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ASSESSMENT OF LEVELS OF Pb, Cd AND Hg IN *SARDINA PILCHARDUS* (WALBAUM, 1792) FROM THE BAY OF BOUMERDES (ALGERIA)

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Abstract: Contaminated commercial fish species, like *Sardina pilchardus* (Walbaum, 1792), is the main way of the exposure of consumers to toxicity by heavy metals (contamination through diet). Then many risks maybe rise in the consumer's populations. The aim of the present study is to evaluate the concentration of three potential toxic heavy metals (Pb, Cd and Hg) in this fish, and to verify possible health risks to consumers from the studied region. For this purpose, one hundred specimens of this fish were sampled during two seasons, Spring and Summer (2017) from the Bay of Boumerdes (Algerian coast). Our results show that the higher mean concentrations of Cd were recorded in the liver. Mean concentrations of these three studied metals in the muscle did not exceed the recommended limits in this fish, and no risks related to its consumption can be suspected.

Keywords: *Sardina pilchardus*, Toxic heavy metals, Muscle, two seasons, Bay of Boumerdes.

INTRODUCTION

The nutritional composition of the fish is interesting, mainly because of its content of long chain polyunsaturated fatty acids (AGPI-LC) of the n-3 series, but also because of its vitamin D and iodine content. Unfortunately, fish are also carriers of various chemical contaminants, especially those potentially toxic (heavy metals). Consumption recommendations must therefore take into account both these nutritional benefits and the chemical risks (without exceeding the toxicological reference doses) (Medale, 2009; U.E, 2006; OMS/FAO, 1995).

The marine environment in which these fish live receives a considerable amount of pollutants (FAO, 1971). The main sources of marine pollutants are biological and chemical. In the case of heavy metals, they enter the aquatic environment through natural sources and anthropogenic sources (Bryan, 1984). Heavy metals are cumulative in the human body (fish consumers) and can cause irreversible damages (Aubert et al., 1989). For example, the ingestion of cadmium-contaminated food (Bernard and Lauwerys.,1986; Nordberg et al., 1973), rapidly causes vomiting, often bloody, associated with intense abdominal pain, diarrhea and myalgia (Chahid, 2016).

Haemodynamic disorders and renal toxicity maybe caused by this metal (Cd). Consumption of fish contaminated with MeHg affects the main target human organs: the nervous system, kidneys and blood (Chahid, 2016).

The Mediterranean Sea is strongly impacted by human activity and inputs of contaminating products, intense urban development and large concentrations of coastal tourism. These activities caused degradation and modifying of its ecosystems (D'Adamo et al., 2008), and also the disappearance of certain animal and/or plant species and consequently lead to the dysfunction of the trophic chain (Gold, 2002).

In recent decades, Algerian coastline has known heavy industrialization and rapid urbanization, affecting therefore the quality of marine water. In the Bay of Boumerdes particularly, the Sebaou River drains many pollutants like discharges from the plastics, pigments, batteries, mirror manufacturing and PVC industries (Ghobrini et al., 2017). The industries installed in the studied region are many: cement works, thermal power stations, fertilizer factories, etc. (Tireche, 2006). *Sardina pilchardus* accounts the largest share of marine catches and the most important in terms of commercial interest and biomass (Pinnegar et al., 2003). Studies previously conducted on this species in the Bay of Algiers (Ouabdesselam et al., 2017; Benguendouz, 2018), reported that the toxic metals (Pb, Cd and Hg) measured in its muscle do not exceed the regulatory limits.

The aim of this study is to determine the levels of Pb, Cd and Hg concentrations in the muscle and liver of *Sardina pilchardus* (Walbaum, 1792), to complete previous obtained data, especially for Hg scarcely studied until now, and to verify if the contamination by these three heavy metals present healthy risks for the consumers in the studied region.

MATERIALS AND METHODS

Fish samples were obtained in the fishing ports from fishermen whom fishing activity occurs in the Bay of Boumerdes (Figure 1).

