J Environ Occup Sci 2014; 3(1):47-52 **ISSN: 2146-8311**

GESDAV

Journal of Environmental and Occupational Science

available at www.scopemed.org

Original Research

Concentrations of heavy metals in muscle, liver and gill of *Sardina pilchardus* (Walbaum, 1792): Risk assessment for the consumers

Adil Chahid[1,2], Mustapha Hilali [2], Abdeljalil Benlhachemi [2], Issam Meftah Kadmiri [3], Taoufiq Bouzid [1]

[1]Laboratoire Régional d'Analyses et de Recherche Agadir /Office National de Sécurité Sanitaire des Aliments (LRARA/ONSSA), BP 474 Agadir Morocco
[2]Laboratoire Matériaux et Environnement, Faculty of Sciences Ibn Zohr University, Agadir Morocco
[3]Moroccan Foundation of Advanced Science, Innovation and Research, Biotechnology Centre Rabat Morocco

Received: February 12, 2014

Accepted: February 20, 2014

Published: February 28, 2014

DOI : 10.5455/jeos.20140220085459

Corresponding Author:
Adil Chahid,
Laboratoire Régional d'Analyses et de Recherche Agadir /Office National de Sécurité Sanitaire des Aliments (LRARA/ONSSA), BP 474 Agadir Morocco
chahidadilster@gmail.com

Key words: Sardina pilchardus; heavy metals; daily intake; Morocco.

Summary

Aim: Present study was aimed at investigating the three heavy metals namely cadmium, lead and mercury in liver, gills and muscles of sardine fish (*Sardina pilchardus* Walbaum,1792) which was collected from five locations in south of Morocco.

Method: Mass fraction of heavy metals in fish samples were determined using graphite furnace and cold vapor atomic absorption spectrometry.

Results: Generally, the levels of Cd, Hg and Pb in muscle of sardine were lower than those in livers and gills. This study reveals that the concentrations of heavy metals in sardine are below the maximum acceptable concentrations for human consumption.

Conclusion: Metal concentrations in the edible parts (muscle) of sardine were assessed for human uses according to provisional tolerable weekly and daily intake. The estimated values of all metals in muscle of fish in this study were below the established values. Therefore, it can be concluded that cadmium, lead and mercury in pilchard pose no health risk for consumers.

INTRODUCTION

Fish is a healthy food because of its nutritional benefits related to its proteins of high biological quality, desirable lipid composition, valuable mineral compounds and vitamins [1].

Scientific data indicate that fish consumption reduces the risk of coronary heart diseases, decreased mild hypertension and prevents certain cardiac arrhythmias [2]. At the same time, fish products are often contaminated by a variety of chemical contaminants [3].

Among the wide range of toxic substances contaminating the aquatic environment; a major concern has been focused on heavy metals. Mercury, lead and cadmium are non-essential elements occupying top positions in all lists of toxicants [4].

The Atlantic Ocean is rich in high value fish species with the Northeast Atlantic and Eastern Central Atlantic being included in the top of the principal marine fishing areas in the world. Morocco, maintains its position among the three major seafood producers in Africa [5].

Among the species, there is sardine widely distributed in the referred areas and that represents the most important pelagic groups captured [6]. The total catch reported for this species to FAO for 1999 was 901427 tonnes. Morocco is the country with the largest catches country with 429732 tonnes [7]. Currently, Morocco is the largest producer of sardine, with nearly half of the global landings.

In terms of international trade, the sardine is sold in two main forms namely canned sardines and fresh and frozen sardines. In the market of canned sardines, Morocco is by far the largest exporter of this product [8].

The purpose of the present study was first, to determine levels of cadmium, lead and mercury in muscles, livers and gills of sardine from five locations in south of Morocco, Agadir, Dakhla, Essaouira, Laayoune and SidiIfni. Our second purpose was to assess the public health risks associated with consuming fish harvested from these areas by estimating daily and weekly intakes and by comparing them with the provisional tolerable weekly intakes (PTWI) and provisional tolerable daily intakes (PTDI) recommended by various authorities.

