Heavy metals (Zn, Cd, Cu, Pb, and Fe) assessment in sardines, Sardina pilchardus (Walbaum, 1792) from the Algerian west coast

Kalakhi N.[1]; Bendimerad M.A.[1]; Kerfouf A.[2*]

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Abstract

The sardine (Sardina pilchardus) is the most popular species for fishing of the Algerian coast. Five toxic metals zinc (Zn), cadmium (Cd), copper (Cu), lead (Pb), and iron (Fe) that can affect human health are studied in this fish. Samples are collected monthly between January and December 2019 in the Ghazaouet bay. The collected samples were analyzed by atomic absorption spectrophotometry by sex and for two organs (gonads and gills). For all the male and female samples, Zn and Fe showed the highest values, respectively (1.00±0.406 mg/kg.FW and 0.770±0.28 mg/kg.FW), however the low values concern Cu (0.017 mg/kg.FW). Zn and Cd contents are higher in the gonads, and those of Fe and Pb are higher in the gills. However, Cu concentrations are similar in both organs (gonads and gills). Statistical analysis recorded a significant difference ($p<0.05$) for all metals (Zn, Pb, Fe and Cd) except for Cu ($p> 0.05$) between the two organs. S. pilchardus may represent a hazard for consumers. S. pilchardus may represent a hazard for consumers, but a continuous monitoring of heavy metals is necessary to insure the prescribed worldwide limit.

Keywords: Sardina pilchardus, heavy metals, atomic absorption spectrophotometry, Ghazaouet bay, Algerian West Coast.

1-Laboratory of Valorization of Human Actions for the Environment, Protection and Application in Public Health. Abou Bakr Belkaid University, SNV / STU Faculty, B.P.119 - 13000 Tlemcen, Algeria.

2-Laboratory of Eco-development of Space. University Djillali Liabes, Sidi Bel Abbès, 22000, Algeria

*Corresponding author's E-mail: kerfoufahmed@yahoo.fr

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Introduction

Coastal areas are under intense stress from toxic contaminants, anthropogenic as well as industrial (Benallal et al., 2020; Kaddour et al., 2021). The Algerian West Coast receives significant inputs of anthropogenic pollutants (Remili and Kerfouf, 2013; Dilem et al., 2014; Guendouzi et al., 2021). Few studies have been conducted on this area so far, and most of them showed contamination sediments and plants with heavy metals (Dali-youcef et al., 2005; Benguedda-Rahal et al., 2011). The selected locality as its proximity to Ghazaouet harbor is exposed to chemicals coming mainly from industrial activities (Benguendouz, 2017). The toxicity persistence and bioaccumulation of heavy metals have resulted in the deterioration of aquatic ecosystems (Aissaoui et al., 2022). These heavy metals are concentrated in water and aquatic microorganisms, resulting in their bioaccumulation in all aquatic biological resources. The degree of accumulation in fish tissues is influenced by factors such as habitat, chemical elements present in the water, water conditions (temperature, transparency, pH and dissolved oxygen), age of fish, genus, body mass, and physiological conditions (Copat et al., 2012). Contamination by heavy metals represents one of the most direct impacts on biota out of the multitude of anthropogenic disturbances in coastal and estuarine systems (Vasconcelos et al., 2007).

The sardine (Sardina pilchardus) is the most popular species for fishing and the most consumed species by the local population. Seafood products are part of the animal protein resources and a wide variety of mineral salts, among which is sardine (Sardina pilchardus). This fish has a very important commercial and economic interest in Algeria (Hattab and Gaouar, 2018). The study of the discharge of metallic pollutants into the marine environment through biological material such as sardines makes it possible to evaluate the quality of the biological resources and of the marine environment. Therefore, this study aims to determine the concentrations of heavy metals in marine fish, which are common along the Algerian west coast. To assess the accumulation of heavy metals (Zn, Cd, Cu, Pb and Fe), by Clupeidae specimens (Sardina pilchardus, Walbaum, 1792) were sampled. These pelagic species are chosen due to their socioeconomic importance and abundance along the Algerian coastline to reflect the status of its habitat (Mehouel et al., 2019).

Materials and Methods

Presentation of the study area

The Ghazaouet Bay is located in northwestern Algeria at approximately 10 km from the Moroccan border (Fig. 1). The Ghazaouet town hosts an important harbor on the coast for the entire northwestern region of Algeria. Coastal seawaters are continuously exposed to industrial, urban, and agricultural wastes, releasing huge quantities of contaminants and trace metals, especially Zn and Cd, originating from a large industrial complex of zinc electrolysis, near the harbor of Ghazaouet (Benguedda et al., 2011; Belhadj et al., 2017).

