (2016) confirms that the liver is a good indicator of chronic metal exposure, and plays an important role in their storage and inactivation. Ennouri et al. (2013) noted the highest concentrations of Cd, Pb and Hg in the liver and gills of *M. barbatus*. The metals were shown to be eliminated more rapidly from the muscle than from the liver (Yilmaz et al., 2007). Metallothioneins protect cells against metal ion aggression (detoxification role by capturing excess metals of exogenous origin) (Bensakhria, 2018). Fish muscle provides a low metal content due to its low metabolic activity (El Morhit et al., 2012).

Statistically significant differences ($p < 0.05$) were recorded only at the Dellys site between the Hg concentrations of the three size classes of *M. barbatus*. Indeed, the highest Hg concentrations were noted in small and medium sized specimens (Table 2). High concentrations of this metal in young or juvenile fish may be related to the young life stages which generally show a higher sensitivity to metals (Hoang et al., 2004). Metabolic activity in fish is inversely proportional to size (Durville et al., 2003). Our results corroborate with several works that have shown that metal accumulation is higher in young than in older *M. barbatus* (Nussey et al., 2000; Widianarko et al., 2000). However, high Hg concentrations have also been found in older specimens of *Diplodus sargus*, a littoral, benthic and omnivorous fish (Casadevall et al., 2017).

Pb and Cd contaminate mainly the medium and large size classes, considering the liver of *M. barbatus*, and the small and medium size classes for the muscle. The comparison between the size classes shows statistically significant differences in the concentrations of these two metal elements (Table 2). The decreased concentrations in large fish can be explained by a dilution effect with growth and the ionic exchange in the fish media (Pourang et al., 2005). Gaspic et al. (2002) showed that Cd and Pb concentrations in the liver decrease with increasing length in *Merluccius merluccius* (Linnaeus, 1758) and *M. barbatus barbatus*. In fact, if fish growth is faster than metal accumulation, the observed metal elements concentration decreases with age and weight. Negative correlations between length and concentrations of Mn, Cu, and Cd were reported in *Lethrinus lentjan* from Arabian Gulf (Al-Yousuf et al., 2000). However, other studies reported different results with no significant correlation between size and metal concentration, as it was shown for Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in *Pagellus acarne* (El morhit et al. 2013), or for Cd in *Scophthalmus maximus*.

Table 2
Variations in annual mean concentrations of Pb, Cd and Hg (in μg/g weit weight) in liver and muscle, as well as biological parameters of *M. barbatus barbatus*, 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 (n = 223)	Small (n = 49) (Lt < 12 cm)	11.4_a (1.41)	1.18_a (0.16)	1.33_a (0.63)	2.22_a (0.21)	0.32_a (0.02)	$1.04_{a,b}$ (0.97)	$0.28_{a,b}$ (0.12)	0.25_a (0.07)	0.10_a (0.07)
	Medium (n = 125) (12 cm < Lt < 15 cm)	14.2_b (0.83)	$1.22_{a,b}$ (0.10)	1.47_a (0.76)	1.66_a (0.68)	0.15_b (0.10)	0.49_a (0.23)	0.41_a (0.61)	0.26_a (0.19)	0.15_b (0.09)
	Large (n = 49) (Lt > 15 cm)	17.1_c (1.40)	1.26_b (0.11)	2.08_b (0.53)	1.83_a (1.44)	0.28_a (0.19)	2.84_b (6.71)	0.16_b (0.20)	0.30_a (0.18)	$0.13_{a,b}$ (0.08)
	Average concentrations/site/organ				**1.90 (0.77)**	**0.25 (10)**	**1.45 (2.63)**	**0.28 (0.31)**	**0.27 (0.14)**	**0.12 (0.08)**
	Meaning of Statistical Test (P)	***< 0.001***	***0.012***	***< 0.001***	***0.187***	***< 0.001***	***0.003***	***0.014***	***0.388***	***0.008***
Bejaia (n = 144)	Small (n = 64) (Lt < 12 cm)	11.4_a (1.41)	1.18_a (0.16)	1.33_a (0.63)	0.41_a (0.0001)	0.06_a (0.0001)	0.31_a (0.15)	0.21_a (0.25)	0.22_a (0.18)	0.19a (0.05)
	Medium (n = 54) (12 cm < Lt < 15 cm)	14.2_b (0.83)	$1.22_{a,b}$ (0.10)	1.47_a (0.76)	0.47_a (0.29)	0.09_b (0.0001)	0.51_a (0.26)	0.03_b (0.02)	0.28_a (0.22)	0.20a (0.10)
	Large (n = 26) (Lt > 15 cm)	17.1_c (1.40)	1.26_b (0.11)	2.08_b (0.53)	1.07_b (0.45)	0.06_a (0.01)	0.49_a (0.45)	$0.10_{a,b}$ (0.26)	0.33_a (0.04)	0.20_a (0.05)
	Average concentrations/site/organ				**0.65 (0.24)**	**0.07 (0.003)**	**0.43 (0.67)**	**0.11 (0.17)**	**0.27 (0.14)**	**0.19 (0.07)**
	Meaning of Statistical Test (P)	***< 0.001***	***0.012***	***< 0.001***	***< 0.001***	***< 0.001***	***0.061***	***0.005***	***0.176***	***0.808***
Dellys (n = 58)	Small (n = 15) (Lt < 12 cm)	11.4_a (1.41)	1.18_a (0.16)	1.33_a (0.63)	.[a]	.[a]	0.96_a (0.0001)	0.38_a (0.0001)	0.35_a (0.0001	0.21_a (0.0001)
	Medium (n = 29) (12 cm < Lt < 15 cm)	14.2_b (0.83)	$1.22_{a,b}$ (0.10)	1.47_a (0.76)	0.24_a (0.14)	0.03_a (0.02)	0.70_a (0.24)	0.04_b (0.05)	0.14_b (0.03)	0.18_a (0.07)
	Large (n = 14) (Lt > 15 cm)	17.1_c (1.40)	1.26_b (0.11)	2.08_b (0.53)	0.12_b (0.11)	0.03_a (0.01)	0.69_a (0.34)	0.01_b (0.0001)	0.23_c (0.0001)	0.09_b (0.10)
	Average concentrations/site/organ				**0.18 (0.12)**	**0.03 (0.01)**	**0.78 (0.27)**	**0.14 (0.19)**	**0.24 (0.10)**	**0.16 (0.06)**
	Meaning of Statistical Test (P)	***< 0.001***	***0.012***	***< 0.001***	***0.028***	***0.526***	***0.051***	***< 0.001***	***< 0.001***	**0.002**

