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Metallothionein as indicator of trace metals exposure in two fish species from the southern Moroccan Atlantic

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Abstract

Two fish species John Dory (Zeus faber) and Common Hake (Merluccius merluccius) of high commercial value were collected from the southern Moroccan Atlantic coast by bottom trawling at depths between 50 and 200m. A mesological analysis was carried out to determine the environmental conditions of these two species. These organisms were used as bioindicators to assess the level and effect of trace metals (Cr, Mn, Fe, Cu, Zn, Cd and Pb) contamination in the marine environment. To do it, two complementary analytical methods were used; the study of biological responses (metallothionein synthesis, biomarker of exposure to metals trace in the liver and muscle fish) combined with chemical analysis of the same matrices to determine the level of contamination. A significant difference (P <0.05) was observed between the species but also between the accumulation organs. The content of metallothioneins and trace metals is particularly important in the liver in comparison with the muscle for the two species sampled. The study showed that the levels of trace metals in the muscle of these fish do not exceed the maximum limits recommended by the European Community. It has also helped to provide information on the current state of accumulated trace metal levels and metallothionein levels in different accumulation organs, thus supporting the use of these proteins as a biomarker of metals exposure.

Keywords: Trace metal elements, Metallothioneins, Liver, muscle, Fish, South Atlantic coast, Morocco.

Introduction

The Moroccan Atlantic coast extends for about 3000 km. Known for its biological diversity and its considerable fisheries resources, it is subject to many forms of environmental pressures such as continuous urbanization, over-concentration of industrial networks and ecosystems pollution[1].

One way to assess the health of these marine ecosystems is to use biomarkers. In the sense of Lagadic, a biomarker represent an observable and/or measurable change at the molecular, biochemical, cellular, physiological or behavioral level, which reveals the present or past exposure of an individual to at least one polluting chemical substance[2]. The use of biomarkers to estimate the risk due to pollution has increased remarkably in recent years. The "biomarker" approach is currently used by international monitoring agencies as a tool to detect and estimate exposure and effects related to environmental contamination.

The main advantages justifying the wide use of biomarkers in many marine research and monitoring programs are: sensitivity, accuracy, ease of implementation and low cost[2,3]. The most promising biomarkers appear to be parameters for which there is good knowledge about their sensitivity, the effects of confounding factors and the significance of the response. Thus, biomarkers used for many years in ecotoxicology, such as 7-ethoxyresorufin-O-deethylase (EROD), metallothioneins (MT), acetylcholinesterase or even vitellogenin can be cited[3]. Vertebrates and invertebrates are used as sentinel species to assess the level of marine pollution[4]. Nevertheless, fish remain widely used in surveillance programs (BIOMAR, BEEP, FULLMONTI, etc.)[5]. These are sensitive indicators of metals and organic pollutants exposure of the marine environment[6]. In monitoring programs, the fish choice is based on their abundance but more particularly on the importance of their position in the food chain and their high commercial value. In addition, fish require essential metals (copper (Cu) and zinc (Zn)) as micronutrients for endogenous metabolism, which are taken by the surrounding environment (water and sediment) or by food[7]. Non-essential metals are also recovered and accumulated in fish tissues, including lead (Pb) and cadmium (Cd), which are considered the most dangerous metals in the aquatic environment[8, 9]. Among the biomarkers most used in the marine environments biomonitoring using fish are the MT. These exposure biomarkers are low molecular weight cytosolic proteins (about 6-7 kDa) able to bind essential metals as Fe, Mn and Zn, as well as non-essential metals as Cd, Cr and The Pb in ionic form.

The MT contain a large proportion of cysteine residues with free functional groups, thus allowing binding to some metals. They are normally expressed in animal tissues and are involved in the homeostasis of heavy metals. This biomarker is widely used in the international JAMP programs developed under the OSPAR, MEDPOL proposed under the United Nations Environment Program and the Swedish Environmental Protection Agency program NMMPS[3]. Although the development of chemical analyzes has made it possible to detect and quantify many chemical contaminants in the environment, the association of these pollutants can make interpretations of their potentially toxic effects on organisms difficult[10].

The use of these biomarkers in assessing the health of marine ecosystems integrates into the determination of the toxic effects of pollutants especially when they are present at low, sub-lethal concentrations. Indeed, chemical monitoring of organisms makes it possible to characterize the nature and concentration of pollutants; Biomonitoring methods assess not only the presence, but the response of organisms to these pollutants and thus their effects at different levels of organization.

This work falls within this framework. The objective of this study is: i. To determine the levels of trace metals in the liver and muscle (Cd, Cr, Cu, Fe, Mn, Pb and Zn) in two fish species from the coastal zone of the southern Moroccan Atlantic to assess trends the quality of fish habitat. Cd, Pb and Cr are chosen for their toxicity while Fe, Mn, Cu and Zn are selected as vital elements for fish growth. ii. To evaluate the biological responses of fish (via the metallothionein biomarker) to exposure to trace metal elements (TME).

Materials and methods

Study site: The species studied were sampled from 17 stations between Tantan (29°00'N 16°52'W) and Cape Blanc (22°45'N 11°20'W) (Figure-1) during an ecosystemic campaign on board of the R/V Dr Fridtj of Nansen. This study is particularly valuable because this area of the Canary Current ecosystem represents a considerable fishery potential[11]. Sampling was carried out between June and July 2012.

Sampling procedure: *Zeus faber* and *Merluccius merluccius* were collected by bottom trawl of 30 minutes at intervals depth from 50 to 200 m. The stations were set along transects drawn from the coast towards the open sea. After collection, the fish were weighed, length was measured (Table-1), samples (n = 3) were dissected to separate the liver from muscle pieces that were immediately frozen and stored until arrival to the laboratory. In addition, from each sampling station, the parameters of the water quality were determined.

