HEAVY METAL AND TRACE ELEMENT BIOACCUMULATION IN MUSCLE AND LIVER OF THE MERLU (*Merluccius merluccius* Linneaus, 1758) FROM THE GULF OF BEJAIA IN ALGERIA

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Abstract

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This preliminary study aims at assessing a contamination level of Bejaia Gulf by some Metallic Trace Elements (MTEs) such as zinc, iron, copper, nickel, lead and cadmium. The use of hake, *Merluccius merluccius* (Linneaus, 1758) as a bio-indicator of these heavy metals, made it possible to put in an obvious the presence of these micropollutants at the level of two target organs, the liver, detoxifying organ and the muscle, the most prized part by consumer. The MTE assays are performed using a Flame Atomic Absorption Spectrophotometer (FAAS). As for the fish contamination level, it is referred to the guide values of the WHO, FAO and Algerian recommendations. Within the framework of our study, the recorded values show that hake accumulates all metal pollutants being analyzed. Iron and zinc exhibit the highest concentration, the presence of nickel and copper were also recorded. Lead and cadmium have critical concentrations exceeding even Algerian standards.

These results revealed no significant difference in both sexes and in both target organs. However, the study indicates considerable contamination in young fish with significant concentrations according to established standards. These data reveal that *Merluccius merluccius* is a bio-indicator of marine pollution in the Gulf of Bejaia.

Key words: heavy metals bioaccumulation, *Merluccius merluccius*, contamination, Gulf of Bejaia.

Introduction

Metals occupy a prominent place in global economic activity playing a strategic role in some applications. The scientific and technical definition of heavy metals brings back their high density (exceeds 5g/cm³), their high atomic mass, and their toxic power for biological systems (INERIS, 2005). Comparatively to other contaminants, heavy metals are inorganic compounds; they are very toxic minerals, even at low concentrations of which they constitute a serious threat to human health, organisms and natural ecosystems, because of their toxicity, their persistence as well as their bioaccumulation characteristics (DeForest, Adams, 2007; Onistratenko et al., 2009). In addition, many heavy metal ions are come to be known as toxic because of their carcinogenic effects to human health (Fu, Wang, 2011, Mason et al., 2014).

Merluccius merluccius L., 1758, as a fish belonging to the Merluccidae family, has been strongly recommended as a bio-indicator species for these Metallic Trace Elements (FAO/WHO, 1972). This fish is found in the Mediterranean much more in the western than the eastern part (Recasens et al., 1998; Arneri, Morales-Nin, 2000; Morales-Nin, Moranta, 2004), and in the North-East Atlantic from England to Senegal (Sarano, 1986; Lucio et al., 2000; Piñeiro, Saínza, 2003; Murúa et al., 2006; Lannin, 2006; Domínguez-Petit, 2007).

It lives in varying depths of the continental shelf, near the coast for young fish and up to 1000 m deep for adults (Bouaziz, Djabali, 1998). As a result, work has been carried out on the biocontamination of xenobiotics on hake (Storelli et al., 2005; Aksu et al., 2011; Belhoucine et al., 2014). However, although they are more numerous, gaps still persist in this area in particular, because to date, no study has been carried

Fig. 1. Maps of Gulf of Bejaia.

out in the Gulf of Bejaia. The present work aims at evaluating the quality state of the coastal environment by Metallic Trace Elements (Cd, Pb, Cu, Ni, Fe and Zn) accumulated by two target organs (liver and muscle) of *M. merluccius*.

Material and methods

Study area

Bejaia region is located on the southern shore of the Mediterranean, in the eastern sector of the Algerian coast. Administratively, Bejaia is limited by five provinces: Tizi-Ouzou and Bouira in the West, Jijel in the East, Setif and Bordj Bouarredj in the South. In the North, it opens on the Mediterranean with a length of about 100 km (Fig. 1). The Gulf of Bejaia is limited to the East by the Cape Cavale, to the west by Cape Carbon and open to the North on the Mediterranean.

It presents a centre of economic and industrial attraction but it is intensely stifled, particularly by the tourism sector which makes it vulnerable to any pressure.

Sample analysis

Merluccius merluccius was sampled along the Bejaia Gulf by professional fishermen. During the study period (September 2015 to September 2016), the collection of the biological material is carried out on more than 800 individuals of length ranging between 9.8 and 81.9 cm.