MATERIALS AND METHODS

Study area and fish sampling

Twenty sardines were collected randomly from five fishing ports along the Moroccan coastline. From north to south, the sampling stations were at Essaouira, Agadir, SidiIfni, Laayoune and Dakhla. Specimens were collected during the sampling period from January to December 2012. The fish length ranged between 18 and 20 cm and the weight ranged between 40 and 46 g.

They were dissected with plastic laboratory set and samples of liver, gills and muscle were quickly removed, washed with distilled water and stored at -18°C prior analysis.

Reagents

De-ionized water (18 MΩ cm) from EASY Pure II-Bernstead system was used to prepare all aqueous solutions. All mineral acids and oxidants (HNO_3, H_2O_2, H_2SO_4 and HCl) used should be of the highest quality. 2.5 % Ammonium dihydrogen phosphate solution ($NH_4H_2PO_4$) was utilized as a matrix modifier. Standard stock solutions of mercury, cadmium and lead were prepared from concentrated stock solutions of 1000 mg/l and were diluted to the corresponding metal solution. The working solution was freshly prepared by diluting an appropriate aliquot of the stock solutions using 10% HNO_3 for diluting lead and cadmium solutions and 1 M HNO_3 for diluting mercury solution.

Apparatus

All the plastic and glassware were cleaned by soaking (with contact) overnight in a 10% (w/v) nitric acid solution and rinsed with deionized water.

Total mercury concentration was analyzed by an Automated Mercury Analyzer AULA-254. Reducing agent solution of stannous chloride 10 g in 100 ml of 6 M HCl was used. Flow rate of high purity argon gas and reading time were 60 ml/min and 15s respectively. Analytical measurements were established on peak height.

A Shimadzu AA-6300 model atomic absorption spectrometer with deuterium background corrector was used for determination of lead and cadmium concentration by a graphite furnace atomic absorption spectrophotometer using argon as inert gas. Pyrolytic graphite tubes were used and signals were measured at a peak height. Samples were injected to the furnace using an automatic auto sampler.

Chemical analysis

The samples were taken from -20°C and were thawed at room temperature. The samples were dried in an oven at 65°C until a constant weight was obtained, allowed to cool, and then ground in a household food mill. Sample portions between 0.2 and 1g were afterwards digested, for lead and cadmium determination, in Teflon vessels in microwave oven (ANTON PAAR 3000 closed vessel microwave digestion system, Graz, Austria), using 5 ml of 65% HNO_3 (Merck suprapure) at a following microwave digestion program: 4min for 200W, 5min for 400W and as last step 10min for 600W [9]. Then, hydrogen peroxide (2ml, 30%) was added to each digest to breakdown organic matter that may be undigested during the acid digestion and again the same heating program was applied. For total mercury determination, 4ml of HNO_3, 2ml of H_2SO_4 and 1ml of HCl were slowly added. The tube was then placed on top of a steam bath unit for 1 hour at 90°C to complete dissolution. After cooling, 5ml of potassium permanganate ($KMnO_4$) was added and after 30 minutes 5ml of hydroxylamine chlorhydrate was added [10].

The samples were then transferred to clean volumetric flasks, and diluted to 50 ml for muscle and to 25 ml for liver and gill samples with deionized water. Before analysis, the samples were filtered through a 0.45µm membrane filter. Sample blanks were prepared in the laboratory in a similar manner as the field samples. All metal concentrations were determined on wet weight basis as $\mu g.g^{-1}$. Digested samples were analyzed in triplicate for each metal.

Accuracy of these analyses was examined using external reference materials from Food safety laboratory/ NRL for Heavy metals [11]. Recoveries of all the elements are ranged from 94% to 106%.

