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RESEARCH ARTICLE

HEAVY METAL CONTAMINATION IN FOUR FISH SPECIES FROM THE ATLANTIC COAST OF MOROCCO

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Abstract

The purpose of this study is to assess concentration of selected metal (Lead, Cadmium and Mercury) in the muscle of four fish species *Scomber japonicus, Sardina pilchardus, Engraulis encrasicolus, Trachurus trachurus,* collected in December 2012 and 2013 from central and southern zone of the Moroccan Atlantic coast using the surveys conducted by R/V Al Amir of the national institute of fisheries research.. The samples were analyzed by a graphite furnace atomic absorption spectrometry (AAS) for lead and cadmium, and by cold vapor atomic absorption spectrometry (CVAAS) for mercury. The concentration of heavy metal measured in the fish species were expressed as mg/Kg of wet weight (w/w). In our study, the heavy metals content in the muscles of fish species did not exceed the limit allowed by European communities. This results will therefore allow to assess the level of metal contamination between different species of pelagic fish studied, and also attest to the quality of the fish captured along the Moroccan coast

INTRODUCTION

The coastline of Morocco covers a 3500 kilometer stretch. This coastal area is a major attraction for the socio-economic activities. The main industrial activities of the country are localized on the Atlantic coast. The northern area from Tangier to Casablanca represent over than 2/3 of industrial units (food, textile, chemical, mechanical industry…). Then the central sector from Casablanca to Agadir includes more urban areas, and very large industrial infrastructure (Berraho, 2006). Thus, several pollutants are discharged daily into the environment.

These rejected contaminants, end up rapidly in the marine environment, which represent the ultimate repository of all anthropogenic emissions. Among them, there are the heavy metals considered the most harmful pollutant of the aquatic environment due to their capacity to bioaccumulate and biomagnified in marine organisms at different organs. (Harte et al., 1991, Schuurmann and Markert, 1998). The accumulation of heavy metals in these living matters may subsequently affect the quality of the environment and deteriorate the ecological imbalance.

Actually fish are present at the top of the aquatic food web, therefore they accumulate heavy metals from water, sediment and food (Yilmaz, 2007; Zhao, 2012).

Fish are widely used as sentinel species of contamination in the aquatic environment, because it is represent an important part of the human diet, so it is not surprising that many studies have been conducted to establish the risk of contamination by metals in different species of edible fish (Prudente et al., 1997; Kucuksezgin et al., 2001; Lewis et al., 2002).In the present work, levels of three metals (Cd, Pb and Hg) in the muscle tissue of some commercial fish from the Moroccan Atlantic coast were determined. Therefore, four pelagic fish species (*Scomber japonicus,* *Sardina pilchardus, Engraulis encrasicolus, Trachurus trachurus* have been analyzed for heavy metals contents. The three metals were selected because they are, especially considered in the field of monitoring of the quality of marine environment. The aim of this work is to evaluate the current environmental status of the coast, and to compare the metals content in muscles against the recommended maximum permissible limit to assess the quality of fish and the health risk for human.

MATERIALS AND METHODS

Sampling

Four species of pelagic fish (*Sardina pilchardus, Engraulis encrasicolus, Trachurs trachurus* and *Scombrus japonicus*) were caught by trawling according to availability in December 2012 and 2013 at 10 stations along the Moroccan Atlantic coast, from Safi to Cap Blanc (Fig.1). The fish were stored at -20°C in plastic bags prior to analysis. Subsequently, the fish were dissected, and a part of muscle, away from the viscera, was removed. After dissection, the samples were freeze drying by thermo lyophilizer, with water content determined during the lyophilization process. Then each dried fish sample was homogenized by mixing in rotating glass bottle, and stored until analysis.

Analytical procedure

The digestion procedure is carried out according to the official AOAC method: 2000. Aliquots of approximately 0,25g of freeze-dried tissue was digested in Teflon bombs with 5 ml of suprapur nitric acid (65% Merck) and 2 ml of hydrogen peroxide (30% Fluka). Then, they are left at room temperature for at least one hour. The bombs were finally closed tightly and placed in a microwave (Millestone - Start E) for digestion. After cooling the remaining digested solutions were subsequently transferred to 50 ml volumetric flasks and diluted to level with ultra-pure water: Milli-Q.