ww: weit 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 size classes regarding the biological parameters and the metal concentrations in each matrix.
[a]Not detected.

2.6.2. Seasonal variations

The higher concentrations of the three studied metals (Pb, Cd and Hg) recorded in the liver were observed whatever the season (Fig. 2). The highest values of Pb, Cd and Hg were recorded in summer (Fig. 2A), spring (Fig. 2B) and autumn (Fig. 2C) respectively. The same result was obtained for *Sardina pilchardus* sampled in the same sites (see Aissioui et al. submitted).

According to Martinez-Gomez et al. (2012), the evolution of the physiological state during the reproductive season of the fish would influence the accumulation of metals elements. Indeed, Layachi et al. (2007) reported that the minimum values of the vacuity coefficient of *M. barbatus* were observed in winter, with a beginning of fall from the summer. According to EL Bouhali et al. (2008) the diffusion of Pb through the skin barrier is a possible mechanism for the bioconcentration of Pb in fish. This would explain, in our case, the increase in Pb concentrations from autumn to summer (low feeding and reproduction period). The trophic transfer of these metals (autumn/winter) is therefore noted during the reserve accumulation seasons in this species. Merbouh (1997) have noted in *M. barbatus* and *S. pilchardus* an increased hepatic activity occurring before and after spawning, which explains the high accumulation of pollutants with the trophic regime. The probable hypothesis for the accumulation of these metals in warm seasons (in our case early spring and late summer) could be related to the input of pollutants and the metabolic activity of fish. Kargin (1996) notes a difference in contaminant concentrations in the tissues of *M. barbatus* linked to seasonal change, so that the warm season records the highest concentrations: application of fertiliser and increased metabolic activity of the fish (by temperature). According to Khan et al. (2011), native organisms had developed adaptive mechanisms to minimise metal elements internalisation. The environmental factors have a limited effect on the trophic transfer of metals in fish (Pouil, 2017).

2.6.3. Inter-site differences

The highest mean concentrations of Hg in the muscle of *M. barbatus barbatus* were recorded in the bays of Bejaia (0.19 μg/g ww) and Dellys (0.16 μg/g ww) (Table 2). The highest mean concentrations of Pb and Cd were recorded in Algiers for both matrices (Pb: 1.90 and 0.25 μg/g ww; Cd: 1.45 and 0.28 μg/g ww in liver and muscle respectively) (Table 2).