Statistical analysis

Analysis of variance was used to evaluate the analysis data and significant differences among means were determined by One-way analysis of variance (ANOVA) and Tukey test (p=0.05). All statistical analyses were performed with SPSS 13.0 for windows.

RESULTS AND DISCUSSION

Trace elements in gill, liver and muscle

Levels of cadmium, lead and mercury in the muscle liver and gill of pilchard are shown in table 1.

Cadmium levels were lowest in muscle of sardine from Sidi Ifni, Essaouira and Dakhla (0.005 µg/g, mean value) (Figure 1). In muscle of sardine from Laayoune, mean cadmium level was slightly higher (0.008 $\mu g.g^{-1}$). Mean cadmium level in sardine gills ranged from 0.004 $\mu g.g^{-1}$ in Sidi Ifni to 0.041 $\mu g.g^{-1}$ in Essaouira. In liver of sardine, mean Cd concentration ranged from 0.030 $\mu g.g^{-1}$ in Sidi Ifni to 0.082 $\mu g.g^{-1}$ in Dakhla. Literature data reported mean cadmium levels in muscle, liver and gill of pilchard of 0.015 µg/g (dry weight, dw), 0.3 $\mu g.g^{-1}$ and 0.06 µg/g, respectively [12]. In sardine from Mediterranean Sea, mean Cd values (dw) in muscle ranged from 0.55 to1.1 $\mu g.g^{-1}$, in liver from 3 to 4.8 $\mu g.g^{-1}$, and from 1.56 to 2.5 $\mu g.g^{-1}$ for gill [13,14]. From the Atlantic Ocean the values are 0.08 $\mu g.g^{-1}$ ww for muscle, 0.07 $\mu g.g^{-1}$ ww for liver and 0.08 $\mu g.g^{-1}$ ww for gill [15], and 0.024 $\mu g.g^{-1}$ ww for muscle [10].

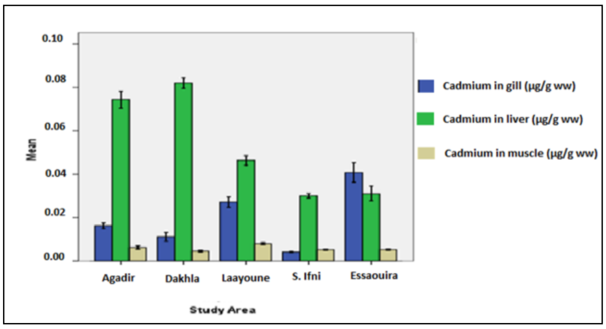

Figure 1. Cadmium concentrations in different organs of sardine (µg/g wet weight)

Lead levels in sardine ranged from 0.016 $\mu g.g^{-1}$ in Dakhla to 0.023 $\mu g.g^{-1}$ in Agadir (not detected in Sidi Ifni) for muscles, from 0.013 $\mu g.g^{-1}$ in Sidi Ifni to 0.092 $\mu g.g^{-1}$ in Agadir for liver, and from 0.004 $\mu g.g^{-1}$ in sidi Ifni to 0.049 $\mu g.g^{-1}$ in Dakhla and Agadir for gill (Figure 2). Literature data showed that lead concentrations (w/w) ranged from 0.1 to 6.8 $\mu g.g^{-1}$ in muscle, from 0.3 $\mu g.g^{-1}$ dw to 39.4 $\mu g.g^{-1}$ ww in liver and from 0.5 $\mu g.g^{-1}$ dw to18.8 $\mu g.g^{-1}$ ww for the gill from Mediterranean sea [12,15]. In fish from Atlantic Ocean, mean lead value in muscle was 0.076 $\mu g.g^{-1}$ ww [10].

