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Quantification of two metallic elements in the Mullet, *Mugil cephalus* Linnaeus, 1758 (Perciformes Mugilidae), fished at the bay of Oran (NW Algeria)

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ABSTRACT

Aware of the problem of marine pollution, the Algerian authorities and the national scientific community are increasingly interested in environmental studies, with a view to assessing risks and protecting our coastal ecosystem. For this reason, several studies are carried out on the studies of the degree of contamination of the Algerian coasts as well as the fishery products. This research is partly rooted in a monitoring mission to conduct an integrated study of the marine environment. The objectives of this work are oriented towards societal questions and to provide elements of assistance to the management of this environment for the local actors. This study focused on the evaluation of the concentrations of the two heavy metals Lead (Pb) and Zinc (Zn) in Mullet, *Mugil cephalus* Linnaeus, 1758 (Perciformes Mugilidae), caught in the Oran bays. This fish reflects very well the quality of its biotope; it is a very abundant species in Algerian coastal waters and very appreciated by the Algerian consumer. The harvesting campaigns were carried out for fourteen months from February 2012 to March 2013, the quantification of heavy metal concentrations was carried out in three organs: liver (the detoxification organ), gonads (the reproductive organs) and flesh (representing the part consumed by humans). Heavy metal concentrations were determined by Flame Atomic Absorption Spectrophotometry as a function of several parameters. The mean concentrations of Lead and Zinc in muscle, liver and fish gonads in Oran Bay are below standards, i.e. the grades are generally low in the two metals studied and do not exceed the normative limits required in fish. This study indicates a limited bioavailability of two metals (Lead and Zinc) in the fishery products. Results of biochemical analysis revealed metallic contents below the maximum allowable doses (D.M.A), which other studies have confirmed. These heavy metals are present at low concentrations in nature and in living organisms.

KEY WORDS marine pollution; fishing products; heavy metals; *Mugil cephalus*; Oran Bay.

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INTRODUCTION

Ensuring the safety of food of animal origin, offered to consumers, is one of the priority concerns of food safety. Nowadays, and for this purpose, many preventive and repressive programs are regularly carried out in the national sphere and internationally.

Given the industrial and human activities, several compounds and toxic elements are thrown into the marine environment, causing deterioration in the quality of marine waters, nuisance in aquatic organisms, and especially a threat to human health. In this study we found it necessary to raise awareness of the most feared pollutants for the marine environment and their involvement in the bioaccumulation phenomena in food chains and beyond to raise the importance of knowledge about biochemical risks and marine pollution to preserve human health and environmental quality.

It has become essential to monitor the quality of the marine environment, especially since it alone covers 71% of the planet's surface. Monitoring programs require targeted monitoring and controls, limited over time to identify infringements and nonconformities. Also, to take corrective measures to establish an adequate control plan (Baghdadi, 2012).

Aquatic environments are today the receptacle of effluents and industrial and urban waste often toxic to living organisms (Taleb & Boutiba, 2007).

Contamination of fish requires monitoring and analysis of potentially hazardous toxic elements. These metallic elements have natural trace components of the aquatic environment, but their levels have increased due to several activities (industrial, agricultural and mining). As a result, fish are exposed to high levels of these metallic elements (Ünlü & Ümgüm, 1993; Kalay & Canli, 2000).

This study constitutes a first non-exhaustive approach to better know through the fish the state of contamination of the natural environment and to provide answers to the questions raised by the laboratory study of the network of environmental monitoring of Oran-Algeria (LRSE).