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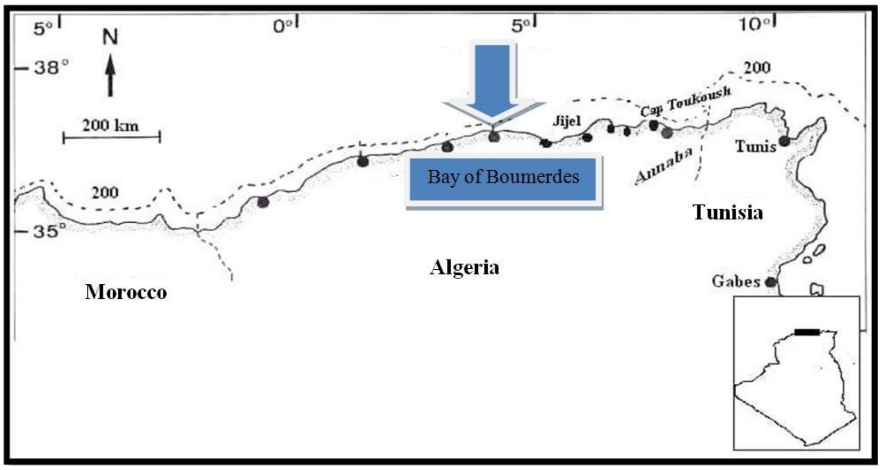

Fig. 1. Location of the study site (carte in Hamida, 2005 modified).

One hundred specimens of *Sardina pilchardus* of the medium size class most commercialised on the Algerian market (12cm<Lt<15cm) were sampled from the Bay of Boumerdes during Spring and Summer (2017), due to the availability of this species in this period. The fish were obtained from landings (boats using the seine net for sardine fishing). Organs and tissues (liver and muscle) intended for metal analysis are taken and then freeze-dried in a freeze-dryer (CHRIST Betta 1-8) in order to stop any chemical or biological transformation. The lyophilisates have been crushed and mineralised by microwave (SPEEDWAVE TWO V.2.0).

The Cd and Pb determinations were performed by ICP/MS Agilent Series 7700 (Inductively Coupled Plasma Mass Spectrometry) at the geochemistry laboratory of the Sonatrach Research and Development Center (CRD). The Hg determination was performed by NIC Mercury Analyzer at the Thermodynamics Laboratory at the same center. 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 reports: 2018a for Pb, Cd and 2018b for Hg) on the basis of statistical exploitation of the results:

- The calculation of the values assigned to the material (mean m) and the standard deviation for the proficiency assessment (standard deviation used for the calculation of the z-score) were evaluated with an improved version of the algorithm A of ISO 13528.
- The calculation of our measurement uncertainty was based on inter-laboratory test data in accordance with NF ISO 11352 (2013).

The results were presented in tables in the form of "mean ± standard deviation" for all the variables analysed. These statistical processes were carried out using IBM SPSS 24 software.

RESULTS AND DISCUSSION

Heavy metal levels in the liver and muscle of *Sardina pilchardus*

The mean concentrations of the three toxic metals (Pb, Cd and Hg) measured in the liver are higher than those recorded in the muscle (Table 1). The mean concentration of Cd 1.15±1.41 µg/g and Pb 0.35±0.09 µg/g of fresh weight in the liver of the *S. pilchardus* exceed the recommended value (0.25 and 0.3 µg/g of fresh weight respectively). However, the mean concentrations of Hg in liver (0.15±0.11 µg/g of fresh weight does not exceed the recommended limits (0.5 µg/g).

The liver is a good indicator of chronic exposure to metals and plays an important role in their storage and inactivation. Our results agree with those of (Chahid, 2016), which clearly showed that the liver of *S. pilchardus* has higher concentrations than in other organs. These results reflect the role of the liver, an accumulating and detoxifying organ, for the Pb and Cd of certain marine fish. (Ennouri et al., 2017) reported in *Liza ramada* (Risso 1810), that the liver is the preferential accumulation organ for Hg. He notes that the concentrations in the liver are higher than those found in muscle, and mentioned that the liver is the main vector of potentially toxic substances for the consumer.

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). Metallothioneins protect cells against metal ion aggression (detoxification role by capturing excess metals of exogenous origin) (Bensakhria, 2018).

For the muscle, the edible part of this fish, the mean concentrations of these three toxic metals are below the regulatory thresholds (Table 1). Our results corroborate with the work of (Hamida et al., 2018). According to this work, the Pb concentrations measured in *S. pilchardus* muscle of Boumerdes Bay sampled in 2017 are similar (vary from 0.021 to 0.055 µg/g fresh weight). Our results are also in agreement with the work of (Benguendouz, 2018), the values of Pb and Hg measured in *S. pilchardus* from the Bay of Algiers do not exceed the regulatory standard. These metals are maybe eliminated more rapidly from the muscle than from the liver (Agusa et al., 2007). According to El Morhit (2012), the fish muscle presents a low metal content due to its low metabolic activity.

