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Impact of some biotics and abiotics factors on the accumulation of heavy metals by a biological model *Merluccius merluccius* in the bay of oran in Algeria

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Abstract

Our study focused on the assessment of contamination by three trace metals (Cd, Pb and Zn) an abundant demersal fish in Algerian waters and appreciated by a large fish-eating population, hake *Merluccius merluccius* (L. 1758) , caught in the bay of Oran. Monthly sampling was spread over a year from January to December 2010. Two organs have been identified: the liver and muscle. Metal concentrations were determined by atomic absorption spectrophotometry with flame based on three parameters (sex, size and month). From this study, it appears that the hake (*M. merluccius*) bioaccumulates three metals pollutants sought, the highest concentrations are those of zinc, lead concentrations more or less important, and the lowest concentrations are those of cadmium. The processed results showed no statistically significant difference between the levels of trace metals in both sexes and at both organs considered. The use of hake as bio indicator in the assessment of contamination by these micro, helped to highlight their presence at two target organs (liver and muscle). These results only reveal the existence of a close relationship between marine pollution and many other industrial and urban wastes, the Oran Bay and surrounding areas.

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Introduction

The marine environment is a significant challenge in terms of economic development. In recent decades, the pollution of the oceans around the world has become a growing concern internationally.

The Mediterranean Sea is increasingly threatened by different sources of pollution is due to increased coastal anthropogenic contributions of its neighboring countries in the process of industrialization, which may reduce its economic potential to adversely impact on human health indirectly, through their incorporation into the food chain (Christensen *et al.* 2003, Henry *et al.* 2004).

The Algerian coast intensively exploited and densely populated is practically affected by various pollutants. Oran region is certainly one of the areas where the deterioration of the quality of coastal waters is the most noticeable. The city of Oran is an economic and industrial attraction. The coastline is characterized by Oran massive urbanization that concentrates 936,927 inhabitants, which represents 77.5% of the total population of the province. Added to this is the environmental problem of various kinds such as industrial, agricultural, domestic, etc...

However, the coast of Oran became vulnerable and exploited, being the preferred container inputs toxic and corrosive contaminants, whether from rivers, atmosphere or urban and industrial outfalls. Among these pollutants, chemicals that can cause degradation of water quality include heavy metals that cause a sharp increase in metal content to supplant natural sources (Grimes, 2010).

The presence of such pollutants in the marine environment is a serious problem for the Man who stands at the top of the food chain. Consumption of a large number of marine species exposed to a multitude of health risks. Awareness of the degradation of our quality of life by these contaminants has led to interest in their fate in the environment.

The present work aims to assess the state of the quality of the coastal environment by studying contaminants (Cd, Pb, and Zn) in the liver and muscle organs hake *Merluccius merluccius*.

Materials and methods

Study Sites

The Algerian basin is located south of the western Mediterranean basin at latitude of 35° to 40° North to longitude 2° to 7° West 45 East. It is located east of the Alboran Sea, between Algeria to the south, the Balearic Islands in the North West and North East Sardinia (Benzohra and Millot 1995).

The Oran Bay (Fig.1) is located north west of Algeria and South West of the Mediterranean, it belongs to the Coast Mountains Tel Septentrional (Jebel Murdjadjo and Khar) (Leclaire, 1972). Oran coast is lined with cliffs that are located including Cape Falcon (Boutiba, 2007). This bay is characterized by the absence of Oued except rare coastal wadis of modest importance. The Gulf of Oran in the central part of the Oran coast and opens from west to east, it is bordered on 30km high land and draw a half circumference just regular meadows from Cape Falcon to Cape of needle. It is between the bay Andalusian and the Gulf of Arzew.

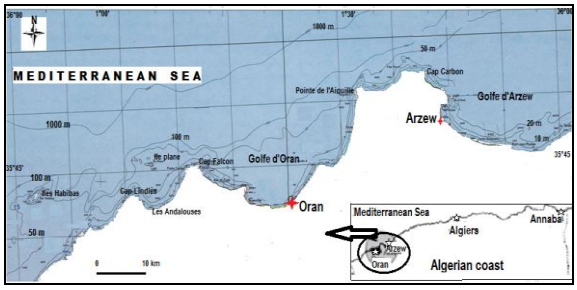

Fig. 1. Geographical position of the study area: the Bay of Oran.