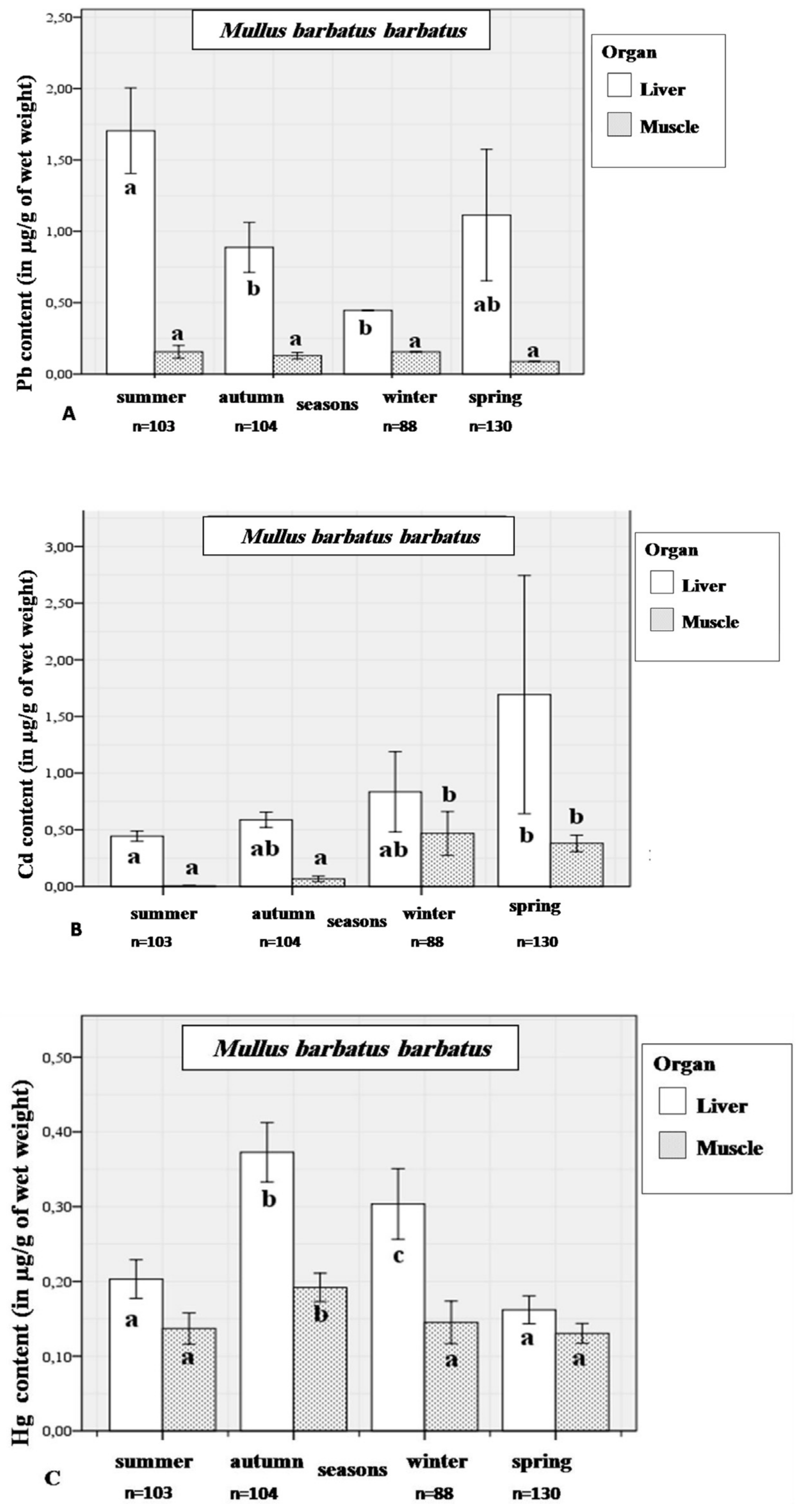

Fig. 2. Variation in the average concentrations (μg/g wet weight) of Pb (A), Cd (B), Hg (C) and 95% of standard deviation (error bars) in the liver and in the muscle of *M. barbatus barbatus* sampling on the Algerian coast, according to the seasons (The letters a, b, c indicate significant seasonal differences for the same matrix).