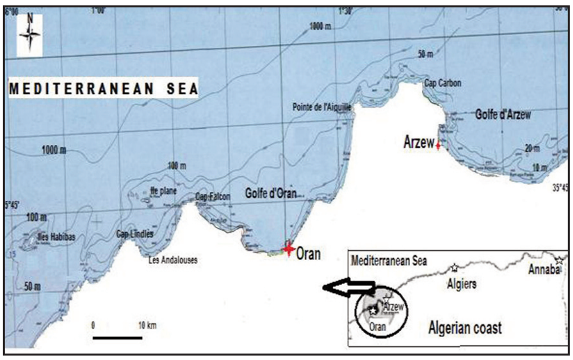

Figure 1. Geographical position of the study area: the Bay of Oran (Belhoucine et al., 2014)

Some heavy metals are essential in minimal amounts for normal growth and development of these fish such as zinc, and others do not have biological significance such as lead (Nemesok & Hughes, 1988; Kalay & Canli, 2000).

Monitoring of metal concentrations in the environment does not provide direct information on bioavailable concentrations in ecosystems and understanding of contamination processes. It is in this context that Goldberg (1975) proposed monitoring contaminant concentrations in living organisms, such as fish to monitor environments and their levels of contamination. It is the principle of "quantitative bio-indicators" based on the fact that marine organisms accumulate contaminants (Garrigues et al., 2002). It is in this context that we chose our coastal species, *Mugil cephalus* Linnaeus, 1758 (Perciformes Mugilidae) which is located in the study area (Medifaune, 2002); this type of fish is also appreciated by the population of the Algerian coast.

For other references see Goldberg et al. (1978), Denton & Burdon-Jones (1981), Legorburu et al. (1988), Attrassi & Saghi (1992), Yilmaz (2003) and Yadav et al. (2012).

MATERIAL AND METHODS

Study area

The Algerian basin is located to the south-western Mediterranean basin between latitudes 35°00.00' and 40°00.00' N and longitudes 2°00.00' and 7°00.00' W and 45°00.00' E. It is located east of the Alboran Sea, between Algeria to the south, the Balearic Islands in the north-west and north-east of Sardinia (Benzohra & Millot, 1995). The Bay of Oran (Fig. 1) is located in north-west of Algeria and south-west of the Mediterranean, it belongs to the coastal mountains Tel-Septentrional (Jebel Murdjadjo and Khar) (Leclaire, 1972). The coast of Oran is bordered by cliffs which are located, in particular, at Cape Falco (Boutiba, 2007). It occupies the central part of the Oranan coastline and opens from west to east; it is bordered on 30 km of elevated land and draws a semi-circular almost regular from Cape Falcon to Cape Aiguille.

Sample collection

The samples were taken along the Oran Bay by calling local fishermen and harvesters directly after entering the harbor. Harvesting campaigns were carried out monthly for 14 months from February 2012 to March 2013. Each harvest consists of 10 to 15 samples, harvesting of the fishes is done so that the samples are diversified by different sizes and weights , In order to be able to reflect the variation of the concentrations of the metal elements present in their environments. The samples used for the determination of the heavy metal content were packed in plastic bags and kept in a cooler equipped with Pb storage batteries. The samples were sent directly to the Environmental Monitoring Laboratory (LRSE) of the Department of Biology at the University of Oran for sample preparation and subsequent analysis.

Each sample was placed in an individual packaging guaranteeing its integrity and bearing an identification code ensuring its traceability. It was also be accompanied by a sampling form.

Sample processing

The destination laboratory is initially responsible for preparing the samples (Homogenization and constitution of the samples to be analyzed).

The aim of this section is to describe the method for the determination of heavy metals in mugil by atomic absorption spectrophotometry. The principle of the method consists firstly in wet mineralization because any search for a mineral toxicant fixed in the organs always requires mineralization which results in a complete degradation of the organic matter (Amiard et al., 1987). We have opted for wet mineralization because it minimizes the loss of volatile organometallic compounds during drying (FAO, 1977), followed by an atomic absorption spectrophotometric assay (Casas et al., 2007; Ghanjaoui, 2009; Boumehres, 2010).

In order to obtain a homogeneous solution, the protocol used can be summarized as follows:

Weigh 1 g of fresh sample into the tube.