Collection of samples

Hake is a demersal species bathypelagic (Fig. 2) attending the depths from 50 to 1000m. The range of this species extends over the North Atlantic coast to the British Isles and also encompasses the entire

Mediterranean basin and the Black Sea (Lloris and Matallanas 2003).

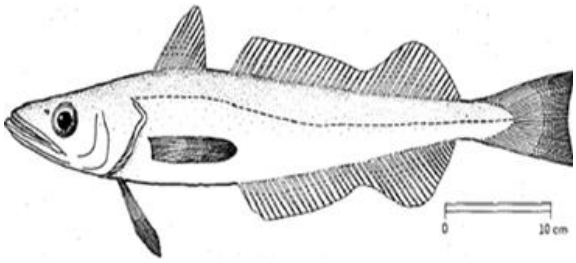

Fig. 2. Hake *Merluccius merluccius* L., 1758.

During the year 2010, and because of one sample per month, 530 individuals (265 males and 265 females) between 13.6 to 57cm *M. merluccius*, processed the same day. Both organs were removed: liver, and muscle.

Analysis of Lead (Pb), Cadmium (Cd) and Zing (Zn) Heavy Metals

The first step of our technique is to group individuals into lots of size classes and sex. In a second step, we proceed to mineralization (wet combustion) is deposited 1g fresh weight of each sample (liver, muscle) in a flask to which was added 1 ml of nitric acid (HNO_3) to 65% purity the temperature at 95°C is heated for one hour, after cooling, the full content of up to 4 ml double-distilled water, the solution is ready to mix with the Atomic Absorption Spectropho tometry flame (SAA) (Amiard *et al.* 1987).

Data analysis

The statistical treatment of the results was performed on a PC using Microsoft Excel version 2007. To compare the average concentrations of heavy metals between two independent groups from the same population the Student t test (test of equalities expectations: two observations of equal variance) was used to estimate the significance of differences between the concentrations of metal pollutants. This difference was considered significant at a probability (p) less than 5% ($p<0.05$).

To test the homogeneity of the concentrations of heavy metals in relation to different size classes of individuals, analysis of variance was used.

Results and discussion

The intercalibration exercises

Each series of samples of our mineralization is automatically accompanied by a hand, of a mineralization white, comprised of reactant-containing solutions of mineralization (nitric acid) and suffer from the same experimental conditions as the sample, and secondly, by a series of samples of inter-calibration of a standard biological material *Fucus sp* 140/TM coded, provided by the International Atomic Energy Agency, Monaco (IAEA, 1995), allowing, thus defining the coefficients variation for each of the desired metals: lead (Pb), cadmium (Cd) and zinc (Zn) and check the correctness and accuracy of the protocol analytic of these results are summarized in Table 1. These intercalibration exercises have shown that our analyzes were carried out in satisfactory conditions, the analytical technique used was reliable and accurate.

Table 1. Results obtained from the inter-calibration exercises expressed in ppm dry weight.

Trace elements	Reference values (A.I.E.A, 1995, Monaco)		Value found
	Min -Max	Mean	
Cadmium	(0,50 - 0,57)	0,53	0,52
Lead	(1,91- 2,47)	2,19	2,15
Zinc	(45,3 - 49,3)	47,3	48,5

The average water content in the muscle of hake is 75.15%. We adapted this mode of expression, because it allows a good comparison with different values from the literature.

Changes in mean levels of heavy metals in organ function, gender, seasonal and by size class and body. We based on the results shown in Fig. 3, we see that cadmium levels bioaccumulate in the liver of females are (0.30±0.08ppm WW (Wet Weight)), and those measured in males of the order (0.23±0.05ppm WW). These cadmiums levels in the liver were significantly

higher in females compared to the rates in males (P<.05). However, no significant differences was observed in the levels of Cd in the muscle compared to that observed in the liver in both sexes (Fig. 3).