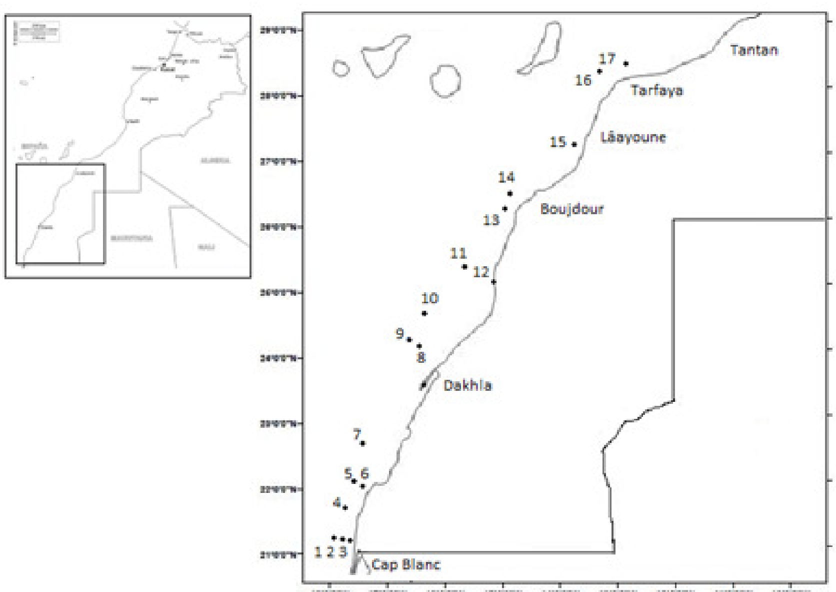

Figure-1: Location of sampling stations.

Table-1: Biometric characteristics of the fish species studied.

Fish species	n	Lenght (cm)		Weight (g)	
		Average ± SD	Min - Max	Average ± SD	Min - Max
Zeus faber	5	3.71 ± 6.55	24 - 55	889.53± 271.63	203 - 2880
Merluccius merluccius	5	31.21 ± 7.77	23 - 64	259.96 ± 182.52	101 - 1041

Measurement of physicochemical parameters: The temperature, dissolved oxygen, salinity and fluorescence parameters were determined onboard, using a CTD SBE 911plus / 917plus equipped with temperature, conductivity and pressure sensors, Chelsea Aqua3 fluorimeter and oximeter 43.

Determination of trace metal elements: The fish samples were first lyophilized in a Power Dry LL3000 lyophilizer Thermo, according to the method of Company et al.[12]. The wet / dry ratio is equal to 4. The freeze-dried liver and muscle samples were digested[13]. Approximately 0.25 g (± 0.01 g) of the test sample weighed accurately in the digestion containers to which was added 5 ml of concentrated pure HNO_3 and 0.5 ml concentrated H_2O_2. After one hour, the containers were closed and placed in the microwave oven (Multiwave Eco, Anton Paar) for 30 minutes. The bombs are allowed to cool, quantitatively transferred to 50 ml tubes, diluted to the mark with Milli-Q water and stored in a refrigerator until ICP-MS analysis. The heavy metal levels are expressed as weight of metal / g of wet weight.

To ensure quality control, calibration was performed using standards, reagent blanks and certified reference materials that were analyzed simultaneously[14]. The ICs (Cd, Cr, Zn, Pb, Cu, Fe, Mn) contained in liver and muscle fish were determined by ICP-MS (Element II, Thermo).

Biochemical determination of metallothioneins: The MT were measured according to the method of Viarengo et al. (1997)[4]. 1 ± 0.3 g of tissue from the frozen samples was homogenized in 3 volumes of 20 mM Tris-HCl pH 8.6 buffer containing 0.5 M sucrose, 0.001 mM PMSF 0.6 and 0.01% mercaptoethanol in an ice bath, using an ultra-Turrax (IKA T25). The homogenate obtained was centrifuged at 30000 g for 20 min. After, 1.05 ml of absolute ethanol and 80 µl of chloroform were added to 1 ml of the supernatant obtained. The resulting mixture was centrifuged at 6000 g for 10 min at 4°C. To the volume of pre-determined supernatant were added 3 volumes of absolute ethanol. After incubation for one hour at -20°C., the mixture is centrifuged at 6000 g for 10 min at 4°C. The pellet containing the MT is washed with 4 ml of ethanol, chloroform and homogenization buffer. After centrifugation at 6000 g for 10 min at 4°C., the residue obtained is dissolved in a solution composed of 150 µl of 0.25 M NaCl and 150 µl of HCl-EDTA. For each MT-enriched fraction, 4.2 ml of a 0.2M sodium phosphate solution, pH 8, containing 2M NaCl and 5,5'-dithiobis 2-nitrobenzoic acid, 0.43 mM. After centrifugation at 3000 g for 5 minutes, the absorbance was measured on a spectrophotometer at 412 nm. The content of the metallothioneins was estimated using L-Glutathione reduced> = 98% with the Ellman reagent. The results are expressed in µg / g of fresh tissue.

Statistical processing of data: For the mesological analysis, Principal Component Analysis (PCA), a descriptive statistical method, was used. The objective is to extract the most pronounced trends from the database (variables, stations) and thus to bring out a global perception of the facts[15,16].

The PCA was performed using the Statistica software (version 6). A raw data matrix with 8 variables was used. The variables selected for this statistical study are temperature (T), salinity (Sa), dissolved oxygen (O_2), chlorophyll a (F), nitrogen compounds (NO^{3-}, NO_2 and NH^{4+}), orthophosphates (PO_4^{3-}) and silica (SiO^{2-}).

To compare the contamination and its effects in the two fish species studied, a variance analysis followed by a Student's T-test was applied to the data to determine whether there was a significant difference in the accumulation of TME in the organs (on the one hand) and the response by metallothioneins in both species (on the other hand).

Results and discussion

Mesological analysis: Structure of variables: Examination of the numerical results of this PCA shows that the 8 values indicate that the F1 axis explains more than half (53.79%) of the total variance of the data compared with 16.92% for the F2 axis. Thus, 70.71% of the information in the data table is extracted by the factor plane F1xF2.

As a result, the analysis of the results of the PCA will be done by limiting itself to these first two axes. The correlation circle (Figure-2) shows that 7 of 8 variables taken into account in the ACP contribute to the definition of the factor plane F1 x F2. The temperature (T) contributes positively to 15.18% to the formation of the F1 axis while the nitrates (NO^{3-}), orthophosphates (PO_4^{3-}) and silica (SiO^{2-}) which describe the trophic conditions (dissolved nutrients) of the study medium contributed negatively to the formation of this axis, respectively, at 17.49%, 22.01% and 19.87% (59.36% of the total variance for these last three parameters). For the F2 axis, the structuring variables are salinity, dissolved oxygen, chlorophyll a (F), and to a lesser degree temperature.

Their relative contributions are respectively 33.07%, 26.55%, 24.48 and 11.31%; Representing a total contribution of 95.41%. The structure of these variables around the F2 axis can be explained by the fact that algal development is dependent on environmental factors such as temperature, salinity etc.

Structure of Individuals: Based on the factorial map F1 x F2 (Figure-3), it can be seen that the stations are positioned according to the variables that characterize them.