Add 1 ml Concentrated Nitric Acid.

Select the program for a temperature of 95 °C for one hour.

Cooling of the mineralizer by a water pump.

Fill or adjust the sample to 4 ml with distilled water.

The final solution is poured into the Atomic Absorption Spectrometry (SAA) buckets which allow the detection of low levels of metals and mineral elements in the solutions.

Statistical analysis

All the data was collected according to the matrices studied in Excel files and then processed with the software Statistica. To compare the mean concentrations of heavy metals according to several parameters (organs, sex, months), an analysis of variance was applied to test the influence of the variables (season and year).

The Student t-test (Testing Expectations of Equalities: Two Observations of Equal Variance) was used to estimate the magnitude of the differences in concentrations of metal pollutants.

The purpose of the analysis of the variance is to test the presence of significant or not significant differences between averages. This analysis is applied to estimate the contribution of each factor to the variance of the dependent variable.

The purpose of the Principal Component Analysis (ACP) is to summarize the data structure described by the quantitative variables, while obtaining correlated or uncorrelated factors.

RESULTS AND DISCUSSION

Variations of the percentages of the average concentrations of heavy metals present in the sample

The coastal marine environment has a certain specificity compared to other environments: it is the ultimate receiving environment for terrestrial pollution (the biologically richest area), and the environment where numerous activities are developed (fishing and marine cultures, tourism, etc.). This medium is therefore vulnerable to a multitude of exogenous substances (Mahyaoui et al., 1989; Boumehres, 2010). Today, marine biodiversity is weakened by various types of pollutants (Moukrim et al., 2000; Banaoui et al., 2004; Marchand, 2008; El Morhit, 2009). Chemicals are among the pollutants involved in the erosion of marine biodiversity. In this case, flora and fauna can be considerably impoverished not only quantitatively, but also qualitatively (Ramade, 1993; El Morhit, 2009).

The analyses revealed the presence of very heterogeneous values of trace metales. The most important contamination concerns zinc, which represents 94%, as opposed to lead contamination, which represents 6% (Figure 1). The level of heavy metals in the different species depends mainly on dietary habits (Amundsen et al., 1997; Romeo et al., 1999; Mormede & Davies 2001; Watanabe et al., 2003).

According to other authors, differences in metal concentrations are related to feeding and eating habits of benthic and pelagic fish species (Bustamente et al., 2003). Our species is a demersal detritivore regularly feeding on zooplankton mainly from industrial chemical releases, dead plants detritus, fish, crustaceans and Algae (Farrugio, 1975; Blaber, 1976; Tung, 1981; Cardona, 2000).

Bustamente et al. (2003) showed that benthic fish usually accumulate higher concentrations of heavy metals than pelagic fish. IIt is important to underline that there areseveral industrial areas (Sonatrach, Chemecal ammoniac industries, and water purification stations) around the study site, which leads to the existence, in the marine environment, of trace elements which are transported to the ecosystems by atmospheric means (ie direct diffusion into the air) and through water currents (Arnac & Lasssus, 1985; Maanan et al., 2004; Marchand, 2008).

The presence of Lead in coastal ecosystems is mainly due to a strong anthropogenic influence (Sunda, 1989; Vasquez et al., 1994; Boumehres, 2010).

The high concentration of zinc is probably related to the presence of the Zinc electrolysis factory in Ghazaouet which is part of Algeria's northwestern coastline; this factory uses seawater for the cooling of the sulfuric acid manufacturing facilities and certain facilities of the central thermal power factory. Water, which has been used for various purposes, is directly discharged into the sea (Bakalem, 1980).

Content of the heavy metals analyzed in the fish studied (in ppm of the wet weight):

Table 1 shows the concentrations of the two pollutants accumulated in *M. cephalus*. The Zinc concentrations range from 6 part(s) per million (ppm) to 25.9 ppm of the fresh weight of *Mugil cephalus*, our results are confirmed by other similar works (Bat et al., 2012; Bouhadiba et al., 2015).