According to Tireche (2006), Hg is found in the discharges of the pharmaceutical and chemical industries and in the effluents of textile factories near the Dellys site. Existing cement factories are also a source of Hg. Furthermore, the mining activity recorded at the Dellys Bay may therefore generate higher concentrations of geochemical background in soils, sediments and water (Aranguren, 2008). In the Bejaia region, the main sources of mercury emissions are industry and household waste incineration (DIPI, 2015). It should be noted that waste dumps (batteries, metallic mercury, products derived from dentistry, pharmacy, pesticides used in agriculture, etc.) are often located upstream of watercourses (Soummam wadi, Agrioun wadi, Souk Elthnine

Table 3
Variations in annual mean concentrations of Pb, Cd and Hg (in µg/g weit weight) in liver and muscle of a pelagic species, *Sardina pilchardus* (Aissioui et al.,submitted) and a benthic species, *Mullus barbatus barbatus* in the three study sites.

		Algiers Bay						Bejaia Golf						Dellys Bay					
		Pb		Cd		Hg		Pb		Cd		Hg		Pb		Cd		Hg	
Species	Size (cm)	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
Mullus barbatus barbatus (n = 425)	Range Min-Max	**1.90 (0.77) 1.66– 2.22**	0.25 (0.10) 0.15– 0.32	1.45 (2.63) 0.49– 2.84	**0.28 (0.31) 0.16– 0.41**	**0.27 (0.14) 0.25– 0.30**	0.12 (0.08) 0.10– 0.15	0.65 (0.24) 0.41– 1.07	0.07 (0.003) 0.06- 0.09	0.43 (0.28) 0.31– 0.51	**0.11 (0.17) 0.03– 0.21**	**0.27 (0.14) 0.22– 0.33**	0.19 (0.066) 0.19– 0.20	0.18 (0.125) 0.12– 0.24	0.03 (0.015) 0.03– 0.03	0.78 (0.27) 0.69– 0.96	**0.14 (0.19) 0.01– 0.38**	**0.24 (0.10) 0.14– 0.35**	**0.16 (0.056) 0.09– 0.21**
Sardina pilchardus (n = 872)	range Min-Max	0.35 (0.16) 0.29– 0.41	0.23 (0.26) 0.21– 0.25	1.17 (1.16) 0.95– 1.53	0.13 (0.093) 0.04– 0.31	0.23 (0.13) 0.18– 0.29	0.1 (0.036) 0.09– 0.11	0.38 (0.32) 0.35– 0.41	0.09 (0.01) 0.09– 0.09	0.67 (0.22) 0.57– 0.73	0.043 (0.02) 0.01– 0.08	0.20 (0.03) 0.14– 0.32	**0.22 (0.036) 0.08– 0.50**	**0.23 (0.02) 0.05– 0.49**	0.033 (0.0034) 0.01– 0.06	**0.82 (0.49) 0.62– 1.05**	0.015 (0.005) 0.01– 0.02	0.21 (0.066) 0.05– 0.46	0.13 (0.053) 0.06– 0.20
	P	**0.001**	**0.545**	**0.155**	**< 0.001**	**0.043**	**0.084**	**0.607**	**0.843**	**0.251**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.044**	**0.032**	**< 0.001**	**< 0.001**

Note: The values in brackets represent the standard deviation.

wadi, etc.) as well as certain mining activities (lover of the Soummam wadi), which allows this metal to be drained (runoff) into the coastal marine environment. Numerous factories (agro-food) are located in the catchment area of the Soummam river. The effluents from the textile and electrolysis factory in the Kherrata region located in front of the Souk Elthnine wadi in Bejaia.

At the Algiers site, the high Pb concentrations could be explained by the very high input of domestic wastewater and the activity of manufacturing Pb-acid batteries. The port activity through its wide use of Pb in Algiers, in fishermen's nets, the high consumption of fuel by motor boats as well as the nature of the paint on the hulls of the various boats are at the origin of high concentrations of Pb in this zone (Mzoughi and Chouba, 2005). Ship repair activities in the port of Algiers cause severe pollution of the seabed through the use of Pb-based paint pigments. According to Tireche (2006), Pb emissions were for a long time dominated by automobile transport due to the presence of Pb in gasoline.

Regarding Cd, the presence of the thermal power plant at the port of Algiers may explain the high Cd levels recorded in the Bay (e.g. cadmium-laden fly ash). The values found in the analysis of fly ash samples by Moufti and Mountadar (2004) are higher than international standards for some metals (Cd, Al and Ti). Two wadis flow into the Bay of Algiers, the Wadi El Hamiz and the Wadi El Harrach. The latter drains domestic and industrial wastewater, especially from the city of Algiers, only 8% of which is treated and discharged directly into the bay (PAC, 2005). In Dellys, the high average Cd concentrations (0.78 μg/g ww in the liver) could be attributed to the nearby agricultural activity and discharges from a fertiliser industry. Cadmium is a minor constituent of fertilisers used in agriculture.