Table 1.

Mean concentrations of Pb, Cd and Hg (µg/g of fresh weight) in the muscle and the liver of *Sardina pilchardus*

Site	Size (cm) (Min-Max)	Metal	Liver (µg/g)	Muscle(µg/g)	Maximum Recommended levels	
					Concentration(µg/g)	References
Boumerdes (n = 100)	13.12±1.55	Pb	0.35±0.09	0.17±0.09	**0.3**	(UE) No 2015/1005
		Cd	1.15±1.41	0.10±0.07	**0.25**	(UE) No 488/2014
		Hg	0.15±0.11	0.07±0.02	**0.5**	(CE) No 629/2008

S. pilchardus is planktonophagous; the accumulation of the three heavy metals studied in its liver and muscle could be due to the ingestion of contaminated zooplankton with heavy metals. Moreover, micro-plastics with fixed heavy metal are shown to be ingested by zooplankton (Cole et al., 2013). The transfer of these heavy metals through the different levels of the food chain could play a very important role in their bioaccumulation in the muscle and liver of fish. Heavy metals first accumulate in zooplankton (secondary production) and then pass to fish species (e.g. *Sardina pilchardus*) to reach the upper levels of the food chain. Nowadays, it is well accepted that the presence of micro-plastics (with heavy metal adsorption properties) in the surface layers of seawater and oceans amplifies and concentrates these metals in pelagic species (e.g. *Sardina pilchardus*). The major pathway for cadmium in fish is via the diet (Dallinger et al., 1987). Cadmium is bioconcentrated, the most important factor is bioavailability (the extent to which a substance can be taken up by the tissues of organisms) as it governs the entry of cadmium into the food chain. Indeed, once cadmium has entered the base of the food chain (phytoplankton), environmental factors have little nfluence on the contamination of superior organisms. Since phytoplankton is present in surface waters, it will be exposed to the cadmium dissolved in this compartment (Verge, 2006). In the marine environment, concentrations of lead and mercury especially in the methylated form (MeHg) increase as one evolution through the trophic chain (Chahid, 2016). MeHg is the predominant form of mercury that biomagnifies in the aquatic food chain (Renner, 2004). Previous evidence suggested that nearly all of the mercury (> 85 %) in the muscle tissue of fish occurs as MeHg (Krystek and Ritsema, 2005; Hight and Cheng, 2006). In the seawater column, microplastics promote the amplification of heavy metal concentrations in marine organisms, particularly in pelagic fish such as *S. pilchardus*. According to (Thompson et al., 2004), low-density microplastics are found in surface waters. They are ingested by zooplankton (Cole et al., 2013) and by adults and larvae of fish (Collignon et al., 2012; Browne et al., 2013; Lusher et al., 2013; Rochman et al., 2013b).

Our results were compared with previous studies conducted on the same fish species from different regions of the Mediterranean Sea (Table 2).

Aissioui et al. (2022) recorded in *Sardina pilchardus* from Dellys during four seasons (spring, summer, autumn, winter) a lower average concentration of Pb and Cd than those recorded in the present study (only spring, and summer). However, the average concentrations of Hg are higher in Dellys than those found in the present study.

Comparison between the different areas of the Mediterranean

Pb levels in *Sardina pilchardus* from Boumerdes recorded by (Ouabdesselam et al., 2017) are higher than those recorded in the Mediterranean Sea (Tab.2). In the present study, Recorded Cd levels are higher than those reported in different regions from the Mediterranean Sea (Tab.2). For Hg, the results of Aissioui et al. (2022) are similar to those of (Benguedouz, 2018) (0.129 µg/g) in Algeria and (Naccari et al., 2015) in Italy (0.132±0.108 µg/g).

Table 2.

Concentration of the three studied heavy metals in *Sardina pilchardus* (expressed in µg/g fresh weight) in different areas of the Mediterranean Sea.