Thus, stations located at Dakhla Bay (coded 40, 41), the coast stations located to the north and south of Cap Boujdor (coded 46, 47), would be marked by the thermal factor, the Tantan station 78) would be a well-oxygenated station, the coastal stations at Tarfaya (coded 67, 76) would be marked by a richness of nutrients and an abundance of phytoplankton.

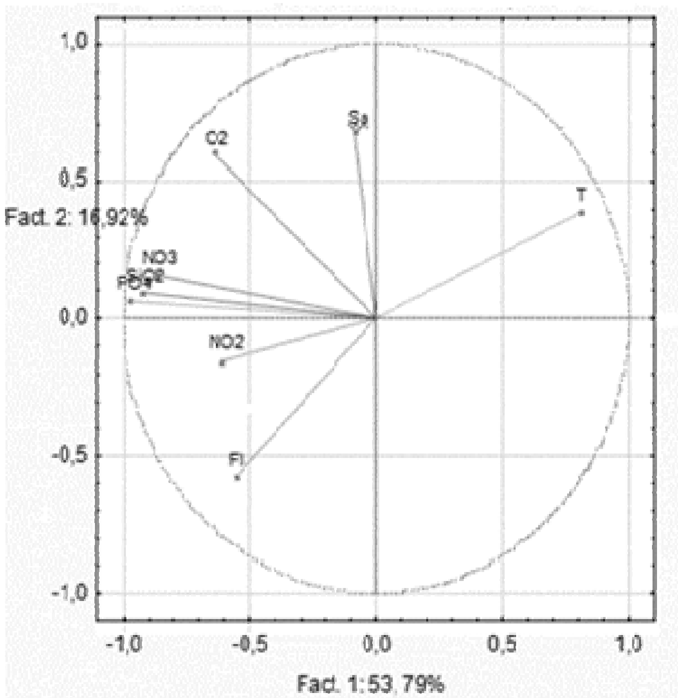

Figure-2: Projection of the variables on the factorial plane F1 and F2.

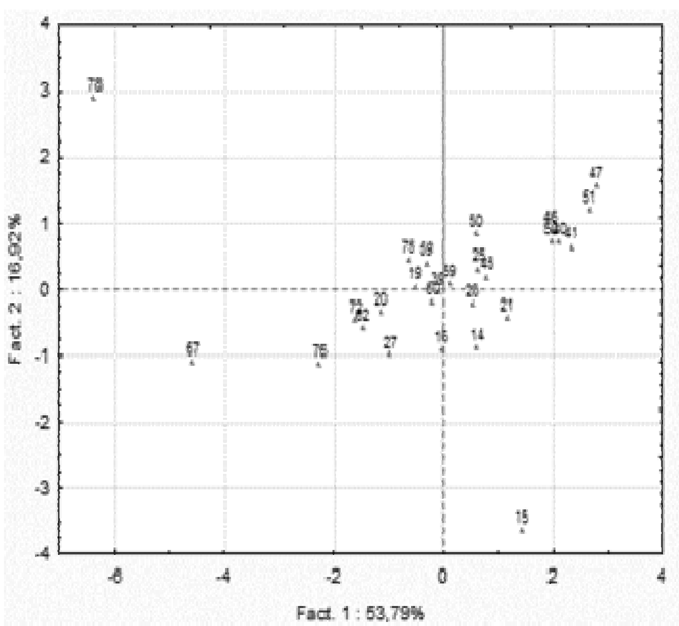

Figure-3: Stations projection on the factorial plane F1 and F2 observations with the sum of the square cosines>=0.00.

Trace metals in fish: The mean values of the TME concentrations in the organs of *Z.faber* and *M.merluccius* are shown in Table-2 and Figures-4, 5, 6 and 7.

Iron: For *Z. faber*, the mean concentration in the liver is 18.59 ppm fresh weight. The maximum level is 55.19 ppm and is recorded north of Cape Boujdor and the minimum value (0.01) is recorded south of Tantan. For muscle, the average grade is 1.82 ppm. The maximum value is observed south of Tantan while the minimum value is observed south of Cape Boujdor. In *M.merluccius*, the mean concentration in the liver is 34.16 ppm. The maximum value (106.75 ppm) is observed at Cape Blanc and the minimum value (0.02 ppm) noted south of Tantan. For muscle, the average is 2.73 ppm. The maximum value (6.28 ppm) is observed at Laayoune level and the minimum value (1.03 ppm) is observed at Boujdor level.

Zinc: For *Z.faber*, the mean content in the liver is 9.63 ppm. It is at Cape Barbas that the maximum (22.15 ppm) is recorded. The minimum (0.01ppm) is observed south of Tantan. In the muscle of this species, the average rate is 2.31 ppm, with a maximum recorded at Cape Barbas and a minimum of 0 ppm north of Dakhla.

The mean concentration in the liver of *M.merluccius* is 24.19 ppm. The maximum (121.99 ppm) is found at Cape Boujdor and a minimum (0.02 ppm) south of Tantan. For muscle, the mean rate is 4.45 ppm with a maximum (10.63 ppm) at Laayoune level and a minimum (2.90 ppm) north of Cape Boujdor.

Copper: For *Z.faber*, the mean value in the liver is 1.28 ppm. In Cape Boujdor, this value is maximum (3.82 ppm) and is close to 0 south of Tantan. For the muscle, the average is 0.10 ppm; The maximum (0.26 ppm) is noted north of Boujdor and the minimum of 0 ppm at Cintra bay (between Dakhla and Boujdor).

For *M.merluccius*, the average in the liver is 3.27 ppm. The maximum (9.47 ppm) is recorded at Cape Barbas and the lowest (near 0 ppm) south of Tantan. In the muscle, the average value is 0.22 ppm. The maximum recorded is 0.33 ppm at Tarfaya level and the minimum is 0.13 ppm south of Tantan.

Manganese: For *Z.faber*, the mean value in the liver is 0.48 ppm. Cape Boujdor has the highest value (1.56 ppm), while the station south of Tantan has the lowest value (0 ppm). For muscle, the average is 0.09 ppm. The maximum value (0.34 ppm) is recorded at Laâyoune and the south of Cape Boujdor and the minimum value (0 ppm) in Cintra Bay.

For *M.mercluccius*, the mean content in the liver is 0.69 ppm. 2.07 ppm was recorded as the maximum value at Cape Barbas level while the minimum value (close to 0 ppm) was noted south of Tantan. For muscle, the average is 0.15 ppm. The maximum (0.42 ppm) is noted at Cape Barbas and the lowest (0.06 ppm) south of Tantan.