Biological sampling is transported directly to the laboratory. A series of measurements are carried out on each individual, which makes it possible to separate them into lots of size and sex classes. Tissues of muscle and liver for each sample are taken. They are dried in an oven for 24 hours at 90 °C, then ground and passed through a sieve. The obtained subsamples are weighed and stored in labeled pillboxes ready for chemical analysis.

In a second step, the mineralization (dry combustion) is carried out: 2 g of dry weight of each sample (liver, muscle) are deposited in the tubes of the mineralizer, 1 ml of nitric acid (HNO_3) is added at 65 °C at 65% purity and 20 ml of double distilled water, then the whole is heated at a temperature of 100 °C for 20 minutes. After cooling, the contents are

Fig. 2. Variations of mean heavy metal concentrations expressed in mg/kg DW according to the sex of *Merluccius merluccius* from Bejaia Gulf.

completed with a maximum of 20 ml of bidistilled water. After filtration, our solution is ready to be analyzed by flame atomic absorption spectrophotometer (SAA) (Amiard et al., 1987).

Data analysis

The statistical treatment of results was performed using Statistica software version 2013. To compare concentrations (mean $\pm$ confidence interval) of heavy metals between two independent groups of the same population, the ANOVA test is applied to estimate the importance of differences between the concentrations of metallic pollutants either by target organ, by sex or size class. This difference is considered significant with a probability (p) of less than 5% ($p < 0.05$).

Preparation of calibration solutions

The stock solutions are of the order of 1000 ppm (1 g/l). In 1 liter of demineralized water, a certain quantity of chemical substances (CuSO_4 , PbNO_3 ,) are dissolved in such a way as to

obtain 1 g of each element (Cu, Fe, Zn, Ni, Pb, Cd). A small amount of nitric acid being used as a stabilizer is added to the solutions prepared for the calibration series in order to be in the same conditions as the unknown solutions to be assayed. From the stock solution of 1 g/l, the standard solutions are prepared according to the well-defined ranges for each element.

Results

All results of targeted MTE analysis (Cu, Fe, Zn, Pb, Cd) obtained from hake organ samples (muscle, liver) are expressed graphically. A comparison is made between concentrations of these metals, as well as their average variation by sex, between organs independently and finally by size classes.

Analysis of MTE according to sex

The results shown in Fig. 2, explain that essential MTEs (Zn, Fe) exhibit high levels, much more in females pointing out a

Fig. 3. Variations of mean heavy metal concentrations expressed in mg/kg DW according to the target organs of *Merluccius merluccius* from Bejaia Gulf.

values of 20.57 ± 1.39 mg/kg DW and 27.51 ± 7.22 mg/kg DW respectively, for zinc and iron. However, the average concentrations in terms of essential MTEs, being recorded at low-dose (Ni, Cu) in both sexes show a significant differences at 5% level, with a higher value for copper element in males (0.73 ± 0.36 mg/kg DW). On the other hand, the mean nickel concentrations are the same for both sexes, posting up a value around 0.28 ± 0.1 mg/kg DW (Fig. 2).

Finally, non-essential MTEs (Pb, Cd) have highly significant values ($p < 0.05$) with a predominance of a higher Cd level in females recording value of 0.23 ± 0.09 mg/kg DW, while Pb dominates in males displaying a rate of 0.67 ± 0.06 mg/kg DW (Fig. 2).

Analysis of MTE according to the organs

Depending on organs, MTEs show that hake presents a highly significant differences ($F = 9.36$ and $p = 0.04$) (Fig. 3). The val-

ues being recorded in essential MTEs in the liver show concentrations approximately twice as much in females than in males and almost twice as higher in the liver as in muscle, displaying a values of 20.63 ± 3.72 mg/kg DW and 37.16 ± 10.73 mg/kg DW respectively, for the zinc and iron.

The rest of the MTEs, indicate that they are more present in the liver of males with average concentrations exhibiting a very highly significant difference ($p < 0.05$) recording values of 0.47 ± 0.02 mg/kg DW and 0.34 ± 0.27 mg/kg DW respectively, for Cu and Ni (Fig. 3).

Finally, Cd levels always significantly high in the liver, recording an average of 0.342 ± 0.13 mg/kg DW. However, the comparison of Pb levels in the same context shows the existence of a very highly significant difference between the samples, always higher in the liver displaying a value of 0.76 ± 0.01 mg/kg DW. (Fig. 3).

These contaminants are marked in the liver with high values relative to the muscle.

Fig. 4. Variation of mean heavy metal concentrations expressed in mg/kg DW according to the size classes of *Merluccius merluccius* from Bejaia Gulf.