Regions		Pb(µg/g)	Cd(µg/g)	Hg(µg/g)	References
Italy (Sicily)		< LD	0.087±0.031	0.132±0.108	(Naccari et al.,2015)
Algeria	Jijel	0.017	-	0.129	
	Algiers	0.016	-	0.091	
Algeria (Boumerdes)		0.99 ± 0.11	00	-	(Ouabdesselam et al., 2017)
Algeria (Boumerdes)		0.055±0.021	0.031 ± 0.017	-	(Hamida et al.,2018)
Algeria (Dellys)		0.03 ± 0.003	0.015 ± 0.005	0.13 ± 0.05	(Aissioui et al., 2022)
Algeria (Boumerdes)		0.17±0.009	0.10±0.07	0.07±0.02	**Present study**

< LD = Detection limit of the device

These differences can be attributed to sources of contamination from neighboring industries in the different regions of the Mediterranean Sea. Boumerdes Bay receives many pollutants from the Sebaou River. This last is threatened by human activity (domestic, industrial and agricultural) draining chemical substances and therefore altering marine water quality of the Bay of Boumerdes (Ghobrini et al., 2017). The same authors qualified the degree of pollution of the Sebaou River as yellow class, according to the National Water Resources Agency (ANRH): The general water quality is defined according to four quality classes determined from a double entry grid (quality class / measured parameters). Class III: Poor quality water, can only be used after very thorough treatment. It is represented in yellow. This study reveals lower Pb concentrations in the water of Sebaou River but very high Cd concentrations mainly coming from the plastic, pigment, battery, mirror manufacturing and PVC industries (Ghobrini et al., 2017). The industrial establishments contribute to air pollution (cement works, thermal power stations, fertilizer factories, etc.) (Tireche, 2006). To this can be added the leaching of soil from active or abandoned industrial sites, contaminated by various substances (nitrogen compounds, hydrocarbons, metal salts containing Cu, Pb, Zn, Cd, Hg, etc.), which leads to delayed toxicity through bioaccumulation (disorders of the metabolism of species) (Boeglin, 1999). According to (UNEP, 2010), the sources of these three toxic metals are: -natural discharges from the earth's crust (weathering and erosion of rocks, degradation of minerals and forest fires, and transport of metals to the aquatic environment (leaching, rivers, wadis, etc.) (Pays, 1999), they are released into the atmosphere, water and land (Pb, Cd and Hg) and arrive by runoff in aquatic environments; - waste incineration or open burning of waste containing (Pb, Cd), road network; - various industrial processes linked to human activities, industrial activity (cement production: Pb and Hg), phosphate fertilizers, acidification of agricultural land, contaminated landfill sites. A report by the Commission for Environmental Cooperation indicates that mercury very often ends up in aquatic systems where it accumulates in living organisms (Agence européenne pour l'environnement, 1997). Metals transported by the atmosphere can sooner or later contaminate aquatic environments (Pays, 1999; Morlot, 1999).

CONCLUSION

This study enabled us to determine the concentrations of three toxic heavy metals (Pb, Cd and Hg) in a species of major economic interest *S. pilchardus* at the level of the central Algerian coastline. The concentrations of measured toxic heavy metals in the edible part of *Sardina pilchardus* do not exceed the regulatory values, which do not indicate a risk of toxicity for human health (fish consumers). *S. pilchardus* from Boumerdes Bay is therefore healthy for consumption. The contamination of marine organisms like fish species by different types of pollutants provides information on the quality of the aquatic environment. Our results show that studied pollutants do not affect until now the water quality of Boumerdes Bay. In contrary, in some regions of the Mediterranean Sea, the recorded concentrations of some heavy metals, like Cd, are in an alarming average concentration in *S. pilchardus* from Oran and Turkey. Then, we urgently recommend the implementation of a control system for contaminants that can spoil seafood products and thus protect the health of consumers.

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AUTHORS CONTRIBUTIONS

Conceptualization Ideas, S. A. and R. Z.; Methodology Development, S. A., R. Z. and L. P.; Validation Verification, S. A. and R. Z.; Investigation Conducting a research and investigation process, Resources Provision of study materials, S. A.; Writing, Writing - Review & Editing, S. A., R. Z. and L. P.; Visualization Preparation, S. A., R. Z. and L. P.; Supervision, S. A. and R. Z.; Project administration, S. A. and R. Z.; Formal analysis, L. P.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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