Chrome: For *Z.faber*, the mean value in the liver is in the order of 0.01 ppm. Cape Barbas records the highest value (0.03 ppm) and the station south of Tantan the lowest value (0 ppm). For muscle, the mean rate is 0.01 ppm, the maximum concentration (0.05 ppm) is recorded south of Tantan and the minimum (0 ppm) at Cape Barbas, off Cintra Bay and at Tarfaya Town.

In the liver of *M. merluccius*, the mean value in the liver is of the order of 0.02 ppm. Cape Boujdor recorded the highest grade (0.09 ppm) and south of Tantan was the lowest (0 ppm). In the muscle, the average content is 0.02 ppm. 0.04 ppm is a maximum value recorded at Cape Barbas and 0.01 ppm and a minimum value recorded at Cape Boujdor.

Cadmium: For *Z.faber*, the mean value in the liver is about 0.06 ppm. The highest grade (0.19 ppm) is recorded north of Cape Blanc and the lowest (near 0 ppm) south of Tantan. For muscle, the mean is close to 0 ppm. The maximum value is of the order of 0.01 ppm and is observed in Laâyoune, while the minimum (0 ppm) is observed at Cintra and Tarfaya bay.

For *M.merluccius*, the mean in the liver is 0.73 ppm. 4.34 ppm is a maximum value and is found at Cape Blanc and Cape Barbas, while the lowest value is south of Tantan (0 ppm). For the muscle the average is close to 0 ppm. The maximum value is nearly 0.01 ppm and is recorded at Cape Blanc, while the minimum is 0 ppm and is recorded at Cintra Bay.

Lead: For *Z.faber*, the mean value in the liver is close to 0.01 ppm. The station north of Cape Blanc is the maximum (0.03 ppm) and that south of Tantan is the minimum (0 ppm). For muscle, the average content is close to 0 ppm, with a maximum (near 0.03 ppm) South of Cape Boujdor and Laâyoune, and a minimum (close to 0 ppm) for most of the stations surveyed.

For *M.merluccius*, the average grade is 0.03 ppm, 0.23 ppm is a maximum value observed at Cape Blanc and Cape Barbas, while 0 ppm has been reported as the minimum value for most stations. In muscle, the mean is close to 0 ppm, the maximum (0.03 ppm) is recorded at Cintra Bay. With the exception of this station, as well as that at Tarfaya, most stations record a minimum of 0 ppm.

The concentrations of TME in the liver and muscle (Figure−4, for *Z.faber* and 6, 7 for *M.merluccius*) are plotted.

Table-2: Descriptive statistics of ETM in liver and muscle fishes.

Metal			Cr	Mn	Fe	Cu	Zn	Cd	Pb
Z.faber	Liver	Average (ppm/ww)	0.007	0.479	18.594	1.278	9.626	0.057	0.005
		Min (ppm/ww)	0.000	0.000	0.009	0.001	0.009	0.001	0.000
		Max (ppm/ww)	0.032	1.555	55.198	3.825	22.150	0.187	0.029
		SD (ppm/ww)	0.008	0.445	15.716	1.044	6.032	0.045	0.008
	Muscle	Average (ppm/ww)	0.013	0.089	1.815	0.101	2.307	0.002	0.003
		Min (ppm/ww)	0.000	0.000	0.002	0.000	0.000	0.000	0.000
		Max (ppm/ww)	0.050	0.338	7.084	0.262	5.318	0.006	0.029
		SD (ppm/ww)	0.015	0.107	2.138	0.088	1.838	0.002	0.010
M.merluccius	Liver	Average (ppm/ww)	0.018	0.690	34.158	3.265	24.194	0.728	0.031
		Min (ppm/ww)	0.000	0.001	0.019	0.002	0.016	0.000	0.000
		Max (ppm/ww)	0.095	2.066	106.750	9.474	121.986	4.343	0.230
		SD (ppm/ww)	0.025	0.497	34.799	3.216	28.436	1.484	0.075
	Muscle	Average (ppm/ww)	0.019	0.152	2.733	0.224	4.449	0.004	0.003
		Min (ppm/ww)	0.004	0.056	1.033	0.126	2.895	0.000	0.000
		Max (ppm/ww)	0.038	0.416	6.276	0.326	10.632	0.008	0.029
		SD (ppm/ww)	0.009	0.097	1.369	0.057	1.775	0.003	0.008

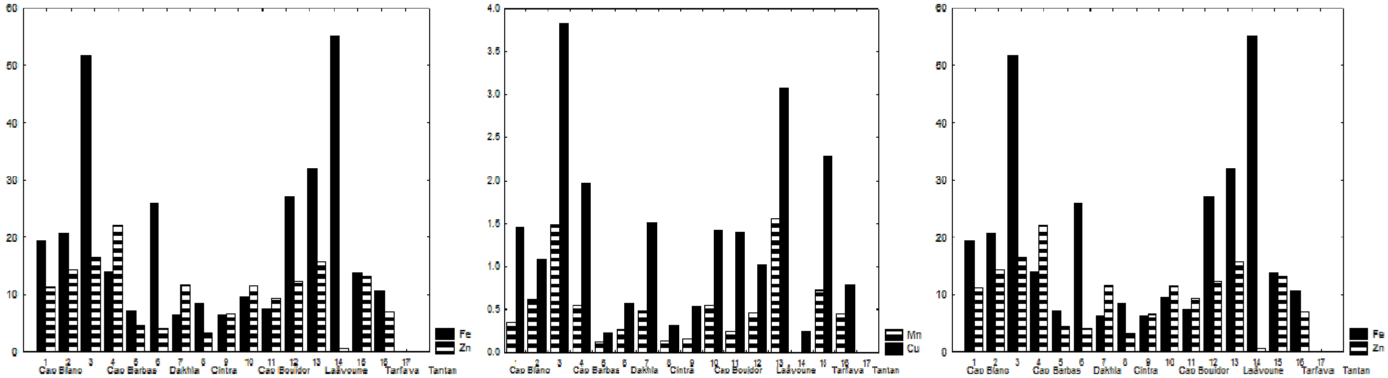

Figure-4: Levels of ETM in liver of *Zeus faber* (ppm/ww).