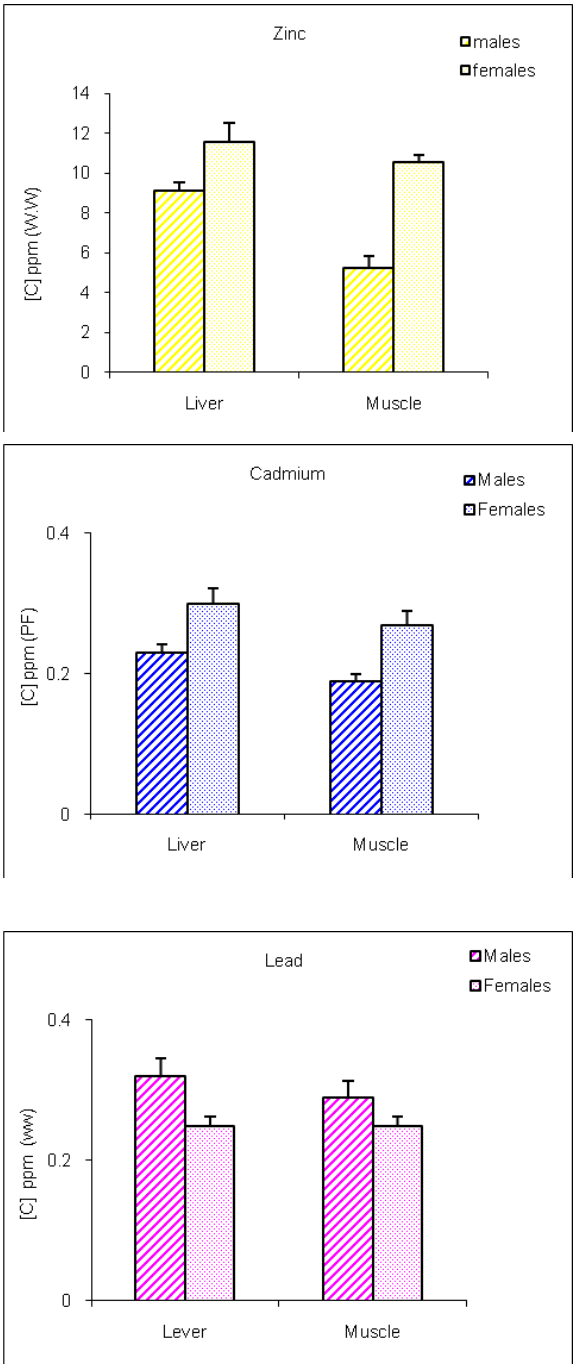

Fig. 3. Changes in mean levels of heavy metals (Cd, Pb, Zn) (mean ± sd ppm WW) liver and muscle *Merluccius merluccius* caught in the Bay of Oran.

Comparison of lead levels showed no significant differences between the liver and muscle samples from hake both sexes, they are generally of the same order of magnitude (Fig. 3).

Finally, these results show that the concentration of Zn in the liver was 1.2-times more significant in females versus males and 2-fold higher in the muscle (Fig. 3).

The zinc concentrations recorded at the two target organs revealed a significant difference ($p<0.05$) with a dominance of a higher rate in females (11.58±0.96) and (10.54±0.36) respectively in the liver and muscle tissue.

The levels of metal pollutants livers of bioindicator organism are greater than those observed in the muscles, expressed as total concentration, or individual for Zn, Pb and Cd One possible explanation is the presence in the physiological state of these elements in liver tissue as enzyme co-factors, but also the fact that they are subject to more rapid elimination from the muscle, as described by several authors for zinc and cadmium (Marcovecchio and Moreno 1993, Cinier *et al.* 1999).

According Ramade (1979), teleost fish, metallic elements are particularly concentrated in the liver but also in the kidneys and more modestly in the muscles. Thus, Powell *et al.* (1981) had already shown that heavy metals were concentrated in the organs of teleost decreasingly: Liver> Kidney> Muscle.

Many studies emphasize the affinity of heavy metals such as Cu, Zn and Cd in the liver with storage capacity and regulation of these metals have been widely described in the literature in fish (Kalay and Canli 2000; Usero *et al.* 2003, De Boeck *et al.* 2004, Oliveira Ribeiro *et al.* 2005, Belhoucine *et al.* 2008). The difference in concentration of the metal between the sexes is, without doubt, that synthesize more females than males metallothionein (Webb, 1997).

Seasonal variations seem to govern the distribution of heavy metals. Indeed, we found that these fluctuate

significantly in the liver and muscle of hake. This bioaccumulation of trace metals in this specimen of western Algeria experienced a sharp seasonal variation (Fig. 4).