In addition, lower Lead concentrations were found between 1.02 ppm and 0.5 ppm, as also proved by Yilmaz (2008).

These results are in agreement with the experiments in the field of bioaccumulation of heavy metals in the organs of the species which is the subject of our experiment carried out at the LRSE (Environmental Monitoring Network- University of Oran1: Bouhadiba et al., 2015), this is similar for other species studied in the same laboratory: *Osilinus turbinatus* (Von Born, 1778) (Belhaouari et al., 2011) and *Merluccius merluccius* Linnaeus, 1758 (Belhoucine, 2014); the same is at the international level for other *Mugil* species (Uluozlu et al., 2007; Bat et al., 2012).

We found significant differences between Zinc and Lead concentrations (Statistica, $p < 0.05$).

Moreover, no trace above the detection threshold was found in the flesh of the individuals analyzed for Zinc. For Lead, all sampled sectors show little inter-individual variation.

Comparison of the heavy metal contents in **Mugil cephalus** *with respect to the maximum permissible doses (D.M.A)*

The values recorded in Table 2 show that the mean concentrations of Zinc and Lead are not very high respect to the Eligible Maximum Dose standards and therefore cannot cause acute toxicity.

Variation of the average concentrations according to the organs of **Mugil cephalus**

Analysis of metals in the three organs (ie, gonads, liver and muscle) shows a gap between the maximum zinc and minimum lead concentrations (Figure 2). Lead, a toxic metal, has many sources and is generally present in small quantities (Harrison et al., 1981; Rooney et al., 1999; El Morhit, 2009; Bouhadiba et al., 2015). The highest levels could be attributed to releases from industries loaded into this element that may be in the vicinity of these sites.

The Zinc concentrations recorded in the three organs are higher in the gonads than in the liver and muscle, respectively (24.08 ppm, 13.93 ppm and 13.85 ppm). On the other hand, it was found that

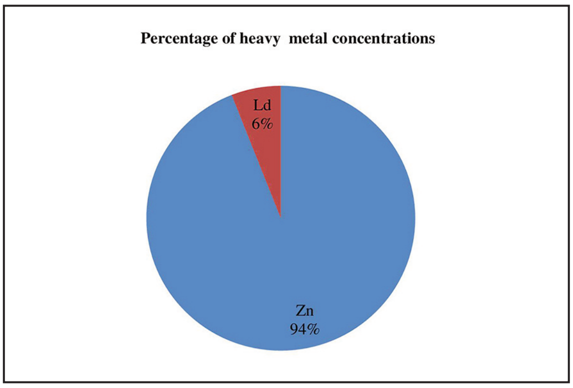

Figure 2. Changes in the percentages of mean concentrations of heavy metals in the sample.

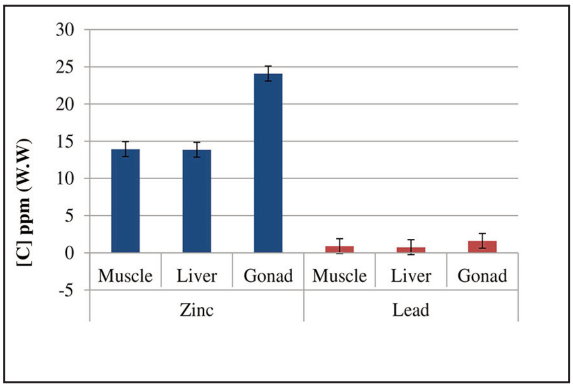

Figure 3. Variation of the mean concentrations of Zinc and Lead (ppm of w.w.) as a function of the organs of *Mugil cephalus*.