Analysis

The concentration of cadmium and lead is determined using a graphite furnace atomic absorption spectrophotometer Shimadzu GPA-EX7i with deuterium background correction using argon as inert gaz. Total mercury levels were determined by an Automated Mercury Analyzer, Aula 254 (Gold trap).

The accuracy of the applied analytical procedure was assessed using (DORM-4 Fish protein certified reference material for trace metal), provided by National Research Council of Canada (Table 1). These standards were digested and analyzed using the same procedure as the fish samples.

The levels of heavy metals reported in mg/kg wet weight for all species were compared to European benchmarks that limit the respective toxicity thresholds in food as follows: Pb = 0.3, Cd = 0.1 and Hg = 0.5 mg/kg wet w, for all studied fish species, except for Anchovy in which the acceptable limit for cadmium is 0.3 mg/Kg w/w. (EC, 2006-2008).

Statistical analysis

The whole data were subjected to statistical analysis. A One way analysis of variance (ANOVA) was used to determine the significant differences in metal levels among species. All statistical calculations were carried out with XLSTAT Version 2015.1.03.15945. Statistical significance was defined as $p \leq 0.05$.

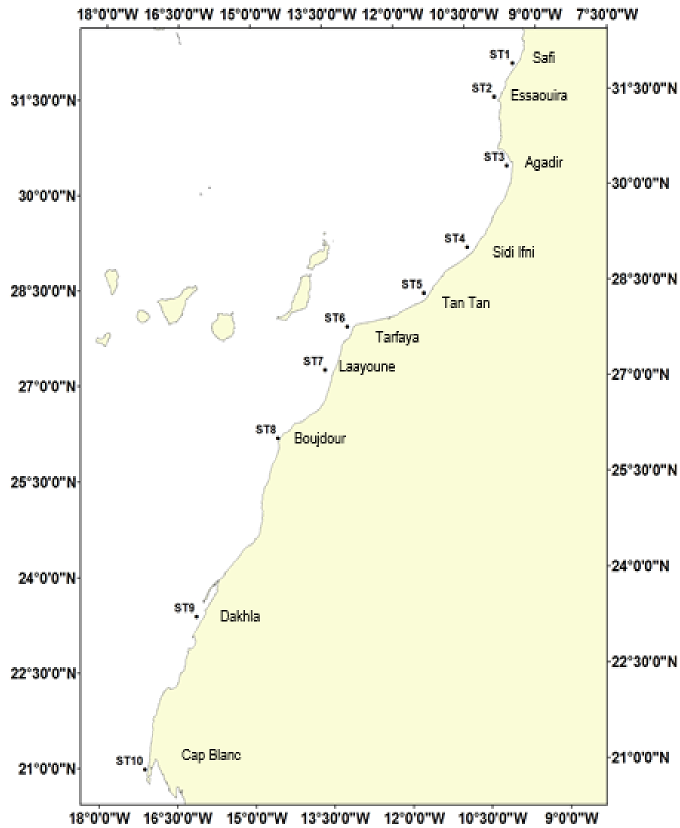

Fig. 1. Location map of sampling stations in the Moroccan Atlantic coast.

RESULTS

Metal determination was performed on the edible part of fish including muscle. Although it provides relatively low metals content due to its low metabolic activity, it can be used as circumstantial biological indicator because of its important mass in comparison with the other organs (Phillips, 1995), to estimate the level of metals that enter to human by the consumption of fish.

The average concentration of cadmium, lead and mercury in each fish species are shown in table 2, then Fig.2, 3 and 4, showed the spatial variation of each trace element contamination along the Atlantic coast. All metal concentrations in samples were converted into wet weight basis by taking the water content of the each fish species into account. The water content averaged 74% in sardine, 76% in anchovy, 77% in horse mackerel and 77% in mackerel. Then measured dry weight were converted to wet weight values by dividing the values by 3.8 for sardine, 4.1 for anchovy, 4.3 for horse mackerel and 4.5 for mackerel.