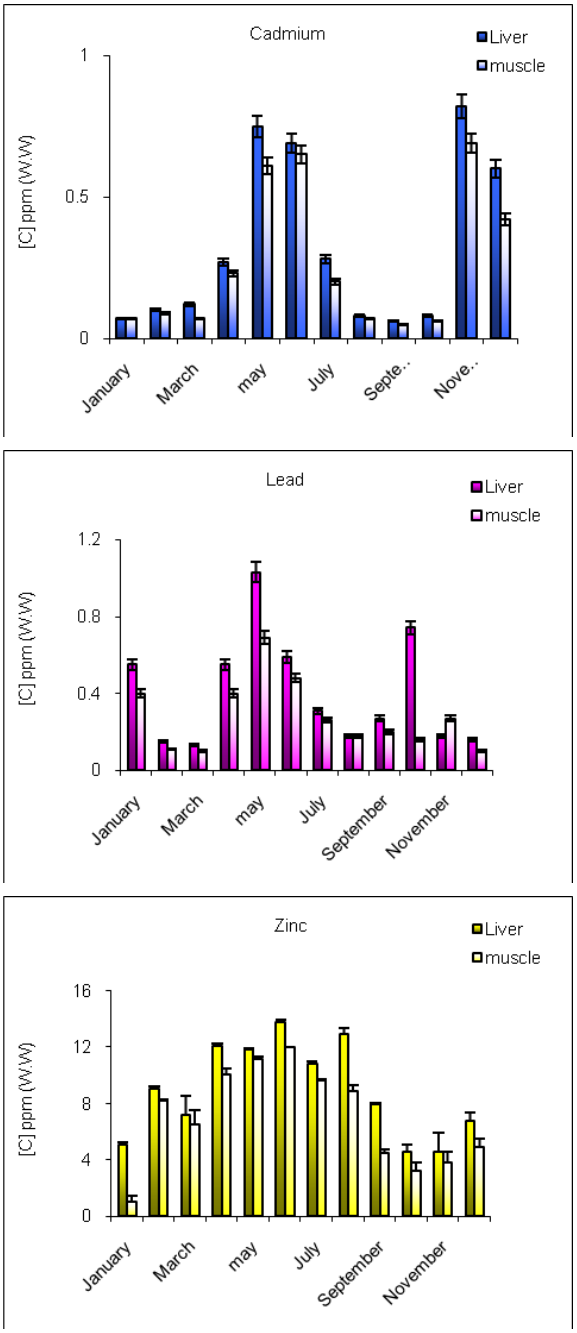

Fig. 4. Changes in concentrations of heavy metals (mean ± sd WW ppm) based on months in *Merluccius merluccius* caught in the Bay of Oran.

The downward trend of trace metals identified during this period corresponds perfectly to the breeding of hake Oran bay. Products accumulated much earlier deported to the external environment. This decontamination females via the egg appears to be related, at least in part, to the mobilization of metals in the liver to the gonads during maturation. The seasonal factor is important and many studies have also shown that metal concentrations in marine species vary seasonally (Bei *et al.*1998, Wright and Mason 1999, Kaimoussi *et al.* 2000, Orban *et al.* 2002).

This tendency to accumulate for zinc is more difficult to explain that this metal is normally regulated by marine organisms. It is possible that high zinc concentrations encountered during the summer, would be attributed to the importance of water activity during this period. In this context, we argue that our data are comparable to those found by Alioua *et al.*(2013) in the sardine *Sardinella aurita* of the golf of Skikda.

In order to study the variation of the contamination in the hake population, centimeter size classes were set at 5cm per class, depending on sex. So we have identified for classes, namely: 17-26cm / 27-36cm / 36-46cm / 47-56cm for females and for males: 13-22cm / 23-32cm / 33-42cm / 43-52cm. 10 individuals of each centimeter class were sampled whenever possible.

Following this study, we found that, overall, the liver and muscle tissues accumulate both, zinc *M. merluccius*, but the largest concentrations are found in the liver for four lots of classes. Changes in zinc seem to be closely related to the phenomena of seasons; however, we do not find significant variations between the two sexes (Figs 5 (a) and (b)).

The difference between the levels for different size classes is more pronounced during the summer months. Decreasing concentrations goes along with the increase in size, so with the age of the individual for males as for females.