2.6.4. Fluctuations of biometric indexes and anomalies

In Algiers Bay, a high polluted site, the highest values of Fulton's K (K = 1.18–1.26%) and hepato-somatic index (HSI = 2.08%) were recorded (Table 2). The use of these parameters to assess the effects of environmental stress on a target organ or whole organism was recommended by many authors (Lloret and Planes, 2003). Their fluctuations can be attributed to various factors such as reproductive status, food availability (Chellappa et al., 1995). In Algiers Bay, these indexes seem to be not affected by the 3 bioaccumulated elements in the muscle and liver. *M. barbatus* capitalises its energy reserves (good condition and overweight) despite the presence of these pollutants in its ecosystem, and this could happen only when favourable conditions are met (food availability, detoxification and neutralisation system, stage of its ontogeny, etc.). On the west coast of Algeria, Taleb and Boutiba (2007) reported an inversely proportional correlation between the bioaccumulated pollutants and the condition coefficient of *Mytilus galloprovincialis* (Lamarck, 1819), particular for zinc, cadmium and iron. The simultaneous influence of the salinity-metal ion couple indicates, in general, a decrease in the tolerance of these ions when salinity increases (Semsari and Haït-Amar, 2001).

We report here that anomalies were observed externally (gill nodules, erosion and blackening of fins) and internally (whitish kidney nodules, whitish to slightly yellowish spots lining the surface of the liver) in some specimens of *M. barbatus* (juveniles and adults) from the two sites, the Bay of Algiers and the Gulf of Bejaia. These anomalies are not very frequent in our samples and their origins could be linked to several factors: quality of the environment (pollutants), physico-chemistry of the environment, pathologies (bacteria, parasites, fungi), etc. Very few studies have demonstrated a direct relationship between potentially toxic elements and marine fish pathologies. Analysis of the Danube sterlet (*Acipenser ruthenus*) revealed a significant presence of sublethal histopathological changes that were most pronounced in the liver and skin and increased accumulation of heavy metals, with the highest concentrations in the liver (Poleksic et al., 2009). Liver of tilapia treated with cadmium showed degeneration of the hepatocytes with nuclear pyknosis in the majority of the cells. Gills showed necrosis and atrophy of the gill lamellae (Kaoud et al., 2011). A recent study confirms the variation in the degree of pollution between the coast and the open sea, where the lowest concentrations are found offshore (Mzoughi and Chouba, 2005). Several researchers also point out that for many ulcerative or nodular diseases observed in fish, it is difficult to specify the etiology of the disorders.

2.6.5. Health risk assessment

Mercury (Hg)

In the 3 studied sites, Hg concentrations in muscle and liver of *M. barbatus* (Linnaeus, 1758) did not exceed the regulatory value threshold (0.5 μg/g ww) (CE, 2008) (Table 3).

The highest concentrations values of Hg were recorded in *M. barbatus* from the eastern Mediterranean basin in the Turkish coast (0.434 ± 0.0127 μg/g) by Keskin et al. (2007) and in *M. barbatus* from the North African Mediterranean basin (Gulf of Bejaia, the present study) (0.19 ± 0.06 μg/g). The western Mediterranean basin presents the lowest concentrations values of Hg in the muscle of *M. barbatus* (Table 4).

Lead (Pb)

Our results show that the Pb concentrations recorded in *M. barbatus barbatus* exceed the regulatory value threshold (0.3 μg/g ww) (CE, 2015) in the liver of specimens of the three size classes in Algiers Bay and in the Gulf of Béjaia and are close to the norm (0.25 μg/g ww) in the muscle of small specimens in Algiers Bay (Table 3). Thereby, 66% and 0% of fish present higher concentration than threshold, considering liver and muscle, respectively.