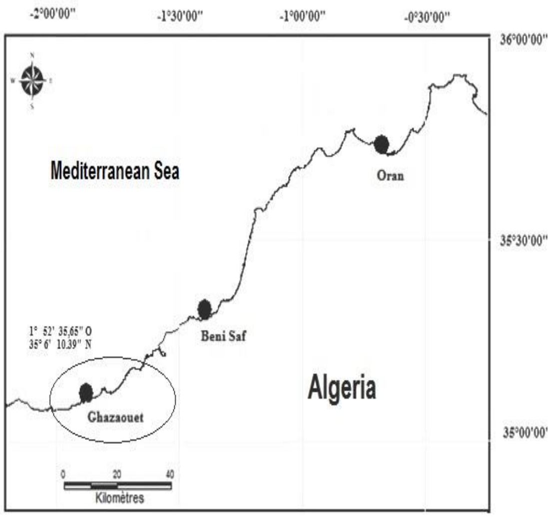

Fig. 1: Geographical location of the bay of Ghazaouet

Sampling

The sardine studied in this work comes from commercial fishing carried out at the port of Ghazaouet (N 35° 06′ 00″W, 01° 52′ 21″). Sampling was carried out (30 individuals/month) between January and December 2019. Due to their importance, the gonads and gills were targeted to analyze the desired metals and to determine the sex macroscopically. Out of 360 fish collected, 132 females and 111 males were macroscopically identified, however, the 117 were considered indeterminate. In order to assess the metal contents (Zn, Cd, Cu, Pb and Fe), we opted for wet mineralization because it allows us to minimize the loss of volatile organometallic compounds during drying. The method adopted is that of Amiard (1987), It consists of depositing 1g of fresh weight of each sample in a measurement glass to which is added 1 ml of nitric acid (HNO3) at 65% purity, then closed with help refrigerants. The apparatus is brought to a temperature of 95°C for one hour. After cooling, the mineralized is recovered in test tubes and the content is then adjusted up to 4 ml of double-distilled water. This solution is ready for assay Flame AAS Perkin Elmer: AANALYST 100 – version 1.10 5s70, fitted with H.C.L. Hallow Cathode Lamp (hollow cathode lamp), specific for each element: 0.05ppm (Zn), 0.10ppm (Pb), 0.05ppm (Cu), 0.03ppm (Cd) et 0.20ppm (Fe).

Data analysis

To compare the means of the heavy metals concentrations according to sex and organs, the Student test was used to estimate the significance of the differences between the concentrations of metal pollutants. A probability level <0.05 was considered significant. All statistical analyzes were performed with the software MINITAB for analysis and statistical treatment of the data, version 16 for Windows (2010).

Results

Overall, the study of the variation in the heavy metal contents accumulated by this species showd that their classification is presented respectively in the following order: Zn> Fe> Pb> Cd> Cu. The average concentrations were calculated relative to the fresh weight (FW) of gonads and gills, expressed in (mg/kg.W) (Tables 1, 2-3).

Table 1: Comparison of trace metals contents in the gonads by sex.

Metals	Sex	N	Mean±SD	P
Zn	♀	131	1,101±0,747	0,001*
	♂	111	1,51±1,07	
Pb	♀	66	0,038±0,017	0,943^{n}
	♂	69	0,038±0,019	

Cu	♀	119	0,015±0,011	0,002*
	♂	105	0,021±0,014	
Cd	♀	110	0,021±0,019	0,001*
	♂	94	0,032±0,026	
Fe	♀	132	0,585±0,294	0,439 ^{n}
	♂	111	0,555±0,311	

N: Number; M: Mean; SD: Standard Deviation; P:p-value; n: Non-significant difference; *significant difference.

Table 2: Comparison of trace metals contents in the gills by sex.

Metals	Sex	N	Mean±SD	P
Zn	♀	132	0,656±0,318	0,011*
	♂	111	0,780±0,420	
Pb	♀	64	0,057±0,019	0,990 ^{n}
	♂	62	0,057±0,019	
Cu	♀	117	0,016±0,011	0,510 ^{n}
	♂	106	0,017±0,010	
Cd	♀	114	0,018±0,021	0,017*
	♂	103	0,026±0,027	
Fe	♀	132	0,967±0,402	0,955 ^{n}
	♂	111	0,971±0,446	

N: Number; M: Mean; SD: Standard Deviation; P:p-value; n: Non-significant difference; *significant difference.