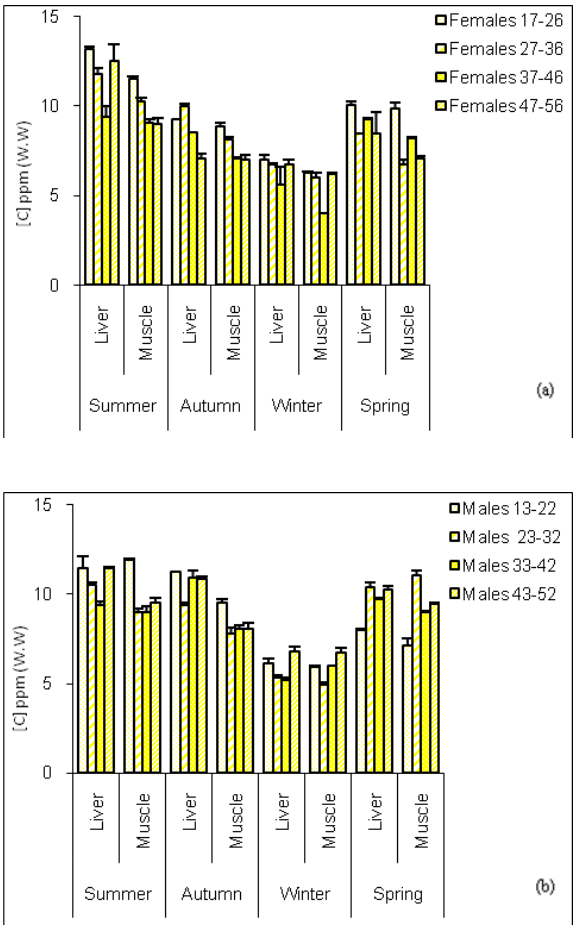

Fig. 5. Monthly Assessment of zinc levels (mean ± sd ppm WW) as a function of size in *Merluccius merluccius* females (a) and males (b).

It appears from the statistical treatment of results found, the absence of a significant difference in the bioaccumulation of zinc between the four groups of different class and the two bodies. The change in mean concentrations of heavy metals seems to be related to the size of individuals. It is the class of the smallest is the most contaminated. This is consistent with the results of Coombs (1980) which states that the development (growth, size and sexual maturity) of a marine organism influences the bioaccumulation of metals contaminants. Our results also coincide with those of Aissi (1980), who analyzed the levels of certain metals in mullet *Mullus surmuletus* the Bay of Algiers and speculates that more than the weight increases in fish more levels down; thus, individuals with small sizes have the highest rates of metals.

According to the same author, the metal concentration seems to decrease slightly with age although the absolute concentration in the animal continues to increase. This is explained by the fact that older people carry a certain amount of travel, and are dynamic, so move away from the coast and therefore any sources of pollution.

The plumbic levels within each size class, showed no significant difference between the two bodies. For cons, the concentration of that toxic metal is increased in the liver of the younger lot of both sexes as that found in the liver of the population of the largest class and accumulates selectively in males than females (Figs 6 (a) and (b)).

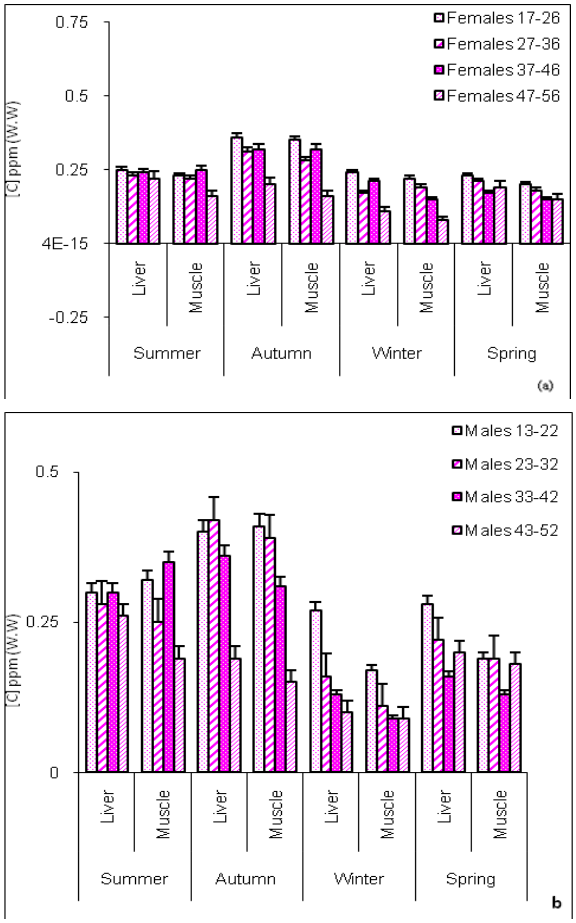

Fig. 6. Seasonal variations lead levels (ppm W.W) in *Merluccius merluccius* females (a) and males (b) by size class and body.