Cadmium is a non-essential and highly toxic metal. About 50% of the cadmium that reach in the ocean comes from human activities. In our study, the concentration of cadmium was found at an average of 0.038 mg/kg wet weight in fish samples, with maximum level of 0.12 mg/kg in muscle of anchovy from Essaouira. The levels were lowest (0.001mg/kg w/w) in muscle of mackerel from Laayoun and in sardine from Laayoun and Dakhla. Howerver no species exceed the maximum levels set by commission regulation (EC) N°629/2008 (0.1 mg/kg w/w for muscle meat of *Sardina*, *Scomber* and *Trachurus* species, and 0.3 mg/kg w/w for muscle meat of *Engraulis* species).

The average of lead in fish species was 0.016 mg/kg wet weight in fish species. Actually, lead represent one of the most ubiquitous and useful metal, it's detectable in practically all phases of the inert environment and all biological systems (Castro, 2008).The samples of Sardine collected from Sidi ifni had the highest level of Pb (0.080 mg/kg w/w), then the lowest concentration (0.001 mg/kg w/w) was found in muscle of sardine collected from Dakhla and Cap blanc, also in muscle of anchovy from Essaouira and in mackerel (0.002 mg/kg w/w) from Laayoun. However no species exceed the maximum levels set by the commission regulation (EC) N°1881/2006 (0.3 mg/kg for muscle meat of fish).

Mercury levels were found in average of 0.0143 mg/kg w/w in fish samples. The highest levels of mercury (0.042 mg/kg w/w) were found in muscle of sardine from Dakhla then the lowest concentration (0.004 mg/kg w/w) were found in muscle of sardine from Laayoun and in anchovy (0.007 mg/kg w/w) from Tarfaya. Mercury is a known human toxicant and the primary sources of mercury contamination in man are thought eating fish (Kalay, 1999). In the present study, no species exceeded the limits set by the Commission regulation (EC) 1881/2006 (0.5 mg/kg for most muscle meat of fish).
Statistically, Anova test didn't indicate any significant variation in the metal concentrations in muscle between the four studied species (ANOVA, p>0.05) (Table3).