Analysis of MTE according to size classes

Figure 4 highlights the relationship between size and sex of hake individuals and the mean levels of targeted MTEs. In the most cases, the latter are accumulated much more in liver and muscle of juvenile and adult individuals, both in male and female populations. The average concentrations of essential MTEs show that Zn has higher values on one side in females, and on the other hand in the size class of more than 25 cm for males and females. Likewise, the Fe also records higher average values in female than male population, either for juveniles or adults (Fig. 4).

From these results, it seems that the average concentrations in Cu and Ni are higher in liver of male and female adults, but they pile up much more in muscles of juvenile females. As inorganic element, the mean concentrations of Cd accrue in female livers as well as in juvenile male muscles; this accumulation tends to decrease with age. Also, Pb concentra-

tions decrease with the size of individuals in male population, unlike females that stack this element with age, first in liver, then in muscle.

Discussion

Most of the time, a waste discharge is never considered as a unique source because different types of pollution are mixed and interact with each other (synergistic effects). Sources as wadis or sewers reject organic wastes and detergents, some of which are accompanied by heavy metals (chemical pollution), various microorganisms such as bacteria and viruses (biological pollution). The whole flow ultimately into the aquatic ecosystem (Physical Pollution) (Gravez, Bernard, 2006). In addition, several previous studies have shown that heavy metals are considered as contaminants in coastal and marine environments around the world, stemmed from industrial, agricultural and anthropogenic discharges (Ruilian et al., 2008;

Table 1. Comparison of heavy metal content in the muscle in *Merluccius merluccius* from the Maximum Permissible Dose (MPD).

Metals Species	Lead (Pb)	Cadmium (Cd)	Cooper (Cu)	Nickel (Ni)	Zinc (Zn)	Fer (Fe)
Fishes	0.5 mg/kg WW (a) 0.3 mg/kg WW (b) 0.12 (d) 0.3 mg/kg Wt (e) 0.3-6 mg/kg DW (f)	0.1 mg/kg ww (a) 0.05 mg/kg ww (b) 0.05 mg/kg ww (c) 0.181 mg/kg ww (d) 0.1 mg/kg dw (e) 0.15-3 ppm DW (h)	0.15 mg/kg ww (b, e)	0.5 mg/kg ww (a) 0.3 mg/kg bw (b)	30 mg/kg ww (a) 48.5 mg/kg DW (d) 5 mg/g Dw (g)	50 mg/kg ww (b)
Present study <i>M.merluccius</i>	0.6	0.203	0.62	0.282	17.18	22.16

Notes: (a) – OMS-IPCS (1998); (b) – JORADP No25 (2011); (c) – CEE (1982); (d) – IAEA (2004); (e) – FAO/WHO (2011); (f) – GIPPM (1973); (g) – NHMRC (1992); (h) – IOPR (1996); DW – dry weight; WW – wet weight.

Belhaouari et al., 2011; Onivogui et al., 2013; Abi-Ayad et al., 2018).

Heavy metals are considered as serious pollutants of the aquatic environment because of their persistence, remanance and biomagnification in the food chain (Yigit, Altindag, 2006; Guardiola et al., 2016), thus the aquatic animals may be exposed to high concentrations by these micropollutants (Kalay, Canli, 2000; Henry et al., 2004; Ribeiro et al., 2005). It must be pointed out, there are two categories of metals, those which are indispensable for life (Zn, Cu, Ni, ...) whose deficiency, as well as excess, can cause diseases, and those which are very toxic (Cd, Pb, Hg), they are not considered as essential metals (Altindag, Yigit, 2005). Their positive role in cells, to date, is not known but they are increasingly using in industrial sectors.

Unlike other contaminants, heavy metals are inorganic compounds, highly toxic minerals, even at low concentrations either for plants or animals. They are seen as major contaminants for both terrestrial and aquatic environments (Chen et al., 2000; Lafabrie et al., 2007). The regulation of these metals has been widely described in fish literature

Generally, Lead is present in small quantities. The industry discharges, leaching from gas stations and farmlands, road traffic, lixiviation phenomenon in dumps, and atmospheric deposition contribute to the high lead load (El Morhit et al., 2013).

According to Cossa et al. (1993), Lead levels in *Platichthys flesus* muscle tend to decrease with size in less contaminated areas, while (Benguedda et al., 2011; Pouil et al., 2017) showed that the accumulating power of various metallic elements is different not only from one species to another, but in food webs as well.