Moreover, Cossa *et al.* (1992) suggest that lead levels in the muscle *Platichthlys flesus* tend to decrease with the size in less contaminated areas.

The study of graphs 7 (a) and (b) highlight the seasonal influence on the behavior of cadmium depending on the size and sex in females and males, respectively. For male and female individuals, cadmium appears to reveal substantial variations in the different periods of the year.

Overall, it appears that the average of the lowest concentrations are observed during the winter period, these values will then increase progressively during the hottest time of the year and will stabilize during the fall season, and it is in spring than most increases concentrations are encountered.

For female and male individuals (Figs7 (a) and (b)), the summer marks an upward trend in average concentrations toxic metal.

From these results, it seems that in most cases older people are less contaminated for three traces elements. In contrast, small individuals seem to be the preferred target of these heavy metals. It seems that the average concentrations of inorganic elements tend to decrease with the size of fish. This is likely to put in relation to the much more intense physiological activity in young people who focus more metals in their tissues (Fig.7).

Level of contamination by trace elements of *Merluccius Merluccius* compared to the Maximum Permissible Dose (MPD)

Location level metal contamination of *M. merluccius* from the maximum permissible dose (MPD) estimating quantities of heavy metals in ecosystems and organisms is an important part of the work and research carried out in ecotoxicology (Huang *et al.* 2007). These xenobiotics are problematic because of their persistent nature and toxicity. This is because the regulations impose on the low thresholds.

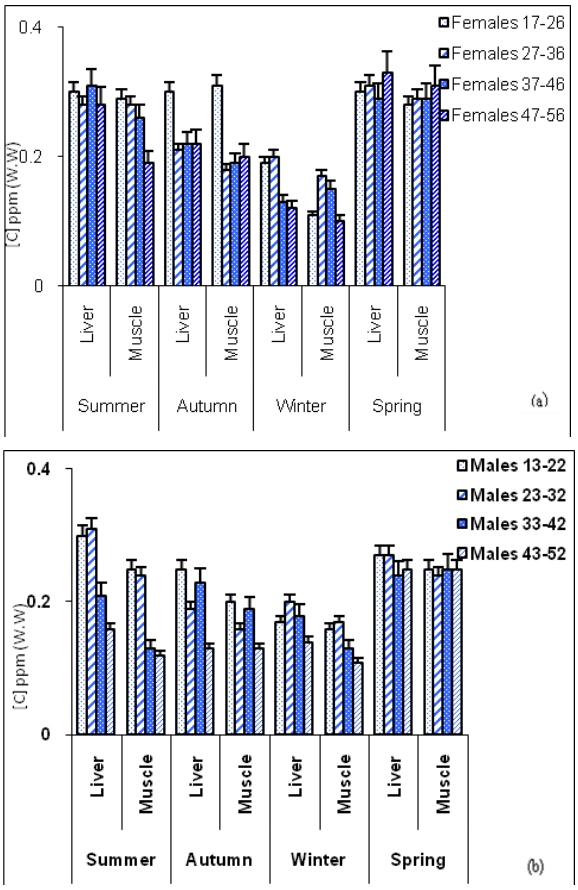

Fig. 7. Monthly evaluation of cadmium concentrations (ppm WW) *Merluccius merluccius* females (a) and males (b) by size class and body.

By comparing our results of heavy metals in the hake *M. merluccius* from the tolerated threshold (Table 2) muscle; we could deduce that the values recorded in the Bay of Oran remain below the critical values of contamination.

Comparing the levels recorded in this category of fish studied shows that the levels of trace elements (Zn) are higher compared to those toxic elements (Pb and Cd). This is typically related to their toxicity. However, the levels of zinc (16,27ppm DW (Dry Weight)) does not constitute a danger for the consumer because they are well below the Maximum Permissible Dose (MPD) (5 mg/g).