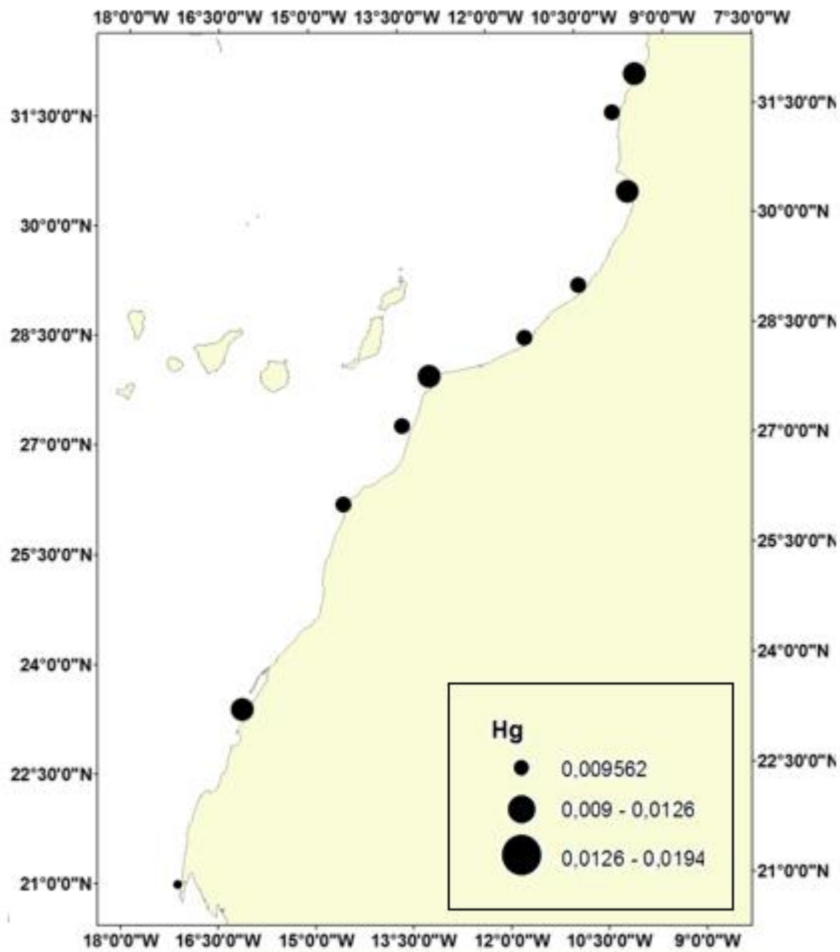

Fig.2. spatial variation of mercury contamination along the Atlantic coast.

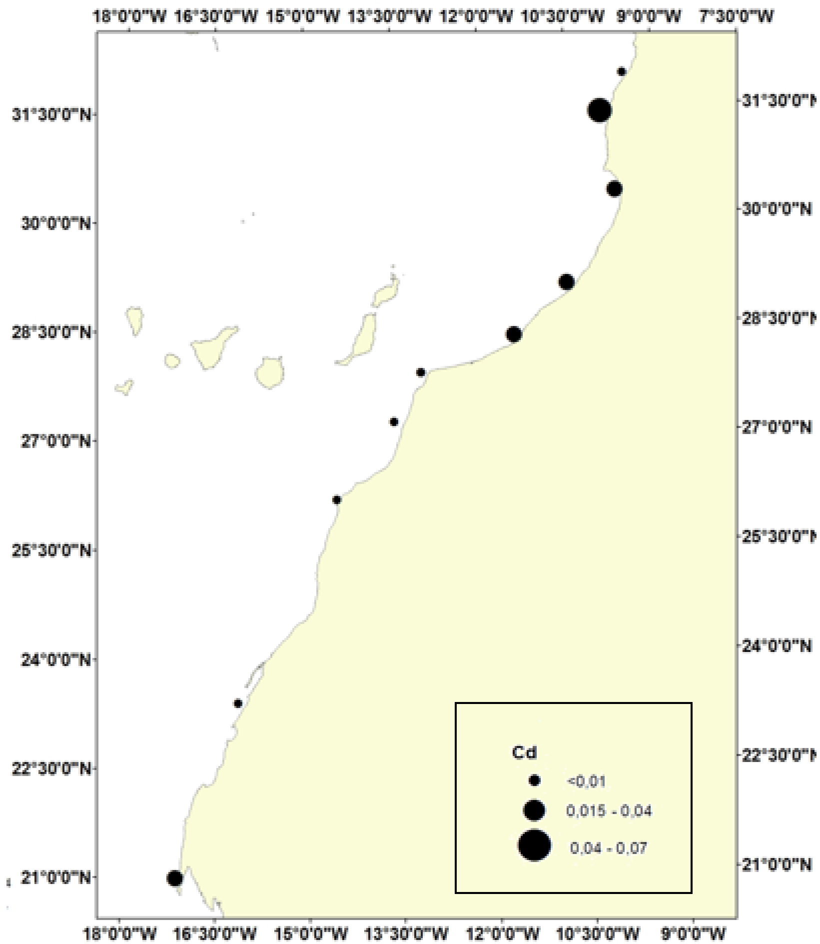

Fig.3. Spatial variation of cadmium contamination along the Atlantic coast.