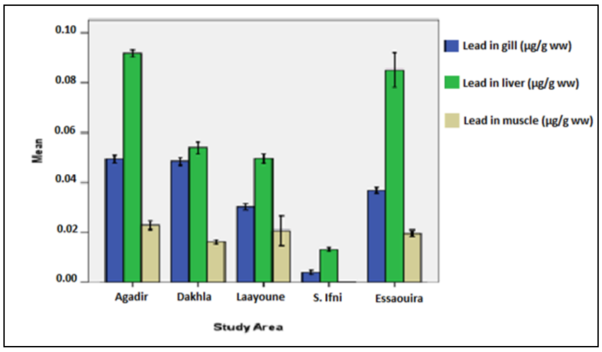

Figure 2. Lead concentrations in different organs of sardine (µg/g wet weight)

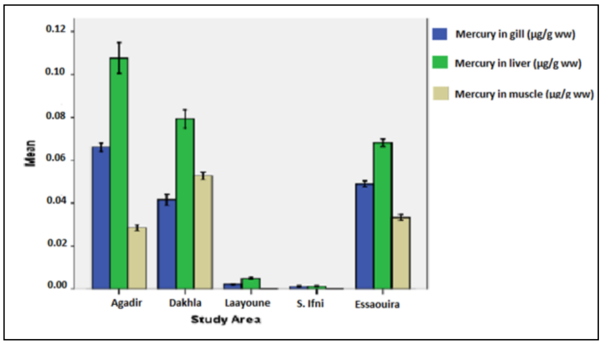

Figure 3. Mercury concentrations in different organs of sardine (µg/g wet weight)

Figure 3 show the distribution of total mercury mass fraction in gill, liver and muscle of sardine. In all locations, the lowest Hg levels were obtained in muscle. Among samples, the lowest Hg level in muscle of sardine was obtained in Sidi Ifni and Laayoune (under detection limit). In other samples, mean mercury levels in muscles of sardine ranged from 0.029 to 0.053 $\mu g.g^{-1}$. Mean mercury level in sardine gill ranged from 0.001 to 0.066 $\mu g.g^{-1}$ and from 0.001 to 0.108 $\mu g.g^{-1}$ in liver. Similar values for mercury content were reported in literature. In sardine from Mediterranean Sea, mercury levels in muscle, liver and gill were 0.14 $\mu g.g^{-1}$, 0.16 $\mu g.g^{-1}$ and 0.1 $\mu g.g^{-1}$ respectively [12]. In muscle of fish from Atlantic Sea, mercury content was slightly higher (0.084 $\mu g.g^{-1}$ w/w) than in Moroccan sardine investigated in this paper [10].

Results of our study showed that concentrations of heavy metals in muscle of fish were generally lower than those in livers and gills. This is in accordance with the previous records on metal accumulation in aquatic animals. It is well known that target organs of heavy metals are generally organs like the liver (or hepatopancreas) and the gills that are metabolically active [14]. The levels of heavy metals in the gills reflect the concentrations of metals in the waters, where the fish live, while the concentrations of metals in liver represent storage of metals in the fish body [16]. The difference in accumulation potential between muscle and liver can be explained by the activity of metallothioneins, proteins that are present in liver but not in the muscle, which have the ability to bind certain heavy metals and thus allow the tissue to accumulate them at a high degree [17].

Sardines from areas such as Dakhla and Agadir had the highest cadmium level, particularly in liver, which can be can only be explained by a natural input of dissolved Cd probably due to upwelling [18]. The highest cadmium levels in gill of pilchard were observed in Essaouira, which can be attributed to the discharge at sea of phosphogypsum, Cd-enriched by-product of the industrial transformation of phosphate ore into phosphoric acid [19].

The highest level of lead in Moroccan sardine was observed in liver, particularly in Agadir and Essaouira, due to numerous industrial and domestic discharges from this heavily populated and highly industrialized zone.

Our results showed higher mercury levels in Moroccan sardine from Agadir than in other areas. These results can be explained by high industrial activities, which is in agreement with results published in literature [18].