By comparing the levels recorded by category, we found that the purely toxic elements (lead and cadmium), accumulated in the muscle tissue of *Merluccius merluccius* exceed the tolerated thresholds (Table 1). The average levels present in our samples are 0.6 and 0.203 mg/kg DW respectively for lead and cadmium, far exceeding the recommended standards (CEE, 1982; IOPR, 1996; WHO-IPCS, 1998; FAO/WHO, 2001; IAEA, 2004; GIPPM, 1973; JORADP N°25, 2011). Cadmium and lead, which causes dysfunctions in the renal,

hepatic, gastrointestinal and reproductive systems of living beings (Amiard-Triquet et al., 1993; Mason et al., 2014). In WHO (1993) reported that cadmium, after mercury, is the most toxic of heavy metals for aquatic animals. Its presence is explained by the very frequent use of phosphate fertilization, the main source of this element, also by the littoral currents (very intense upwelling). Thus, Belhoucine et al. (2014) showed the behavior of cadmium which reveals significant variations during different periods of the year.

According to Amiard et al. (1987), lead can be biomagnified in biological systems, subsequently becoming a contaminating potential for different trophic links. The origin of the contamination by this element would be linked on one hand, to the fuel reserves stored around the Gulf of Béjaia, and on the other hand, to the important human activity (harbour, gear circulation...) that characterizes the peri-urban part of this Gulf, while Gagneux-Moreaux (2006) reported that the most important of lead flow to the ocean comes from the atmosphere.

Our results confirm those of Nsimanda et al., (2015), indicating that adult fishes accumulate more heavy metals compared to juveniles, showing higher levels of lead than cadmium in each part of analyzed fish. The presence of lead and cadmium in coastal ecosystems is largely a reflection of anthropogenic influence, and the bioaccumulation of these elements generally depends on the availability of these metals in the environment whose the contamination remains dependent on several surrounding factors (Nacer, 2013; Kayalto et al., 2014; Chouba et al., 2016).

Sex is one of the predominant biological factors. In 2011 Lacoue-Labarthe et al., showed that from a certain stage of embryonic development of individuals, sex plays an important role in the accumulation of different Metallic Trace Elements (Cd, Cu, Zn...), while Webb in 1997, announced that the sex undoubtedly, plays an important role in the distribution of MTEs.

Other authors attested that the loss of contaminants is caused by reproduction (Belhoucine et al., 2014). Spawning causes significant decontamination in females, since from their first reproduction, a clear decrease in metal pollutant concentrations is observed, While Mellon-Duval et al. (2010)

and El Habouz et al. (2011), reported that the accumulation of these elements is the cause of the difference in growth between males and females. Eventually, the downward trend of the identified MTEs corresponds to the breeding season of *Merluccius merluccius*, which spreads throughout the year (Alik et al., 2016). This decontamination by female population via the egg seems to be related at least, in part, to the mobilization of metals in liver for the gonads during maturation.

The high nickel content in hake from the Gulf of Bejaia suggests that potential sources of contamination by this element would be largely, either domestic discharges or surrounding and industrial agglomerations (discharges that Soummam Wadi carries to the Gulf, Cevitale unit, Eghzar Amokrane industrial area ...). However, the hypothesis that the hydrodynamism in this part of the Gulf favoring this distribution, cannot be ruled out.

The tendency for zinc accumulation is more difficult to explain because this metal is normally regulated by marine organisms since it enters in several biological processes. Following this study, we found that, the liver and muscle tissues accumulate both zinc, but the highest concentrations are found in the liver. However, we found significant variations between the two sexes and according to the considered size classes. It is to mention that many studies highlight the affinity of Zn, Cu and Cd in the liver (Yilmaz, 2009; Bashir et al., 2013...). This could be explained by the role of these elements in hepatic physiology as enzymatic cofactors, but also the fact that they are subject to rapid elimination by muscle (Marcovecchio, Moreno, 1993; De Conto Cinier et al., 1999).

Several studies have also shown that concentrations of these Metallic Trace Elements in marine species vary seasonally (Bei et al., 1998; Orban et al., 2002). Öztürk et al., (2009) argued that these variations codify the distribution of heavy metals playing an important role in bioaccumulation by hake. In this context, we believe that our data are comparable to those already observed, that changes in zinc seem to be closely related to seasonal phenomena. In addition, it is possible that high concentrations of zinc recorded during the summer, are attributed to the importance of water activity during this period.

The variation in the mean concentration of heavy metals appears to be related to the individual sizes; this is consistent with the results of (Coombs, 1980; Oualietal, 2018) which stated that the development (growth, size and sexual maturity) of marine organism influences the bioaccumulation of contaminants.