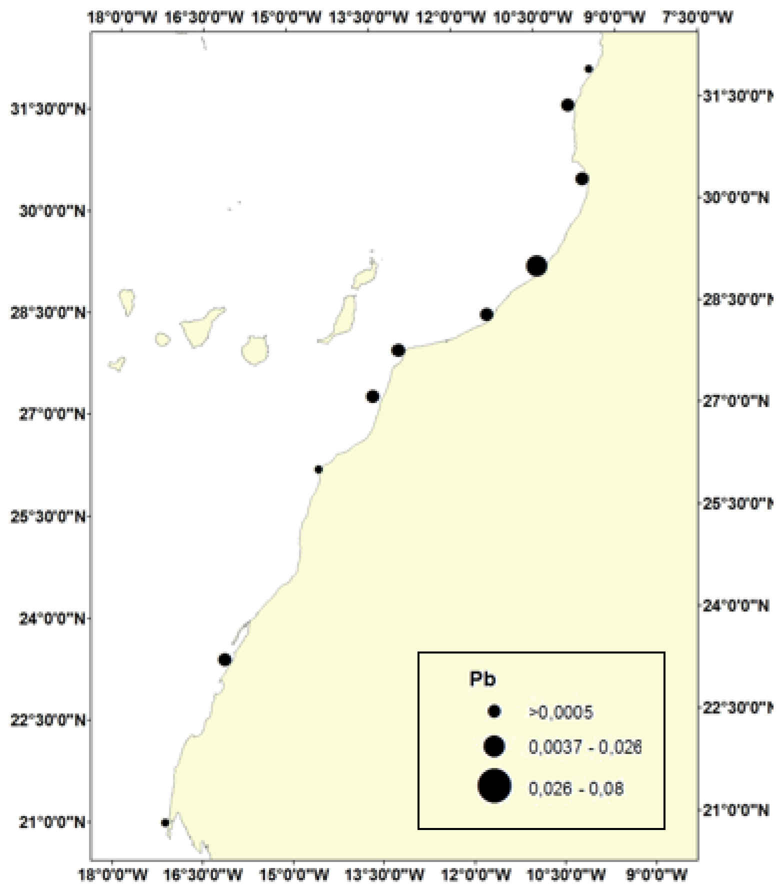

Fig.4. spatial variation of lead contamination along the Atlantic coast.

Table1: Concentration of metals found in standard references materiel DORM-4.

Metals	Certified value	Measured value	% recovery
Cd	0,306	0,276±0,02	92,11%
Pb	0,416	0,370±0,02	92,42%
Hg	0,410	0,319±0,02	79,74%

Table2: Contents metallic means (mg/kg wet weight) of Cd, Pb and Hg in different fish species.

		Mercury		
Region	*Sardina pilchardus*	*Engraulis encrasicolus*	*Trachurus trachurus*	*Scombrus japonicus*
Central	0.010 ± 0.03 (0.008-0.014)	0.013 ± 0.005 (0.009-0.016)	0.016 ± 0.006 (0.01-0.023)	0.018 -
South	0.016 ± 0.003 (0.004 - 0.042)	0.007 ± 0.02 (0 - 0.007)	0.015 ± 0.004 (0-0.029)	0.019 ± 0.02 (0.016-0.024)
Total	0.013 ± 0.003 (0.010 - 0.016)	0.01 ± 0.004 (0.006 - 0.013)	0.0155 ± 0.001 (0.015 - 0.016)	0.018 ± 0.001 (0.018 - 0.019)

Lead

Region	*Sardina pilchardus*	*Engraulis encvrasicolus*	*Trachurus trachurus*	*Scombrus japonicus*
Central	0.033 ± 0.030 (0.005 - 0.080)	0.006 ± 0.005 (0 - 0.01)	0.005 ± 0.005 (0.004 - 0.015)	0.020 ± 0.000 (0.020)
South	0.005 ± 0.003 (0.001 - 0.010)	0.025 ± 0.020 (nd - 0.040)	0.005 ± 0.006 (nd - 0.0094)	0.025 ± 0.03 (nd - 0.061)
Total	0.019 ± 0.020 (0.005-0.033)	0.015 ±0.010 (0.006 - 0.025)	0.005 ± 0.0002 (0.005 - 0.006)	0.022 ± 0.003 (0.020 - 0.025)

Cadmium

Region	*Sardina pilchardus*	*Engraulis encvrasicolus*	*Trachurus trachurus*	*Scombrus japonicus*
Central	0.026 ± 0.020 (0.009 - 0.060)	0.057 ± 0.050 (0 - 0.12)	0.022 ± 0.01 (0 - 0.035)	0.040 -
South	0.008 ± 0.010 (0.001 - 0.029)	0.016 ± 0.020 (0 - 0.051)	0.004 ± 0.006 (0 - 0.012)	0.01 ± 0.008 (0.001 - 0.018)
Total	0.017 ± 0.010 (0.008 - 0.020)	0.037 ± 0.020 (0.016 - 0.057)	0.013 ± 0.13 (0.004 - 0.022)	0.025 ± 0.02 (0.010 - 0.040)