Our results are in agreement with those of Bachouche et al. (2017) who reported in *M. barbatus barbatus* from the central Algerian coast, high concentrations of Pb (0.57 μg/g dw in muscle and 17.2 μg/g dw in liver). The highest mean Pb concentrations were recorded in Algeria, in the present study in Algiers bay (0.25 ± 0.10 μg/g ww) and western coast of Algeria (Oran) (0.189 ± 0.15 μg/g ww) by Bensahla talet (2014) and in Turkish coast (0.22 ± 0.08 μg/g ww) by Tepe et al. (2008). In *M. barbatus barbatus* from the western Mediterranean Basin, Pb concentrations remained low, not exceeding 0.07 μg/g ww (Table 4). Regarding four benthic fish species (Table 5), *Diplodus sargus* and *M. merluccius* are the species presenting the highest values of Pb (Ayad, 2010; Belhoucine, 2012) compared to *M. barbatus* and *Mullus surmuletus*, sampled in the same site (Oran).

Cadmium (Cd)

Cd concentrations recorded in the liver were above standard in specimens of the three size classes of *M. barbatus* in the three studied sites (which represents 100% fish) and exceed the regulatory value (0.05 μg/g ww) (CE, 2014) in the muscle of the three size classes in the Bay of Algiers, and in small specimens in the Gulf of Bejaia and Dellys Bay (Table 3), representing 66% of fish. Our results corroborate the work of Atoui et al. (2019) who reported in the Algerian eastern coastline high Cd concentrations in the flesh of *M. barbatus barbatus* at the port areas of Skikda 2 and Skikda 1 (0.76 ± 0.032 and 0.3 ± 0.016 μg/g dry weight respectively). Our results are in agreement with those of Bachouche et al. (2017) who reported in *M. barbatus barbatus* from the central Algerian coast, high Cd concentrations (0.58 μg/g dw in muscle and 2.2 μg/g dw in liver). In western Algeria, Bentata-Keddar (2015) found that Cd concentrations are above European standards, varying in *M. barbatus* muscle between 0.034 and 0.098 μg/g ww and in liver between 0.023 and 0.097 μg/g ww. The highest Cd values were recorded respectively in the western Mediterranean basin on the Spanish coast (1.24 ± 0.11 μg/g

Table 4
Concentrations of the three Potentially toxic elements in μg/g weit weight in the muscle of *Mullus barbatus barbatus* according to the different regions of the Mediterranean basin.

Study site		Pb (μg/g ww) mean ± (min–max)	Cd (μg/g ww) mean ± (min–max)	Hg (μg/g ww) mean ± (min–max)	Reference
Mediterranean Basin occidental	Spain (Catalonia)	0.002–0.07	0.001–0.01	0.14–0.36	Falco et al. (2006)
	Spain (Valenvia)	< LD	1.24 ± 0.11	0.093 ± 0.018	Martinez-Gomez et al. (2012)
	Italy (Sicily)	< 0.06	0.084 ± 0.069	0.08 ± 0.03	Copat et al. (2012)
	Italy (Sicily)	< LD	0.126 ± 0.125	0.138 ± 0.107	Naccari et al. (2015)
Mediterranean Basin Oriental	Egypt	0.02 ± 0.08	0.02 ± 0.08	–	Soliman and Mahmoud Nasr (2015)
	Turkey (Marmara Sea)	0.035 ± 0.0336	0.012 ± 0.0054	0.434 ± 0.0127	Keskin et al. (2007)
	Turkey (Trabzone)	0.22 ± 0.08	0.02 ± 0.00	–	Tepe et al. (2008)
	Turkey (Mersin)	**0.16** ± **0.03** (0.02–0.42)	< 0.0004	–	Korkmaz et al. (2017)
Mediterranean Basin North Africa	Tunisia	0.04–1.14	0.01–0.38	0.11–0.64	Ennouri et al. (2013)
	Oran (arzew)	0.189 ± 0.15	0.083 ± 0.054	–	Bensahla talet (2014)
	Oran	–	0.059 ± 0.025	–	Bentata-Keddar (2015)
	Beni saf (Ain Témouchent)	–	0.062 ± 0.036	–	
	Algiers Bay	**0.25 ± 0.10** **0.15–0.32**	**0.28 ± 0.31** **0.16–0.41**	**0.12 ± 0.08** **0.10–0.15**	Present study
	Bejaia Golf	**0.07 ± 0.003** **0.06–0.09**	**0.11 ± 0.17** **0.03–0.21**	**0.19 ± 0.06** **0.19–0.20**	Present study
	Dellys Bay	**0.03 ± 0.015** **0-0.03**	**0.14 ± 0.016** **0.01–0.38**	**0.16 ± 0.05** **0.09–0.21**	Present study

< LD Detection limit of the device.
Mean ± (min–max) mean ± standard deviation (minimum–maximum).