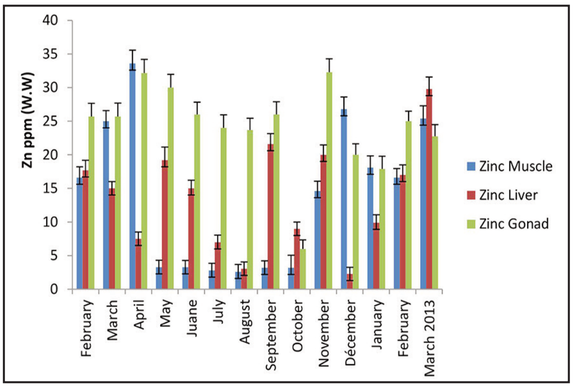

Figure 5. Monthly assessment of mean concentrations of Zinc (ppm of W.W.) in *Mugil cephalus*.

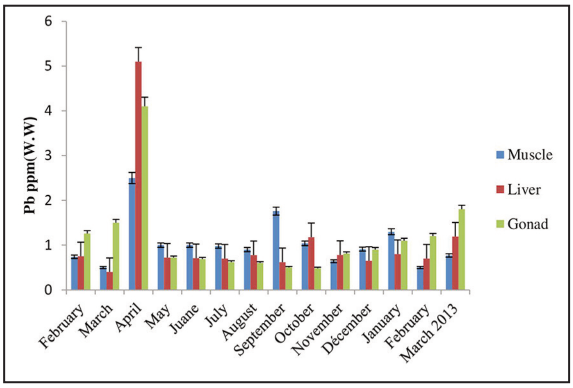

Figure 6. Seasonal variation in mean concentrations of lead (ppm of W.W.) in *Mugil cephalus*.

the differences in Lead concentrations at the three organs are low, which is of the order of (1.61 ppm) for the gonads, of (0.91ppm) for the muscle and (0.76 ppm) for the liver.

The high concentrations of Zinc recorded are explained by the fact that this element is essential in the metabolism of the cells in the enzymatic reactions as cofactor (Adeyeye et al., 1996).

In the literature, the authors state that fish meat usually has the lowest potential for accumulation of heavy metals, while the liver and kidneys have the highest potential (Lafaurie et al., 1981; Lafaurie, 1982; Hilmy et al., 1983; Dallinger et al., 1987; Chen & Chen, 1999), this data coincides with the results obtained in our *Mugil* species. Other studies have shown that heavy metals accumulate mainly in metabolic organs such as the liver which stores metals to detoxify by producing metallothioneins (Kargin & Erdem, 1991; Olsson, 1996).

The results of this study confirm that there are significant correlations between the concentrations of the two heavy metals.

This study presents a spatio-temporal evaluation of the chemical contamination of fishery products by determining the heavy metal content (Maanan et al., 2004; Glasby et al., 2004). The presence of Lead and Zinc in coastal ecosystems is mainly evidence of anthropogenic influence (Sunda, 1989; Vasquez et al., 1994; Boumehres, 2010).

Monthly assessment of mean heavy metal concentrations (ppm of W.W.) in Mugil cephalus

The levels of monthly variation of the metallic trace elements recorded are present in all organs and throughout the year. Size and sex parameters were not considered. Rise and fall episodes were recorded in metallic trace element concentrations. Gonads accumulate more heavy metals than other organs.

1. Monthly evaluation of mean concentrations of Zinc (ppm of W.W.) in *Mugil cephalus*.

The highest mean concentration of Zinc is observed in April with the respective values of gonads, liver and muscle. There was also a significant drop in concentrations at the three organ levels in October.

2. Monthly assessment of mean concentrations of Lead (ppm of W.W.) in *Mugil cephalus*.

The rate of lead appears to be relatively homogeneous throughout the year, with the exception of April where Lead concentrations are high in the three organs (liver, gonad, muscle, 5.1, 4.1, 2.5 ppm). The accumulation of Lead in April is more intensive in the liver. The decrease in Lead was remarkable in May for the three organs (0.72, 0.72, 1 ppm, respectively).