Table 1. The heavy metal concentrations in different organs of *Sardina pilchardus* (µg/g wet weight)

Metals	Regions	Organ metal concentrations(*)		
		Muscle	**Gill**	**Liver**
Cd	Agadir	0.006 ± 0.003^{ab} (0.002-0.019)	0.016 ± 0.006^{b} (0.006-0.039)	0.074 ± 0.017^{c} (0.039-0.097)
	Dakhla	0.005 ± 0.002^{a} (0.001-0.009)	0.011 ± 0.009^{ab} (0.007-0.048)	0.082 ± 0.171^{c} (0.067-0.097)
	Laayoune	0.008 ± 0.002^{b} (0.002-0.010)	0.027 ± 0.011^{c} (0.011-0.057)	0.046 ± 0.010^{b} (0.031-0.068)
	Sidi Ifni	0.005 ± 0.001^{a} (0.003-0.008)	0.004 ± 0.002^{a} (0.002-0.008)	0.030 ± 0.005^{a} (0.020-0.040)
	Essaouira	0.005 ± 0.001^{a} (0.003-0.008)	0.041 ± 0.020^{d} (0.012-0.073)	0.031 ± 0.015^{a} (0.012-0.064)
Hg	Agadir	0.029 ± 0.006^{b} (0.016-0.043)	0.066 ± 0.009^{d} (0.044-0.084)	0.108 ± 0.033^{c} (0.077-0.198)
	Dakhla	0.053 ± 0.008^{d} (0.038-0.068)	0.042 ± 0.012^{b} (0.024-0.069)	0.079 ± 0.019^{b} (0.039-0.100)
	Laayoune	nd[a] -	0.002 ± 0.001^{a} (0.001-0.004)	0.005 ± 0.001^{a} (0.003-0.008)
	Sidi Ifni	nd[a] -	0.001 ± 0.002^{a} (nd-0.007)	0.001 ± 0.002^{a} (nd-0.004)
	Essaouira	0.033 ± 0.006^{c} (0.023-0.048)	0.049 ± 0.006^{c} (0.04-0.063)	0.068 ± 0.008^{b} (0.056-0.087)
Pb	Agadir	0.023 ± 0.008^{b} (0.012-0.040)	0.049 ± 0.008^{e} (0.039-0.068)	0.092 ± 0.006^{c} (0.080-0.100)
	Dakhla	0.016 ± 0.004^{b} (0.010-0.021)	0.049 ± 0.007^{e} (0.039-0.064)	0.054 ± 0.010^{b} (0.035-0.074)
	Laayoune	0.021 ± 0.027^{b} (0.009-0.100)	0.030 ± 0.006^{b} (0.022-0.042)	0.050 ± 0.009^{b} (0.036-0.067)
	Sidi Ifni	nd[a] -	0.004 ± 0.004^{a} (nd-0.010)	0.013 ± 0.004^{a} (0.009-0.020)
	Essaouira	0.020 ± 0.006^{b} (0.012-0.034)	0.037 ± 0.006^{c} (0.020-0.047)	0.085 ± 0.031^{c} (0.050-0.191)

nd: not detected

[*] Mean values and ± standard deviation (n=20).

Values in parentheses indicate the minimum and maximum levels.

Statistical significance was defined as $p<0.05$. Distribution with different letters (a, b, c, d and e) are statistically different.

Table 2. The estimated daily and weekly intake of heavy metals for European pilchard consumed by adult people in Morocco

Metal	PTWI*[a]	PTWI**[b]	PTDI[c]	EWI[d] (EDI)[e]
Cd	7	420	60	1.54 (0.22)
Hg	4	240	34	10.2 (1.45)
Pb	25	1500	214	4.42 (0.62)

*Provisional Permissible Tolerable weekly intake (PTWI) in µg/week/kg body weight.