Table 5
Concentrations of Pb, Cd and Hg in μg/g weit weight in the muscle of benthic fish species caught along the Algerian coast.

Fish species	Sites	potentially toxic elements			Authors
		Pb (μg/g ww) mean ± (min–max)	Cd (μg/g ww) mean ± (min–max)	Hg (μg/g ww) mean ± (min–max)	
Mullus barbatus barbatus	Oran (arzew)	**0.189** ± 0.15	0.083 ± 0.054	–	Bensahla talet (2014)
	Oran	–	0.059 ± 0.025	–	Bentata-Keddar (2015)
	Beni saf (Ain Témouchent)	–	0.062 ± 0.036	–	
	Algiers Bay	**0.25** ± **0.10** **0.15–0.32**	**0.28** ± **0.31** **0.16–0.41**	**0.12** ± **0.08** **0.10–0.15**	Present study
	Bejaia Golf	**0.07** ± **0.003** **0.06–0.09**	**0.11** ± **0.17** **0.03–0.21**	**0.19** ± **0.06** **0.19–0.20**	Present study
	Dellys Bay	**0.03** ± **0.015** **0–0.03**	**0.14** ± **0.016** **0.01–0.38**	**0.16** ± **0.05** **0.09–0.21**	Present study
Merluccius merluccius	Oran	**0.29**±0.28	**0.198**±0.059	–	Belhoucine (2012)
Mullus surmuletus	Oran	0.051 ± 0.062	0.021 ± 0.019	–	(Borsali, 2015)
Diplodus sargus	Oran	**0.32**	0.114	–	Ayad (2010)
	Beni Saf (Ain temouchent)	**0.20**	**0.203**	–	

Mean ± (min–max) mean ± standard deviation (minimum–maximum).

Table 6
Target hazard quotient (THQ) and estimated weekly intake (EWI; μg/kg ww) related to Cd, Pb and Hg consumption in the muscle of *Mullus barbatus barbatus* from different regions of the Mediterranean basin.

Study sites		THQ			EWI			References
		Pb	Cd	Hg	Pb	Cd	Hg	
Mediterranean Basin occidental	Italy (Catania)	8×10^{-6}	0.003	33×10^{-6}	/	0.025	0.024	Copat et al. (2012)
	Italy (S. Agata)	0.000	/	0.62	0.1	/	0.44	Traina et al. (2019)
Mediterranean Basin Oriental	Turkey (Antalya)	2.9	3.0	//	//	//	//	Yipel and Yarsan (2014)
	Turkey (Aliaga)	0.11	0.75	2.98	0.1–3.3	0.01–22.8	0.3–5.7	Pazi et al. (2017)
Mediterranean Basin North Africa	Algeria (Alger)	0.0037	0.017	0.047	0.28	0.317	0.141	Present study
	Algeria (Bejaia)	0.001	0.006	0.073	0.078	0.126	0.219	Present study
	Algeria (Dellys)	0.00045	0.008	0.06	0.033	0.160	0.179	Present study

ww) by Martinez-Gomez et al. (2012) and in the North African Mediterranean basin in the Bay of Algiers (0.28 ± 0.33 μg/g ww; present study). In the eastern Mediterranean basin, Cd concentrations are low in the muscle of *M. barbatus*, not exceeding 0.02 μg/g ww (Table 4). Comparison of scientific work carried out along the Algerian coast on four species of benthic fish (Table 5), revealed that the highest Cd values were recorded in *M. barbatus* muscle sampling in Algiers and Bejaia (present study).

The calculated THQ (Table 6) for Pb, Cd and Hg in the three study sites are < 1 indicating that the concentrations measured in the muscle of *M. barbatus* do not represent an adverse effect on consumer health. Regarding the estimated weekly intake (EWI) for each metal, the values recorded in the 3 sites did not exceed the threshold of the provisional weekly intake (PTWI) set by EFSA, traducing the absence of health risk for the consumer. The high intakes of Pb (0.28 μg/kg ww) and Cd (0.31 μg/kg ww) were recorded in the most polluted site (Algiers). The high intake of Hg (0.21 μg/kg ww) was recorded in the second polluted site (Bejaia). Dellys recorded the lowest EWI.

The average consumption of Pb through the muscle of *M. barbatus barbatus* represents respectively 11.2%, 3.12% and 1.32% of the PTWI in Algiers, Bejaia and Dellys. That of Cd and Hg represent 1.26%, 0.50% and 0.64% (for Cd), and 3.52%, 5.47% and 4.47% (for Hg) of the PTWI in Algiers, Bejaia and Dellys, respectively. These results show that the intake of the various toxic metals contained in the muscle of *M. barbatus barbatus* from Algerian coast does not exceed the safety level (without health risk to the consumer).