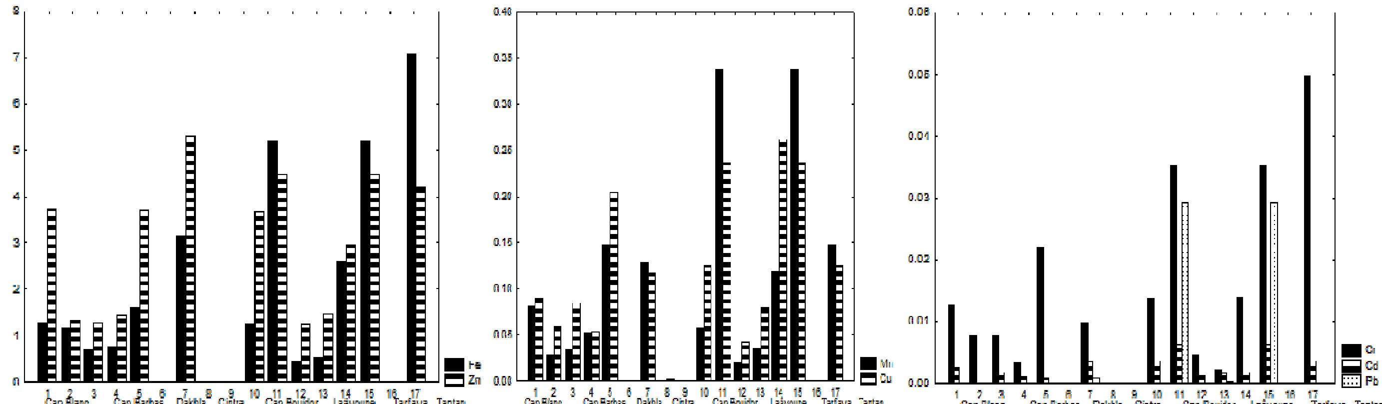

Figure-5: levels of ETM in muscle of *Zeus faber* (ppm/ww) (: Maximum Cd level tolerable[17]).

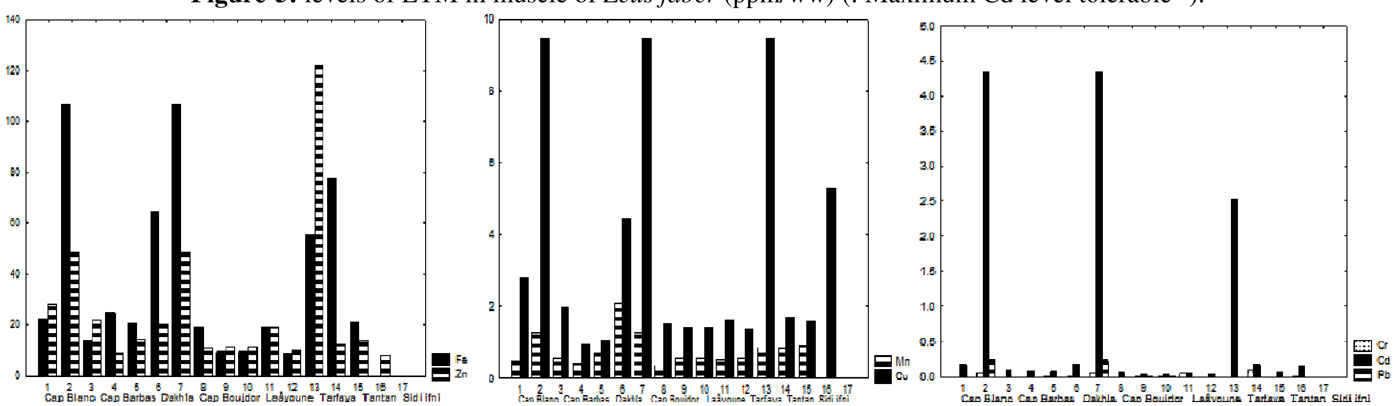

Figure-6: Levels of ETM in *Merluccius Merluccius* liver (ppm/ww).

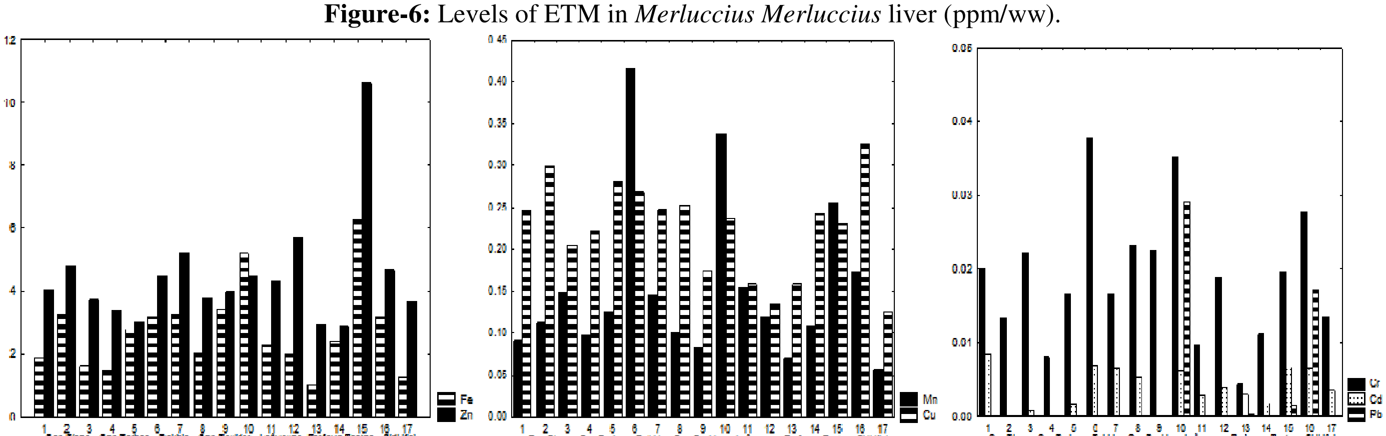

Figure-7: Levels of essential metals in *Merluccius Merluccius* muscle (ppm/ww) (: Maximum Cd level[17]).

Of all the stations surveyed, it is that of southern Tantan which reveals the lowest levels for all TME in the *Z.faber* and *M.merluccius* liver, but the highest values for Fe and Cr in the muscle of *Z.faber*.

At Cape Barbas, the maximum levels of Zn and Cr are observed in the liver of *Z.faber*, while for *M.merluccius* it is Cu, Mn, Cd and Pb which show a high concentration in the liver.

In Cape Boujdor, the values of Fe, Cu and Mn are maximal in the liver of *Z.faber*. The same is true for Zn and Cr in *M. mercluccius*. At Cape Blanc, maximum levels of Cd and Pb are noted in the liver of *Z.faber* and *M.merluccius*. In Laayoune, the Mn and Cd contents are maximal in the muscle of *Z.faber* and those of Fe and Zn in the muscle of *M.merluccius*.