The period of sexual resting of fish is a phase of gametogenesis which is characterized by an increased accumulation of nutritive reserves and stored in the form of carbohydrate, lipid and protein materials (Webb, 1979), and during the period of the laying, these nutrient reserves are drawn. Automatically the heavy metal concentrations fall (release of metals at that time) and the accumulation of reserves will resume slowly only at the beginning of the period of sexual rest (Webb, 1979).

In the Mediterranean, the different populations of *M. cephalus* reproduce between June and October (Faouzi, 1938; Erman 1959; Morovic 1963; Farrugio 1975; Brusle & Brusle, 1977; Brusle, 1981). Which confirms the fall in heavy metal concentrations in specific months during the year.

Seasonal evaluation of mean heavy metal concentrations (ppm of W.W.) in **Mugil cephalus**

1. Seasonal evaluation of mean heavy metal concentrations (ppm of W.W.) in *Mugil cephalus*.

Figures 6 and 7 illustrate an increase in metallic trace elements during the spring season, and its resuming in autumn, which is consistent with the same results of the studies that assert that seasonal variation in grades depends on the season. Higher levels are recorded in spring and autumn (Essadaoui & Sif, 2001).

2. Study of the relationship: Lead and Zinc/ season by ACP (Analysis in Main components).

The projection of the two sampling seasons on the factorial plane (1 × 2) made it possible to identify three distinct groups. In terms of the inertias of the factorial axes of the ACP, the F1 axis accounts for 64.89% of the point cloud information (lead and zinc concentrations according to the seasons) and the F2 axis only provides 35.11 % of information about the similarity between the concentrations of the seasons. The quadrants of the factorial plane make it possible to group the concentrations of the seasons to be compared and the axes divide them into groups and subgroups.

The projection of the variables of the different concentrations of lead and zinc obtained during the

		Mugil cephalus
Zinc	M±E	16.107±5.6
	i-x	6-25.9
Lead	M±E	1.020±0.75
	i-x	0.5-1.02

Table 1. Content of the heavy metals analyzed in the fish studied (in ppm of the Wet weight). M: medium, E: Standard deviation, i: Minimal, X: Maximal.

	Lead	**Zinc**
***Mugil cephalus* (present study)**	1.02 m ppm W.W.	16.10 ppm W.W.
fishes	0.3 - 6 ppm dry.weight (b) 0.5 ppm W.W. (f)	5 mg/g dry.weight (g)

Table 2. Comparison of heavy metal contents in *Mugil cephalus* with respect to the maximum permissible doses (M.P.A). (b) GIPPM, 1973; (f) CSHPF, 1990; (G) NHMRC, 1992.

four seasons is divided into three distinct groups. The two selected factor plots F1xF2 or the inertia rate is 64.35%. The group1 of the positive axis groups together winter 2013 and winter 2012 or the concentrations ranges between 10.65 and 10.87 in ppm of W.W.

Group 2 consists of spring with concentrations of 10.36 ppm of W.W., the third group represents summer and autumn 2012 of the negative side of the factorial axis whose concentrations are respectively 6.73 and 7.87 ppm of W.W.

The results of the seasonal assessment of mean heavy metal concentrations show more than a significant spatial distribution over time. The seasonal variation in the concentration of heavy metals was studied in several Revisions (Philips, 1976, Denton & Burdon-Jones, 1981). However, the results show that the grades vary according to the harvesting period (Mousataid et al., 2005) and seem to depend on the harvest season.

3. The variation of Zinc and Lead concentrations during the sampling period.

The average zinc concentration range is larger than that of lead during the seasonal seasons of sampling, which shows that there is a highly significant difference between seasonal variations in relation to the two heavy metals; 25.9 ppm (zinc) and 1.02 ppm (lead).

***Monthly change in mean concentrations of heavy metals as a function of sex in* Mugil cephalus**

On the basis of the results of figure 10, we can say that the bioaccumulation of the two pollutants is more significant in the male than in the female.