The overview of data acquired on Mediterranean basin revealed that eastern area presented the highest THQ values. In two different regions in Turkey, the THQ of toxic metals are indeed higher than 1, indicating a danger to human health from consumption of contaminated *M. barbatus*. The calculated THQs of Pb, Cd and Hg from the western Mediterranean Sea and North African basins (in the present study) are < 1, indicating that the consumption of *M. barbatus* do not represent a risk for the consumer health, regarding these metals (Table 6).

Considering the estimated weekly intake (EWI), only the values recorded in the Eastern Mediterranean Basin exceed the threshold of the provisional weekly intake (PTWI) set by EFSA (EWICd = 22.8 μg/kg ww, EWIHg = 5.7 μg/kg ww) (Pazi et al., 2017).

2.6.6. Interspecies comparison between M. barbatus barbatus and S. pilchardus

An interspecies comparison of two differently behaving fish species, *M. barbatus* and *S. pilchardus* (sampled at the same sites and during the same period, Aissioui et al. submitted) shows significant differences in the mean concentrations of the three elements (Cd, Pb and Hg). The highest values were observed in *M. barbatus barbatus* (Table 3). This species accumulated these metals more than *S. pilchardus* in liver (Pb and Hg) and muscle (Cd) (Table 3). An exception could be observed for Hg recorded in the Gulf of Bejaia, where *S. pilchardus* accumulated significantly more in muscle compared to *M. barbatus barbatus* (Table 3). In Dellys Bay, *S. pilchardus* accumulated significantly more Pb and Cd in the liver, compared to *M. barbatus. This later species* is a bentho-demersal fish species that is more exposed to contaminants via the sediment, whereas *S. pilchardus* is a pelagic species without direct contact with the sediment. It should be noted that the content of trace elements in water depends on the content of sediments, which are reservoirs of pollutants. Youssao et al. (2011) obtained a positive and significant correlation between the lead content of sediments and that of water in the Nokoué-Chenal lagoon complex in Cotonou. Metals are mainly associated with fine particles (clays, iron oxides and hydroxides, organic matter, sulphides, etc.) in sediments (Diop et al., 2012). On the other hand, amphipod crustaceans, polychaetes, and bivalve molluscs are the main food of *M. barbatus*. Its stomach contains sand and shells due to its bottom feeding (Layachi et al., 2007). As filter feeders, bivalves are known to accumulate chronically numerous of pollutants (Gaitonde et al., 2006). The exposure of pelagic species such as *S. pilchardus* could be associated to atmospheric deposition of Hg, which is dominant at the surface, and anthropogenic inputs favour its abundance at the surface. Cd and Pb are integrated into the zooplankton, which could favour their accumulation.

The results of the present study revealed that the average concentrations of Pb, Cd and Hg measured in the liver of *M. barbatus* are always higher than those recorded in the muscle, regardless of the site and the size of the fish. *M. barbatus* specimens from the two bays, Bejaia and Dellys, showed the highest average concentrations of Hg. The highest concentrations of Pb and Cd were noted in *M. barbatus* from the bay of Algiers.

Globally, these three potentially toxic elements were found in higher concentrations in the muscle of small specimens, compared to medium and large ones. The relationship between some observed anomalies on fish (externally and internally on the body) and the concentrations of the 3 studied metal elements is not evidenced.

The comparison of our data with those obtained in various benthic fish species along the Algerian coast showed that *M. barbatus* contained the highest Cd and Hg values. Compared to a pelagic species sampling in the same sites and at the same period, it was shown that *M. barbatus* generally accumulated more the three metal elements than *S. pilchardus.* This is probably related to the burrowing behaviour of *M. barbatus*, which is thus more exposed to contaminants via the sediments.

These results also showed that the intake of Pb, Cd and Hg contained in the muscle of *M. barbatus barbatus* from Algerian coast does not exceed the safety level. The three elements measured in the muscle of *M. barbatus* (edible part) from the central Algerian coast do not present any risk to the health of consumers of this fish to date, despite its demersal nature in environments where the sediments are loaded with potentially toxic elements and environments located close to the discharge of these metals.

The comparison of these values with literature revealed that the North African and Eastern basins present the highest concentrations of Pb, Cd and Hg, while their concentrations remain generally low in the Western basin.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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