Table3: Means and comparison of heavy metal levels in mixed sites of studied species

	Lead	Mercury	Cadmium
Mackerel	0,024 a	0,019 a	0,016 a
Sardine	0,019 a	0,014 a	0,017 a
Anchovy	0,016 a	0,010 a	0,034 a
Horse mackerel	0,004 a	0,016 a	0,013 a
Pr > F	0,462	0,305	0,467
Significance	No	No	No

*Mean metal concentration of different species from all sites sharing a common letter for a particular metal are not significantly different, p>0.05

DISCUSSION

This work determined the concentrations of Cd, Pb and Hg in *S.pilchardus, E.encrasicolus, T.trachurus* and *S.japonicus* from ten sites in the central and south morocco coast. Even if the metal content in fish muscle is low because of its low metabolic activity, muscle tissues of fish is commonly analyzed because it is the main fish part consumed by humans. Mercury, lead and cadmium represent a toxic elements to humans (Andre et al., 2005). However, according to this work, concentrations of toxic metals are below their maximum permissible level of muscle meat of studied fish species (Hg< 0.5, Pb< 0.3, Cd< 0.1 mg/kg in wet weight) established (EC, 2006- 2008).

One of the most toxic heavy metals in the environment is mercury (Castro-Gonzalez and mendez- Armenta, 2008). High levels of organic or inorganic mercury can damage the brain,kidneys and developing fetus (Vieira et al., 2011).The mean concentration of mercury detected in this study are lower than those reported for sardine from other marine area, varying from 0,084 ppm w/w (Chahid et al., 2013) to 0,041 ppm w/w (Chahid et al., 2014) in the Moroccan Atlantic coast, and 0,018 ppm w/w from the Northeast Atlantic coast (Vieira et al., 2011).Concentrations obtained for horse mackerel are lower than those reported by Storelli, (2008) 0.16-2.41 ppm w/w in Adriatic sea , and by Vieira 2011 0.038-0.337 ppm w/w in the Atlantic sea, and by Stancheva et al., (2013) 0.16 ppm w/w in the black sea. Data obtained in this study for mackerel are also lower in comparison with the results reported by Magalhaes et al (2007) 0.07-0.012 pp w/w for the Azores, and those reported for *Scombrer scombrus* by Falco et al (2006) 0.06-0.15 ppm w/w; in Mediterranean sea and Chahid et al., (2014), 0.041 ppm w/w for the Atlantic sea.

Cadmium is a non-essential toxic metal, it may accumulate from food chain magnification and may induce kidney dysfunction, skeletal damage and reproductive deficiencies (Commission of the European Communities, 2001) in humans. Comparing with other studies, levels of Cd in sardine, horse mackerel and mackerel are higher than those observed in the Northeast Atlantic coast (0,005 ppm w/w for sardine,0,006 ppm w/w for mackerel and 0,0072 ppm w/w for horse mackerel; Vieira et al., 2011) ,and in the Moroccan Atlantic coast (0,0058 ppm w/w for sardine; Chahid et al., 2014),and also in the Mediterranean sea (0,002-0,01 ppm w/w for sardine; 0,003-0,01 ppm w/w for *Scomber scombus*; Falco et al., 2006).However, values are lower than those detected in Adriatic sea (0,02-0,04 ppm w/w for sardine ; Storelli, 2008) and in the Moroccan Atlantic sea (0,024 ppm w/w for sardine, 0,24 ppm w/w for horse mackerel and 0,036ppm w/w for *Scomber scombrus*,Chahid, (2013) and 0,09 ppm w/w; El Morhit, 2013). Data obtained for anchovy are consistent with those observed by Nisbet et al., (2010) from the middle black sea. Actually, the south Moroccan coast is characterized by the existence of upwelling (Orbi et al., 1998; Makaoui et al., 2005). This phenomenon could be the source of the enrichment of the marine environment by cadmium, which occurs mainly in the south, where the anthropogenic pollution sources are almost absent (Benbrahim, 2006). Then the Cd could be readily bioaccumulated in the lower portion of the food chain and bioconcentrated by trophic transfers to high levels in the fish. This was also predicted by physical-biogeochemical modeling in the north-west African upwelling. The model results provide a potential estimation of cadmium bioaccumulation in plankton communities, and agree well with the Cd levels in marine organisms (Auger et al., 2015). Comparing to others fish samples collected from upwelling zone (4.67 to 51.0 ppm, dw; Romeo et al., 1999) and (4.35 to 6.38 ppm, dw; Rejomon, 2010), fish collected from the Moroccan Atlantic south coast shows a lower levels of Cd.