Table 3: Comparison of heavy metals contents according to the organs of all combined sexes

Metals	Organs	N	Mean±SD	P
Zn	Gonads	242	1,287±0,929	3,5e^{-17*}
	Gills	243	0,713±0,373	
Pb	Gonads	135	0,038±0,018	9,5e^{-15*}
	Gills	126	0,057±0,018	
Cu	Gonads	224	0,018±0,013	0,470^{n}
	Gills	223	0,017±0,011	
Cd	Gonads	138	0,037±0,022	1,8e$^{-0,8*}$
	Gills	217	0,022±0,024	
Fe	Gonads	243	0,572±0,301	1,2^{e-28*}
	Gills	243	0,969±0,422	

N: Number; M: Mean; SD: Standard Deviation; P:p-value; n: Non-significant difference; *significant difference.

Two factors (sex and organ) were taken into consideration to reveal their influence on the phenomenon of metallic bioaccumulation in S. pilchardus from Ghazaouet Bay. Zinc in male gonads present higher concentrations (1.51±1.07 mg/kg.FW) than those in females (1.1±0.74 mg/kg.FW), this metal is more accumulated by male gills (0.78±0.42 mg/kg.FW) than in females (0.65±0.31 mg/kg.FW). Cadmium tends to accumulate in the gonads and gills of male individuals (0.032±0.026 mg/kg.FW; 0.026±0.027ppm/FW) compared to female gonads and gills (0.021±0.019; 0.018±0.021 mg/kg.FW). Iron and lead show almost identical levels in both sexes at the level of all organs. Both sexes mark a similar value of lead for gonads (0.057±0.019). The concentrations of Cu measured in the gonads show that males (0.021±0.014 mg/kg.FW) are more contaminated than females (0.015± 0.011 mg/kg.FW). The Zn and Cd contents are higher in the gonads, but those of Fe and Pb are higher in the gills. However, Cu concentrations are similar in both organs (gonads and gills) Noting that statistical analysis reveals a significant difference (p <0. 05) between the two sexes. Except in the case of lead and iron in both organs and Cu in the gills (p> 0. 05).

The samples show that the content of the heavy metals changes by season (Figs 2, 3). The results show that the Zn contents are highest in winter (2.018±1.014) and in spring (1.567±0.796), in the gonads. In the gills, iron has the highest content levels in springer (1.093±0.339) and summer (1.034±0.470).