Mean concentrations by sex indicate that the accumulated organic zinc is greater in males than females. This can be explained by the fact that females draw their nutritional reserves by synthesizing the latter in carbohydrate, lipid and protein materials at the time of egg lying during the summer period.

In the case of Lead, it has a low concentration in both sexes and in all organs.

According to Al-Yousuf et al. (1999) and Canli & Atli (2003), there are factors such as gender and height that can influence the bioaccumulation of heavy metals.

Thus, Powell et al. (1981) had already demonstrated that heavy metals were concentrated in the organs of teleost fishes in decreasing order: Liver> Kidney> flesh.

The t test of Student demonstrated that there is no significant difference between the accumulation of the two heavy metals in male and female subjects.

One of the main results obtained in this study is the demonstration of various concentrations of Zinc and Lead in the mugil on the Bay of Oran. The hypothesis of pollution of the Oran Bay of industrial origin had been put forward to explain the presence of these metals at high concentrations. Our study thus provides a new element of comparison. This study indicates that for Zinc and for Lead, similar concentrations were observed in all fish sampled at the same sampling sites. Information on the con-

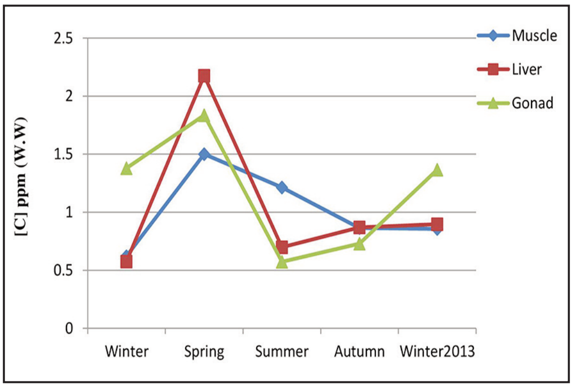

Figure 6. Seasonal variation in mean concentration of lead (ppm of W.W.) in *Mugil cephalus*.

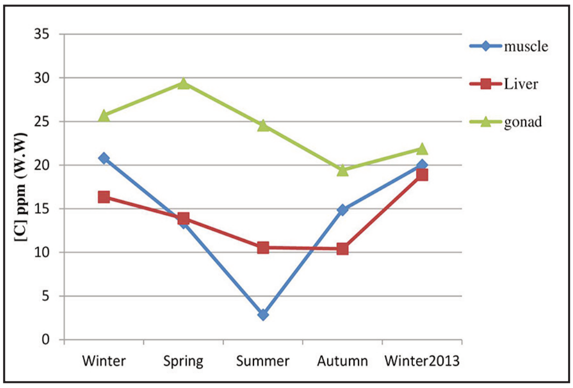

Figure 7. Seasonal variation in mean concentration of zinc (ppm of W.W.) in *Mugil cephalus*.

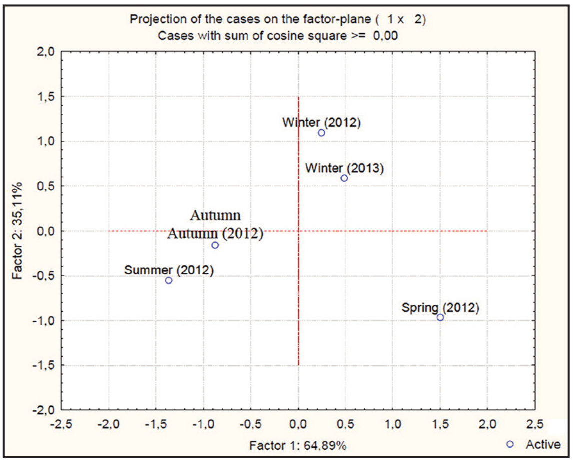

Figure 8. Projection of the sampling seasons in the factorial plane F1xF2.