Lead is a non-essential toxic metal, it may induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults (Commission of the European Communities 2001).Higher levels of lead are observed in muscle of mackerel and sardine from Mediterranean sea (0,01-0,08 ppm w/w; Falco et al., 2006), in sardine from Adriatic sea (0.03-0.09 ppm w/w; Storelli et al., 2008),in sardine, mackerel and horse mackerel from Northeast Atlantic coast (0.0540-0.002-0.0138 ppm w/w; Vieira, 2011) and in horse mackerel from Black sea (0.06 ppm w/w; Stancheva et al 2013) and horse mackerel and anchovy from the Black sea coasts of Turkey (0.60-0.70 ppm w/w; Nisbet et al., 2010).Furthermore, the results found in this study are little bit higher than those obtained in sardine from Moroccan Atlantic coast (El Morhit, 2013),and in mackerel from Atlantic sea (0.009 ppm w/w; Guerin et al., 2010).

In this study, there are some area where showed high levels like concentration of Cadmium in anchovy from Essaouira (Fig.4) which can be attributed to the existence of the discharge at sea of phosphogypsum, the cadmium enriched by the product of the industrial transformation of phosphate ore into phosphoric acid (Chafik2001).

Actually, the variability of heavy metal levels in different species depends on feeding habits (Romeo et al. 1999), ecological needs, metabolism (Canli and Furness, 1993), age, size and length of the fish (Linde et al., 1998) and their habitats (Canli and Atli, 2003; Tuzen and Soylak, 2007).

In our study, the statistical analysis using ANOVA one way show no significant differences of the mean concentrations of Hg, Pb and Cd between the different fish species.

With few exception, the heavy metals levels in the four pelagic fish species from the central and southern Moroccan coast were below the limits for human consumption recommended by EC (2006-2008), and were generally in the same range or below the levels found in the muscle of pelagic fish species from previous studies.

Fish are a major part of human diet. Thus, its quality must be ensured in order to avoid inconvenience to human health. In this work, all analysis revealed generally, the presence of low levels in muscle of the different fish species indicating safe levels for human consumption. We can deduct from our findings that the effect of natural and anthropogenic inputs of metal pollutants in Morocco on these fish species, are still very low. However, it should be noted that despite lower concentrations found and the absence of any risk of acute toxicity; there is an ecological risk which is the cumulative nature of heavy metals through two phenomena including bioaccumulation and biomagnification (Boumehres, 2010; El morhit, 2009). Heavy metals can accumulate at low concentration in different organs of marine biota and reach the toxic threshold affecting the metabolic reactions of organism, or causing disturbance of the ecological balance of affected ecosystems. Therefore, this analysis allow to know the nature of contamination of human activities (Boumehres 2010).

CONCLUSION

Marine pollution and contamination of fishery products is a major concern of the population, local authorities, and scientists, because of its effects on health and the environment. In this study, the samples analyzed are compared with consumption thresholds mentioned previously announced by Regulation (EC) No 1881/2006 of the Commission of 19 December 2006 and Regulation (EC) No 629/2008 of Committee on 2 July 2008 and therefore the products are not dangerous to the consumer. However, the coastal zone and its components represent a dynamic environment, expanding, which continue to be a key and promising sector for the development of Morocco and therefore requires continuous monitoring.

It should also be noted that in recent decades, research in the field of metal contamination attach great importance to the chemical form of polluting elements "concept of speciation," and it has been shown that it's governs toxicology, fate and the metal transfer mode in the environment.

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