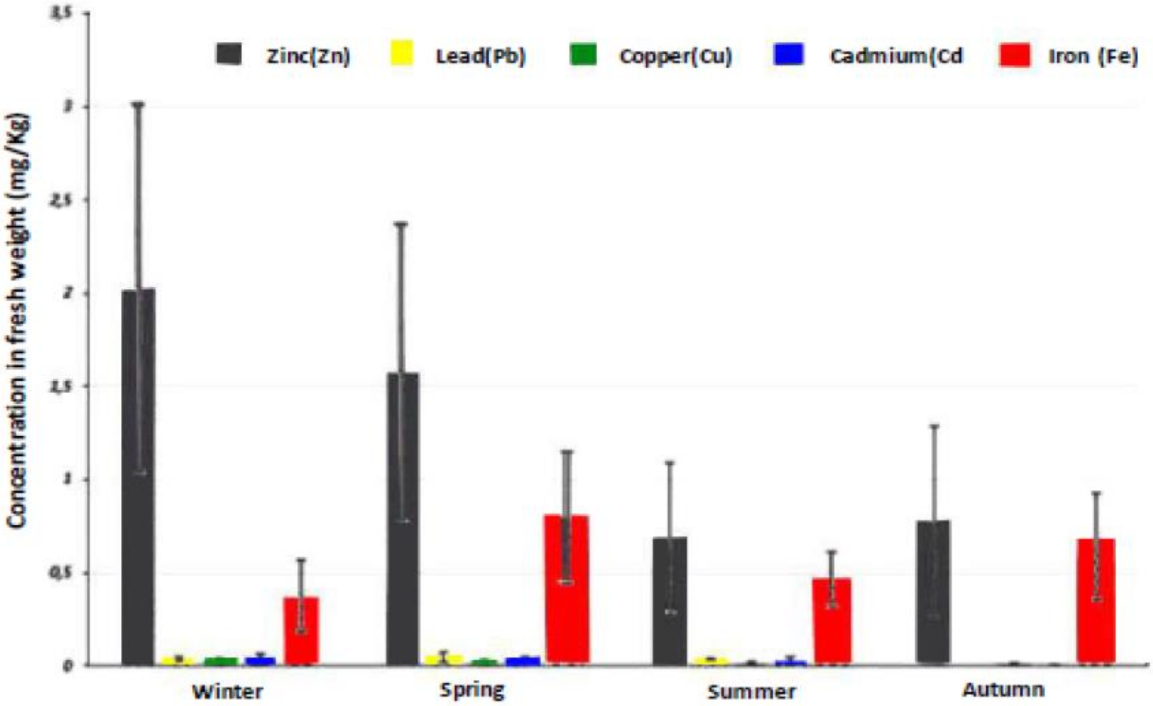

Fig. 2: Comparison by season of heavy metals levels in the gonads

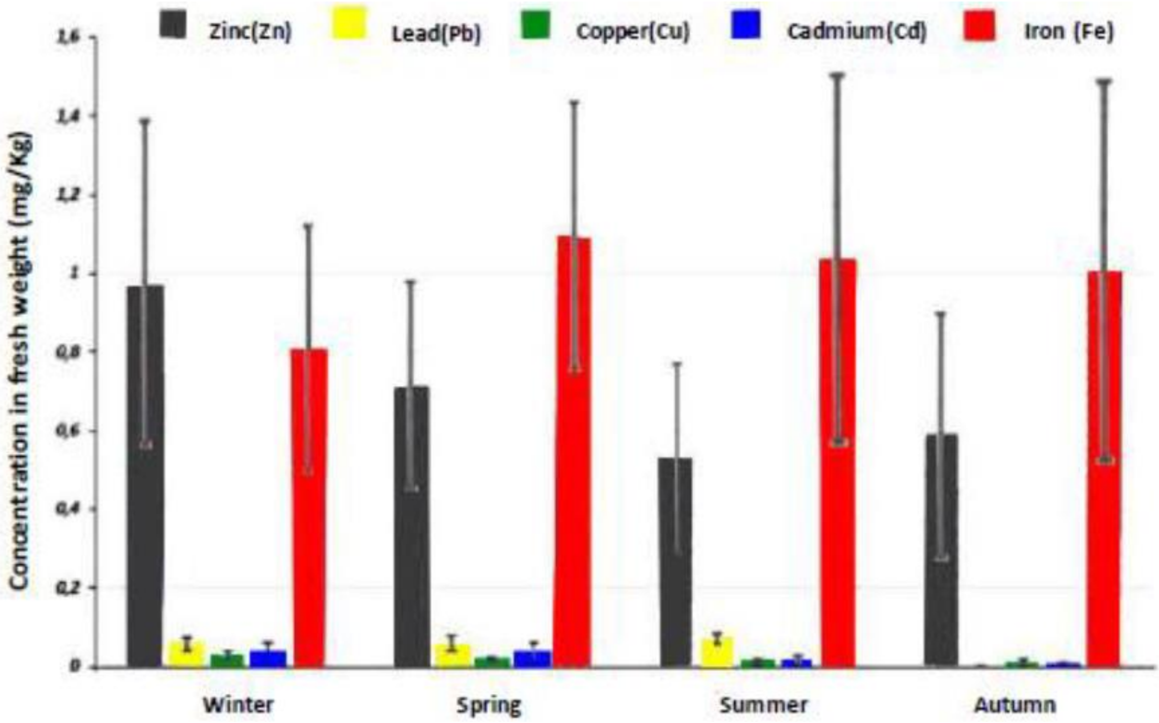

Fig. 3: Comparison by season of heavy metals levels in the gills

Discussion

The comparative analysis of the average concentrations of heavy metals in the two sexes shows that Zinc and Cadmium are bioaccumulated by male individuals than by females in all organs. Nevertheless, Iron and Lead present identical contents in both sexes at the level of all biological matrices. For Copper, the concentrations measured at the gonadal level show that males are more affected than females. On the other hand, at the level of the gills, these contents do not seem to be influenced by the sex factor. The female gonads concentrate more zinc and copper than male gonads in Flathead grey mullet Mugil cephalus (Bouhadiba, 2011). Females are more infected than males, this may be due to their migration to the polluted coast compared to spawning which explains the high contamination with heavy metals via the various sources of pollution (Sidoumou et al., 1991). This is probably due to the phenomenon of accumulation of nutrient reserves during vitellogenesis (Mortet, 1989). Gender is one of the predominant biological factors, partly due to the difference in growth between males and females, and partly due to the loss of contaminants caused by reproduction. Spawning leads to significant decontamination in females since from their first reproduction, a marked decrease in metal concentrations is observed (Bodiguel, 2008).

Table 4: The Mean concentration of heavy metals of different pelagic fishes (Clupeidae) from Algerian coast, Morroco and Tunisia (mean ± standard deviation).