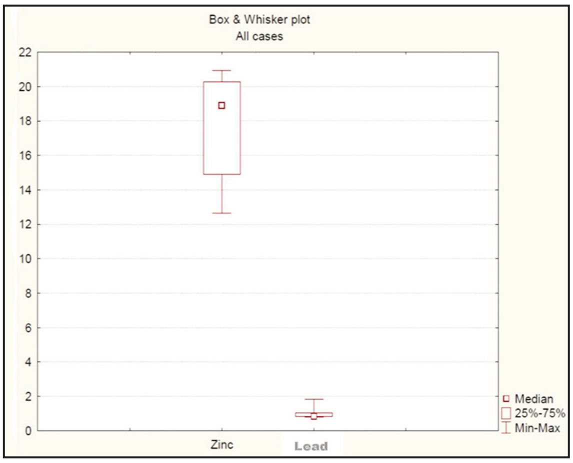

Figure 9. Diagram of the variation of Zinc and Lead concentrations during the sampling period.

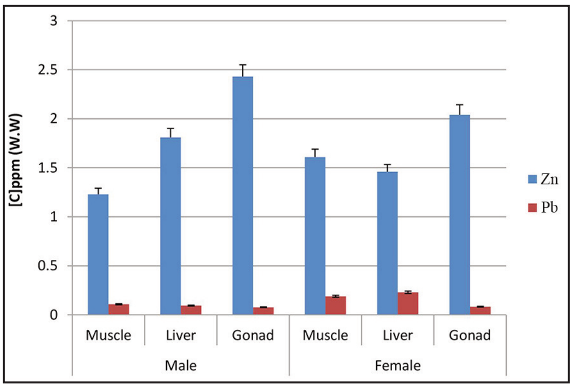

Figure 10. Monthly change in mean concentrations of heavy metals (Lead, Zinc) as a function of sex in *Mugil cephalus*.

centrations of metals in fish is still too fragmentary to be able to know precisely their origin. However, similar levels observed at several geographically distant sites and located on different basins are in favor of a hypothesis of more general contamination origin and not of several local origins. On the other hand, they suggest that industrial chemical activities are the main source of contamination (Baghdadi, 2012).

It also seems probable that the source of the metals found in the fish analyzed in our study is not only the direct pollutant discharges into the Bay but is more related to industrial pollution.

CONCLUSIONS

In Algeria, studies have been carried out on the determination of metallic levels in fish taken from the Mediterranean coast.

The risks of bioavailability and toxicity of these traces metals are feared, as they constitute permanent dangers for the whole food chain and threaten public health (Arnac & Lassus, 1985; Fadil et al., 1997; Boumehres, 2010). As man is the final element of consumption of marine products and being the last phase in the food chain and as this species is highly appreciated by the Algerian consumer; we performed this chemical analysis to demonstrate the level of contamination of metallic elements in fish and at the sampling site.

This allowed us to conclude that the concentrations are below the limit values for fish proposed by FAO (1983). High levels of heavy metals have been observed in the liver and gonads. Although fish liver and gonads are very rarely consumed by humans.

Overall, the interpretation of the results is made difficult by the absence of reference data concerning the concentrations of metals present in fish in natural environments not subject to the effects of anthropization. Our objective was to complement the LRSE study on the search for sources of metallic pollution in the Bay of Oran, to evaluate the quantification of heavy metals in fishes.

The analysis of flesh and gonads and liver of *M. cephalus* makes it possible to assess the state of contamination of the whole food chain and to assess whether this contamination represents a potential risk for human consumption.

The various analyses carried out made it possible to obtain the following elements:

No significant direct impact of the metal releases found in the LRSE study could be observed on the different fish.

No trace of Lead was detected beyond the quantification threshold.

A generalized presence of Zinc in the analyzed parts of fish and lead at equivalent levels in all the sectors studied was highlighted.

The metal content in the flesh does not appear to pose a risk to human consumption due to the large quantities of fish that must be ingested to reach the acceptable doses.

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