The average rates of cadmium recorded in our samples *M. merluccius* (0.55ppm DW) are within the range of those recommended by the IOPR (1996) and

Augier *et al* (1988). Similarly, for medium levels of lead (0.63ppm DW), which also lies within the range of MPD set for the net by GIPPM Fish (1973).

We see only the average doses of heavy metals found in the compared with those from the literature relating to Maximum Permissible Dose hake are not worrisome.

Table 2. Comparison of heavy metal content (ppm) in the muscle in *Merluccius merluccius* from the Maximum Permissible Dose (MPD).

(a) Augier *et al* (1988) - (b) GIPPM (1973) [Interministerial Group Issues Pollution of the Sea] - (c) CNRS (1971) [Group chemists] (d) (WHO 1971) - (e) FAO (1971) - (f) CSHPF (1990) [High Council for Public Hygiene of France] - (g) CNRMS Australia (1992) [National Council for Medical Research and Health] - (h) IOPR (1996) [Paul Ricard Oceanographic Institute].

Traces elements	Cd	Pb	Zn
Fishes	1 ppm D.W (a) 0,15-3 ppm D.W (h) 0,1 ppm W.W (f)	0,3 à 6 mg/Kg D.W (b) 0,5 mg/Kg WW (f)	5 mg/g D.W (g)
This study *M. merluccius*	0,24±0,11 ppm D.W	0,27±0,16 ppm D.W	7,89±0,47 ppm D.W

NB: D.W: Dry Weight ; W.W : Wet Weight

There is currently, no indication that the levels are high enough to cause morbidity or mortality among the fish themselves or ask threats to human health from the consumption of these fish.

This fact does not diminish the potential risk to humans in the medium and long term if urgent measures are not put in place to monitor the safety of seafood, vectors of toxic agents, particularly lead and secondary cadmium, since these metals recorded alarming levels for public health. The reliable assessment of the risks posed by these pollutants on both human health and the environment is a major challenge (Maroni *et al.* 2000, Eason and O'Halloran 2002, Alavanja *et al.* 2004).

Conclusion

Sea, particularly the coast, appears to be the receptacle of all pollutants from land or marine origin. The quality of coastal waters is of concern locally and only a comprehensive policy can overcome the gradual degradation of the marine environment.

The risk of contamination is multiple: a risk to human health from the consumption of contaminated shellfish and marine fish, an economic risk if ban on the marketing of these animals and a negative image of the polluted region.

The origin of metals contaminants detected in the organs (muscle and liver) *Merluccius merluccius* the Bay of Oran result many discards (industrial and municipal). This pollution is due to the pollution load, resulting from anthropogenic activities around the Bay of Oran , which mark the sewage outfalls Broom east (35° 42' 963" N, 000° 37' 266"E) and Fort Lamoune (35° 37' 954' N, 000° 39' 332"W) west of the port of Oran without any treatment (Taleb *et al.* 2006) , knowing that most of the wastewater produced in Algeria from 1970 to 1990 stations are stationary (Bentir, 1996).

Grimes and Boudjakdji (1996) reported that the Oran coast has 11 outlets only. The biggest sewage drains the northern part of the city, and that feeds the harbor in the eastern sector and in the area before the South port. This region is characterized by high pollution which led to the absence of life for benthos, because of its proximity to the main sewer wastewater from a city of Oran.

This dumping of toxic waste from industrial complexes (Arzew) (Refinery - Ammonia Plant - Complex methanol), Factory electrolysis Zinc (Ghazni) and Complex stationer Mostaganem (CELPAP). They constitute a serious threat to the marine environment.

These levels are explained, too, by the fact that the Bay of Oran is under the direct influence of maritime traffic. These circumstances lead to criminalize anti-fouling covering the hulls of boats (Boutiba *et al.* 2003) paints. These antifouling paints are based on copper compounds and / or synthetic chemicals that persist in the long term in the environment (Leroy, 2006).

The release of various metals in seawater is gradually and continuously by the process of leaching paint layers. This contributes in no small way, the emphasis of this pollution if we take into account the considerable overall surface shells of all boats and the effect of gasoline vapor emissions engines that contribute also to the pollution of the sea (Augier *et al.* 1988).

Water carries heavy metals, and incorporate them into the food chain (algae, fish,... etc.). Although heavy metals are usually present in trace amounts, they are nevertheless very dangerous, since their toxicity develops through bioaccumulation in organisms.

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