Comparison of TME levels in liver and muscle: The comparison of the variances by the Pearson T test revealed significant differences ($p<0.05$) for Mn, Fe, Cu, Zn, and Cd levels between the liver and muscle (Tables-4, 5). A TME enrichment factor was calculated for each metal in the organs of the two species studied; this corresponds to the ratio of the mean metal concentration in the liver to that in the muscle (Table-6). The highest enrichment factor was obtained for Cd; in *Z.faber*, the liver can concentrate up to 29 times this metal respect to the muscle and up to 193 times in *M.merluccius*. These results corroborate those obtained by Diop et al. concerning *Solea senegalensis* and *Sardinella aurita*[18], Abeshi et al. in *Merluccius merluccius* and *Mulus barbatus*[19], Bat et al. concerning *Psetta maxima*[20], Henry et al. in *Limanda limanda* and *Pleuronectes Platessa*[21], Kljakovic Gaspic et al. for *Merluccius merluccius* and *Mulus barbatus*[22] (Table-3) and Morhit in *Barbus callensis* and *Pagellus acarne*[23].

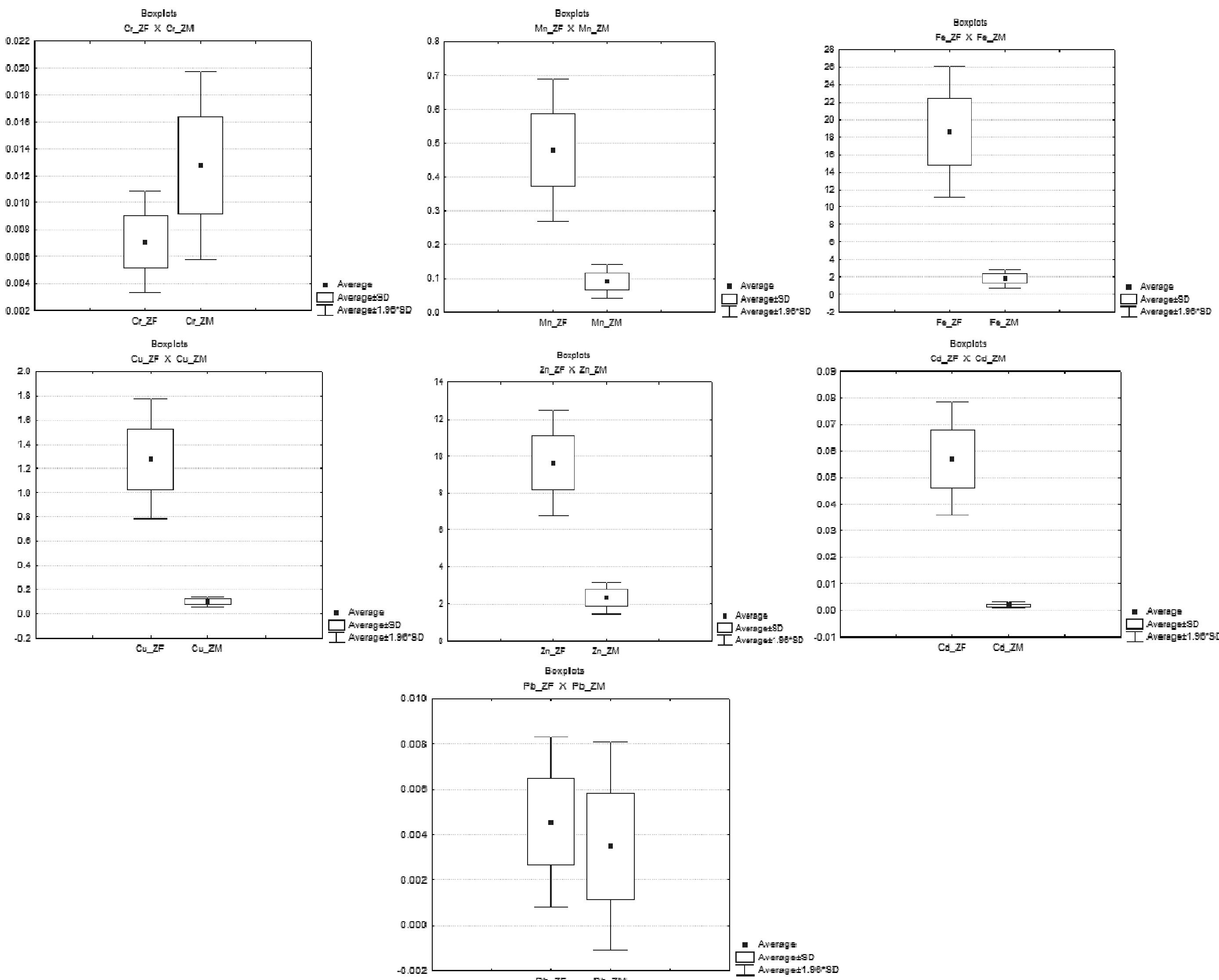

Figure-8: Typical averages and standard deviations of MES concentrations in liver and muscle in *Zeus faber* (ppm/ww).

Table-3: Hepatic metal concentrations in fish species from different coastal areas.