Metallic pollutant levels in liver of bioindicator organisms are higher than those seen in muscle. According to Ramade (1992), Metallic Trace Elements are particularly concentrated in the liver of teleosts also in the kidneys and to a lesser extent in the muscle. In the same context, Powell et al. (1981), already showed that heavy metals were concentrated in the organs of teleost fishes: Liver > Kidney > Muscle.

Pollutants can be immobilized in the sediment or transited to groundwater, surface water, plants, animals and humans (Rouane-Hacene et al., 2008; Ouali et al., 2018). These accumulation processes depend on assimilation rates, excretion and storage of each element.

According to our results, male individuals seem to be far from zones contaminated by these heavy metals. This can

be explained by the fact that males move far from the coast and therefore far from sources of pollution. In contrast, females accumulate these elements with age; also they frequent the shoreline of the coast especially during breeding season. Huang et al. (2007), denoted that these xenobiotics are problematic because of their persistent nature and toxicity. Indeed, the regulation imposes low thresholds.

Trace Elements namely Zinc and Iron show higher levels in comparison to toxic elements; nevertheless, the levels of these elements, being respectively of the order of 17.18 and 22.16 mg/kg DW, do not constitute a danger to consumer health, because they are below the maximum admissible dose (NHRMC, 1992; OMS-IPCS, 1998; IAEA, 2004; JORADP N°25, 2011).

From our results, it can be deduced that the average concentrations of heavy metals found in the hake from the Béjaia Gulf are alarming compared to those cited in literature as well as to allowable doses.

Reliable assessment of risks carried by these pollutants to human health and environment is a major issue (Alavanja et al., 2004). Mainly, the Metallic Trace Elements are transported in the ecosystems by atmospheric way and by means of streams, having various origins, substratum (lead ...), atmospheric pollution (lead, cadmium...), fertilizers (cadmium, lead ...), and urban sludge (mercury, lead, cadmium ...) (Chiffoleau et al., 2001; Ohta et al., 2002; Yi et al., 2011; Cassas, 2006; Borsali, 2015).

Thus, the Gulf of Bejaia is conceived as a final receptacle of many discharges (industrial and municipal), considered as the origin of contaminating metals detected in the *Merluccius merluccius* organs (muscle and liver). This pollution is due to the pollutant load, resulting from human and industrial activities without any treatment. The Gulf of Bejaia which is surrounded by a large hydrological network that drains heavy pollution along the wadis (Soummam and Agrioune), which flow directly to this Gulf causing ecological disasters, is responsible for repeated mortalities of different fish species almost every year.

Hazardous waste spills from industrial complexes in Sétif province (lead-based batteries), the Aïn-zada dam located in the Bordj-Bouaridj province, the Agro-food complex of Eghzar-Amokrane, Tala Hamza Zinc Project, the Cévital complex and the hydrocarbon terminal, represent serious threats to the marine environment.

The high levels are explained too, by the fact that Gulf of Bejaia is under a direct influence of the maritime traffic. However, the paints that cover hulls of boats, considered as antifouling, are based on copper compounds and / or synthetic chemicals that persist in the environment in the long term (Leroy, 2006; Augier, 2008).

It is necessary to take into account some factors such as; the area of Bejaia Gulf, the number of ships with their hulls, as well as their engine effects and gasoline vapor emissions that contribute to the pollution of the gulf. In addition other sources of pollution to be emphasized are; public dumps that pour directly into the gulf, seepage and, also water as vector, contribute to transport a various pollutants then, to integrate them into the food chain (phytoplankton, zooplankton, algae, fish ... etc.). Finally human being placed at the top of the food webs, will be exposed to all sorts of pollution. It should be

noted that climatic conditions also play a considerable role in the fluctuations observed in the environments to be studied.

Conclusion

The bioaccumulation of heavy metals, being studied and analyzed (Cd, Pb, Cu, Ni, Fe and Zn) takes place preferentially in the liver compared to the muscle of *Merluccius merluccius*. In addition, given that the Gulf of Bejaia is significantly polluted, the metal loads far exceed the recommended standards especially for inorganic elements.

These metal concentrations come probably from the industrial complexes installed along the drainage basin of Bejaia region. On the other hand, since hake is placed in higher trophic levels, the uncontrolled and anarchic consumption of seafood can carry a serious risk to population health of the region. To this end, we call on all partners in the professional, researchers and administration sectors to work together to make sure regulations are respected.

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