**Mean weekly fish consumption in Morocco is 0.19 kg per person (ONUAA, 2012)

[a]FAO/WHO, 2004

[b]PTWI for 60kg adult person (µg/week/60 kg body weight)

[c] PTDI, permissible tolerable daily intake (µg/day/60kg body weight)

[d]EWI, estimated weekly intake in µg/day/60kg body weight.

[e]EDI, estimated daily intake in µg/day/60kg body weight.

Human health risk assessment

To evaluate the health risk to Moroccan people through consumption of marine fish, daily intake of heavy metals was estimated on the basis of the concentrations of cadmium, mercury and lead in muscle of sardine and daily fish consumption. According to the Joint Food and Agriculture Organization/ World Health Organization [20], the provisional tolerable weekly intakes (PTWI) for Hg, Cd and Pb are 4, 7 and 25 µg/kg body weight, respectively. The average daily fish consumption in Morocco is 27g per person. This is also equivalent to 192g per person per week [21]. The EWI (estimated weekly intake) and EDI (estimated daily intake) values presented in Table 2 were estimated by assuming that a 60kg person will consume 27 g fish/day which is equal to 192 g fish/week. Average body weight of Moroccan people was assumed to be 60kg [22]. EWI values of metals for an adult (µg/60 kg body weight) consuming 192g fish/week were estimated using the maximum levels for each species [EWI (µg/60kg body weight/week) = maximum levels of metal (µg/kg) multiplied by fish consumption (kg/60kg body weight/week)]. Then, EDI values were calculated from EWI values [23]. Based on this value and the average metal contents it may be concluded that the consumption of Moroccan sardine does not present health risk for consumption because the estimated intakes for cadmium (1.54 µg/kg body weight /week), lead (4.42 µg/kg body weight /week) and mercury (10.2 µg/kg body weight /week) were far below the established safety values.

CONCLUSION

Present study fills a gap by providing information on Hg, Cd and Pb concentrations in the muscles, livers and gills of sardine from the Moroccan coasts of Atlantic Ocean. The concentrations of heavy metals in all organs of Moroccan sardine were considerably lower than the maximum levels set by Commission regulation [24]; these results can be used to evaluate the possible risk associated with consumption of *Sardina pilchardus*. Because PTWI and PTDI values estimated for examined fish and metals were far below the established values by various authorities, it may be concluded that consumption of these species from the Moroccan waters is not a problem on human health.

REFERENCES

1. Vieira C, Morais S, Ramos S, Delerue-Matos C, Oliveira MBPP. Mercury, cadmium, lead and arsenic levels in three species from the Atlantic Ocean: Intra- and inter-specific variability and human health risks for consumption. Food and chemical Toxicology. 2011; 49: 923-932.

2. Kris-Etherton PM, Harris WS, Appel LJ. For the Nutrition Committee. AHA scientific statement. “Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease”. Circulation. 2002; 106: 2747–2757.

3. Storelli MM. Potential human health risks from metals (Hg, Cd and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). Food and Chemical Toxicology. 2008; 46: 2782–2788.

4. Storelli MM, Giacominelli-Stuffler R, Storelli A, Marcotrigiano GO. Accumulation of mercury, cadmium, lead and arsenic in swordfish and blue fin tuna from the Mediterranean Sea: A comparative study. Marine Pollution Bulletin. 2005; 50: 993-1018.

5. Food and Agriculture Organization (FAO). “The State of world Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations”, Rome, 2012.

6. Food and Agriculture Organization (FAO). "The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations". Rome, 2009.

7. Food and Agriculture Organization (FAO). Fisheries and Aquaculture Department, <http://www.fao.org/fishery/species/2910/en>, 2013.

8. Direction des Etudes et des Prévisions Financières (DPEF). Performances des exportations marocaines des produits de la mer sur le marché mondial. Maroc, 2012.

9. Vicente-Martorell JJ, Galindo-Riano MD, Garcia-Vargas M, Granado-Castro MD. Bioavailability of heavy metals monitoring water, sediment and fish species from a polluted estuary. Journal of Hazardous Materials. 2009; 162: 823-836.