Area	Specie	Tissue	Cr	Mn	Fe	Cu	Zn	Cd	Pb	Ref.
Moroccan atlantic coast	Z. faber	Liver (mg/kg)	0.007±0.008	0.479±0.445	18.594±15.716	1.278±1.044	9.626±6.032	0.057±0.045	0.005	Present study
		Muscle (mg/kg)	0.01±0.01	0.45–0.48	18.5–15.72	1.04–1.28	6.03–9.63	0.04–0.06	0.01±0.01	
	M. merluccius	Liver (mg/kg)	0.018±0.025	0.69±0.497	34.158±34.79	3.265±3.216	24.194±28.436	0.728±1.484	0.031	
		Muscle (mg/kg)	0.02±0.02	0.50–0.69	34.16±34.80	3.22–3.27	24.2±28.44	1.48–1.73	0.03±0.08	
Senegalese coast	S. senegalensis	Liver (mg/kg)	2.76±0.64	2.75±0.75	744±222	409±111	52.5±13.4d	1.03±0.53	0.072±0.04	18
		Muscle (mg/kg)	0.39±0.04	0.45±0.04e	5.58±0.84	0.71±0.06	20.9±1.2	0.03±0.01	0.02±0.00	
Oran bay	M. surmuletus	Liver (mg/kg)				1.51	17.56	0.03	0.19	24
		Muscle (mg/kg)				0.21	11.78	0.03	0.04	
Kuwaits marine area	A. latus	Liver (µg/g)	3.52±0.64			112.2±13.88	148.3±20.16			14
	C. arel	Liver (µg/g)	0.84±0.23		46.64±0.02	8.17±0.03	16.78±0.13			
Oran bay	M. merluccius	Muscle (mg/kg)					7,89±0,47	0,24±0,11	0,27±0,16	25
Tirana, Albania	M. merluccius	Muscle (mg/kg)	0.125±0.040					0.35±0.089	0.064±0.098	26
Marmara and black sea	M. merlangus	Liver (mg/kg)	0.15±0.06		38±0.5	10±0.52	30.2±0.3	0.15±0.02	0.03±0.01	27
		Muscle (mg/kg)	1.5±1.07		84.2±12	2.5±0.06	18±1.4	0.03±0.01	0.05±0.01	
	M. barbatus	Liver (mg/kg)	0.18±0.01		3.7±29	15±0.98	109±19	0.7±0.08	0.07±0.02	
		Muscle (mg/kg)	0.33±0.04		20.2±0.7	1±0.18	14.6±1.3	0.05±0.01	0.02±0.01	
Adriatic sea	M. merluccius	Liver (mg/kg)							0.176±0.1	19
		Muscle (mg/kg)							0.10±0.12	
	M. barbatua	Liver (mg/kg)							0.236±0.2	
		Muscle (mg/kg)							0.106±0.1	
Moroccan atlantic coast	P. acarne	Liver (mg/kg)	0.18±0.09	1.07±0.06	74.05±3.45	2.86±1.21	52±1	0.3±0.05	0.12±0.03	28
		Muscle (mg/kg)	0,01±0.003	0,13±0.08	6,29±0.59	0,55±0.63	18,33±3.78	0,11±0.01	0,004±0.0005	
	D. vulgaris	Liver (mg/kg)	0.12±0.05	0.24±0.11	87.61±3.50	3.47±1.81	54.66±2.51	0.22±0.02	0.21±0.05	
		Muscle (mg/kg)	0,016±0.002	0,42±0.54	2,92±0.95	0,42±0.38	105±5	0,06±0.07	0,038±0.037	
Black sea	P. maxima	Liver (mg/kg)				6.66-11.32	37.81-44.11	0.14-0.27	0.21-0.34	20
		Muscle (mg/kg)				1.81-6.11	18.56-35.33	0.016-0.046	0.07-0.21	
Mauritanian atlantic coast	S. aurita	Muscle (µg/g)						600.00	0.34	29

Area	Specie	Tissue	Cr	Mn	Fe	Cu	Zn	Cd	Pb	Ref.
Mid-atlantic ridge	M. mora	Liver (µg/g)	3.35–10.01	0.771–1.171	112.76–399.26	2.368–5.730		1.478–4.578		12
		Muscle (µg/g)	2.87–11.35	0.175–0.620	4.22–41.60	0.156–0.300		0.005–0.006		
Northern iberian shelf	L. boscii	Liver (µg/g)	1.00–2.89		101–222	9.8–27.7	78–163	0.10–1.37	0.002–0.011	7
	T. luscus	Liver (µg/g)	0.43–3.59		68–118	2.3–8.0	8–25	0.02–0.34	0.002–0.05	
Eastern Aegean Sea	P. erythrinus	Liver (µg/g)				nd-21986	2710-78705	1.4-2245	112-8311	30
		Muscle (µg/kg)				nd-383	1352-6693	Nd -9.6	nd-1397	
French coast	L. limanda	Liver (µg/g)		2.9± 1.0		16.8± 8.3		0.13± 0.09	0.04± 0.01	21
		Muscle (µg/kg)		0.87± 0.54		0.94± 0.24		0.003± 0.003	0.07± 0.05	
	P. platessa	Liver (µg/g)		11.0± 10.5		11.1± 10.8		0.12± 0.09	0.09± 0.07	
		Muscle (µg/kg)		0.54± 0.24		1.2± 1.0		0.007± 0.020	0.07± 0.07	
Spanish southern atlantic coast	L. aurata	Liver (mg/kg)	0.01	4.61	337.00	88.90	49.60	0.51	0.32	31
		Muscle (mg/kg)	0.04	2.33	5.40	0.50	6.07	0.03	0.03	
	S. vulgaris	Liver (mg/kg)	0.02	4.42	311.00	68.60	21.30	0.22	0.40	
		Muscle (mg/kg)	0.03	7.06	5.01	0.40	7.81	0.03	0.05	
Adriatic sea	M. merluccius	Liver (µg/kg)						6.5-153	39-298	22
		Muscle (µg/kg)						4.1-14.3	49-141	
	M. barbatus	Liver (µg/kg)						11.2-183	99-970	
		Muscle (µg/kg)						7.6-28.9	57-158	
Mauritanian coast	S. scriba	Liver (µg/g)				12.8± 1.8	166± 48	51.0± 17.0		32
		Muscle (µg/g)				2.03± 0.28	29.25± 7	5.08± 1.6		
	E. costae	Liver (µg/g)				49.1± 14.7	507± 88	4.67± 1.21		
		Muscle (µg/g)				12.28± 3.68	126.75± 22	1.17± 0.3		
Mediteranea n moroccan coast	M. merluccius	Muscle (mg/kg)	0.042-0.320					0.032-0.050	0.022-0.586	33
	Z. faber	Muscle (mg/kg)	0.025-0.075					0.014-0.056	0.033-0.040	
Mediteranea n atlantic coast	M. merluccius	Muscle (mg/kg)						0.02-0.06		UNEP/FAO, 1989 in El Hraiki, 1993
Mediterranea n sea	M. barbatus	Muscle (mg/kg)						0.017-0.05		UNEP/FAO, 1986 in El Hraiki, 1993
Cerified value		Muscle (mg/kg)						0.05	0.30	17
		Muscle (mg/kg)				30.00	30.00			34

Table-4: Test T for trace metals in liver and muscle of *Zeus faber*.

Test t for Independent Samples (ETM_Ave_140417_Nansen12).										
	Average	Average	Value t	dl	p	N Active	N Active	SD	SD	Ratio F	p
	Group 1	Group 2				Group 1	Group 2	Group 1	Group 2	Variances	Variances
Cr_ZF vs. Cr_ZM	0.007	0.013	-1.405	32.000	0.170	17.000	17.000	0.008	0.015	3.427	0.018
Mn_ZF vs. Mn_ZM	0.479	0.089	3.511	32.000	**0.001**	17.000	17.000	0.445	0.107	17.405	0.000
Fe_ZF vs. Fe_ZM	18.594	1.815	4.362	32.000	**0.000**	17.000	17.000	15.716	2.138	54.040	0.000
Cu_ZF vs. Cu_ZM	1.278	0.101	4.633	32.000	**0.000**	17.000	17.000	1.044	0.088	140.383	0.000
Zn_ZF vs. Zn_ZM	9.626	2.307	4.785	32.000	**0.000**	17.000	17.000	6.032	1.838	10.769	0.000
Cd_ZF vs. Cd_ZM	0.057	0.002	5.058	32.000	**0.000**	17.000	17.000	0.045	0.002	502.081	0.000
Pb_ZF vs. Pb_ZM	0.005	0.003	0.353	32.000	0.726	17.000	17.000	0.008	0.010	1.494	0.431

Note: Variables treated as independent samples.