Zn	Cd	Cu	Pb	Fe	Organs	Authors	Area	Date
1.00±0.406	0.048±0.006	0.017±0.0007	0.029±0.001	0.770±0.28	Gonads/gills *Sardina pilchardus*	Kalakhi et *al.,*	Bay of Ghazaouet	Present Study
/	/	/	0.21±0.45 (mg/l)	/	Flesh *Sardina pilchardus*	Merbouh	Bay of Oran	1998
/	0.019 (mg/kg resh wt)	/	/	/	Flesh *Sardinella aurtia*	Benamar et *al.,*	Bay of Oran	2011
42.70±29.54(mg.kg/ FW)	0.0 (mg.kg/ FW)	0.130±0.12 (mg.kg/ FW)	0.30±0.27 (mg.kg/ FW)	/	head / skeleton *Sardina pilchardus*	Ouabdesselam et *al.,*	Bay of Algiers	2017
19.79 ± 4.35 (mg.kg /FW)	0.03 ± 0.02 (mg.kg /FW)	1.31 ± 0.40 (mg.kg/ FW)	0.05 ± 0.02 (mg.kg/ FW)	/	Flesh *Sardina pilchardus*	Hamida et *al.,*	Algeria (Zemouri)	2018
35.6 ± 4.22 (mg.kg/ WF)	0.17 ± 0.06(mg.kg/WF)	0.56 ± 0.25(mg.kg /WF)	0.16 ± 0.25(mg.kg /WF)	/	Flesh *Sardina pilchardus*	Ouabdesselam et *al.,*	Algeria (Corso)	2020
77.46(µg.g/DW)	0.03(µg.g/DW)	/	0.98 (µg.g/DW)	/	Gills *Sardinella aurtia*	Ennourri et *al.,*	Gulf of Tunis	2008
97.33 ±28.3 (mg.kg/ DW)	0.13 ±0.03 (mg/kg dray wt)	0.93 ±0.55 (mg.kg/DW)	0.09 ±0.02 (mg.kg/DW)	33.91± 3.26 (mg/kg dray wt)	Gills *Sardina pilchardus*	El mohrit et *al.,*	The southern Atlantic coast (Morocco)	2013

Similar results have already been mentioned from Sardina plichardus in Algerian coast (Merbouh, 1998; Banamar et al., 2010; El mohrit et al., 2013; Hamida et al., 2018; Ouabdesselam et al., 2017 and 2020) (Table 4). Wu et al. (2007), suggest that metals are first accumulated by target provisional organs such as gills and then transferred to digestive organs such as liver, kidneys and intestines. Zinc and Copper are essential trace elements for the metabolism of fish. They can induce toxic effects by exceeding the lethal dose. Iron (Fe) is essential for cellular respiration in animals. It is also a powerful catalyst for certain biochemical reactions, however it is toxic in high doses (Crichton et al., 2002). Lead (Pb) is classified among the most toxic metals for humans and animals (Roony and Mclaren, 1999). It has no known role in biological systems (Kalay and Kanli, 2000).Cadmium has no known metabolic role and does not appear to be biologically essential or beneficial to the metabolism of living beings (Price and Morel, 1990). Metallic elements do not appear to have a single mechanism, in fact the quantity of metals transferred depends both on the irrigation of the organ considered and on the intracellular binding capacities (Boudou, 1982). According to this author the relative importance between these two parameters determine the target organs of metallic bioaccumulation.

The evaluation of metal concentrations in Sardina pilchardus proves the certain presence of these pollutants in the bay of Ghazaouet. However, the sardine studied, therefore, appears to be a more or less contaminated product which could reflect the quality of the waters of the bay of Ghazaouet (Table 5).

Table 5: Comparison of heavy metal contents in S. pilchardus (mg/kg.F.W) compared to Maximum Admissible Doses (M.A.D).

Metals	Zn	Pb	Cu	Cd	Fe
[C] (mg/kg.FW)	1.00±0.406	0.048±0.006	0.017±0.0007	0.029±0,001	0,770±28
M.A.D. fish	30 mg/kg.FW (b)	0.5 mg/Kg FW(a)	0.5 mg/kg FW(a)	0.1 mg/kg/FW (a)	146 mg/kg FW(c)

Conclusion

The evaluation of metal concentrations in Sardina pilchardus shows the certain presence of these pollutants in the bay of Ghazaouet. However, gender and organ could influence metal accumulation. The sardine studied therefore appears to be a contaminated product, but the concentrations of heavy metals obtained are below compared to Maximum Admissible Doses (M.A.D). It is desirable that this study can be repeated with the study of several parameters that can influence the mechanism of bioaccumulation such as (season, growth, reproduction, etc.).

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