10. Chahid A, Hilali M, Benlhachemi A, Bouzid T.Contents of cadmium, mercury and lead in fish from the Atlantic sea (Morocco) determined by atomic absorption spectrometry. Food chemistry. 2014 ; 147 : 357-360.

11. Agence Française de Sécurité des Aliments (AFSSA-LERQAP), Unité Contaminants Inorganiques et Minéraux de l'environnement (CIME), Equipe Métaux lourds et Eléments minéraux. Maisons-Alfort, France.

12. Hernandez-Hernandez F, Medina J, Ansuategui J, Conesa M. Heavy metal concentrations in some marine organisms from the Mediterranean Sea (Castellon, Spain): Metal accumulation in different tissues. Scient. Mar. 1990; 54(2): 113-129.

13. Canli M, Alti G. The relationship between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and size of six Mediterranean fish species. Environmental pollution. 2003; 121: 129-136.

14. Canli M, Kalay M, Ay O. Metal (Cd, Pb, Cu, Zn, Fe, Cr, Ni) Concentrations in Tissues of a Fish Sardina pilchardus and a Prawn Peaenus japonicusfrom Three Stations on the Mediterranean Sea. Bull. Environ. Contam. Toxicol. 2001; 67: 75-82.

15. El Morhit M, Fekhaoui M, El Morhit A, Elie P, Yahyaoui A. Hydrochemical characteristics and metallic quality in fish in the Loukkos river estuary of Morocco. J. Mater. Environ. Sci. 2013; 4(6): 893-904.

16. Romeo M, Siau Y, Sidoumou Z, Gnassia-Barelli M. Heavy metal distribution in different fish species from the Mauritania coast. The Science of the Total Environment. 1999; 232: 169-175.

17. Visnjic-Jeftic Z, Jaric I, Jovanovic L, Skoric S, Smederevac-Lalic M, Nikcevic M, Lenhardt M. Heavy metal and trace element accumulation in muscle, liver and gills of the Pontic shad (Alosa immaculate Bennet 1835) from the Danube River (Serbia). Microchemical Journal. 2010; 95: 341–344.

18. Banaoui A, Chiffoleau J-F, Moukrim A, Burgeot T, Kaaya A, Auger D, Rozuel E. Trace metal distribution in the mussel Perna perna along the Moroccan coast. Marine Pollution Bulletin. 2004; 4: 378-402.

19. Chafik A, Cheggour M, Cossa D, Sifeddine SBM. Quality of Moroccan Atlantic coastal waters: water monitoring and mussel watching. Aquatic Living Resources. 2001; 14: 239–249.

20. FAO/WHO. "Summary of evaluations performed by the joint FAO/WHO expert committee on food additives (JECFA 1956-2003), (First through sixty-first meetings)". ILSI Press International Life Sciences Institute, 2004.

21. Organisation des Nations Unies pour l'Alimentation et l'Agriculture (ONUAA) (2013-2016). Cadre Programmation par Pays. Royaume du Maroc. <ftp://ftp.fao.org/OSD/CPF/Countries/.../MOR_CPF_2013-2016.doc>, 2012.

22. Lahmam A, Baali A, Hilali MK, Chapuis-Lucciani N, Boetsch G. Obesity, overweight and body-weight perception in a High Atlas Moroccan population. Obesity reviews. 2008; 9: 93-99.

23. Turkmen M, Turkmen A, Tepe Y, Tore Y, Ates A. Determination of metals in fish species from Aegean and Mediterranean seas. Food chemistry. 2009; 113: 233-237.

24. European Union. Commission regulation N° 629/2008/ CE of 2 July 2008. Amending regulation N° 1881/2006/CE, setting maximum levels for certain contaminants in foodstuffs. Official journal of the European Union. 173, 6-9, 2008.