Table-5: Test T for trace metals in liver and muscle of *Merluccius merluccius*.

Test t for Independent Samples (ETM_Ave_140417_Nansen12).											
	Average	Average	value t	dl	p	N Active	N Active	SD	SD	Ratio F	p
	Group 1	Group 2				Group 1	Group 2	Group 1	Group 2	Variances	Varian ces
Cr_MF vs. Cr_MM	0.018	0.019	- 0.159	32.000	0.875	17.000	17.000	0.025	0.009	7.450	0.000
Mn_MF vs. Mn_MM	0.690	0.152	4.375	32.000	**0.000**	17.000	17.000	0.497	0.097	26.204	0.000
Fe_MF vs. Fe_MM	34.158	2.733	3.720	32.000	**0.001**	17.000	17.000	34.799	1.369	646.384	0.000
Cu_MF vs. Cu_MM	3.265	0.224	3.899	32.000	**0.000**	17.000	17.000	3.216	0.057	3128.857	0.000
Zn_MF vs. Zn_MM	24.194	4.449	2.858	32.000	**0.007**	17.000	17.000	28.436	1.775	256.702	0.000
Cd_MF vs. Cd_MM	0.748	0.004	2.068	32.000	**0.047**	17.000	17.000	1.484	0.003	286023.804	0.000
Pb_MF vs. Pb_MM	0.031	0.003	1.516	32.000	0.139	17.000	17.000	0.075	0.008	89.483	0.000

Note: Variables treated as independent samples.

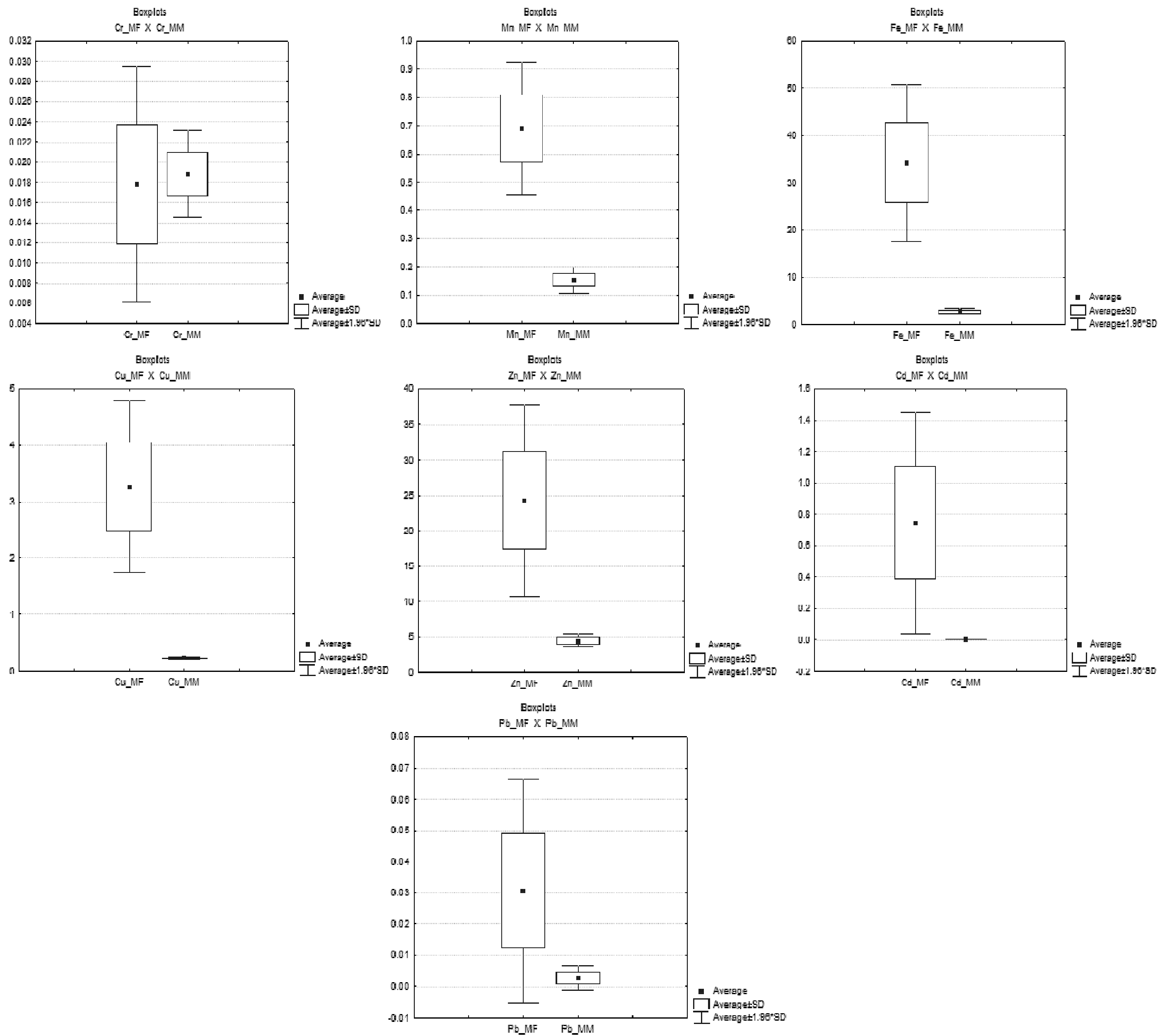

Figure-9: Typical mean and standard deviation of TME concentrations in liver and muscle in *M.merluccius* in (ppm/ww).

Table-6: TME enrichment factor (ratio of liver concentrations / muscle concentrations) of fish in the southern Moroccan Atlantic.

Specie / Metal	Cr	Mn	Fe	Cu	Zn	Cd	Pb
Z.faber	0.555	5.360	10.245	12.653	4.172	29.362	1.308
M.merluccius	0.946	4.525	12.497	14.557	5.438	193.675	10.843

Comparison between levels of contamination in species and organs: The comparison of Pearson T-test variances for the same organ between the two species (Table-7) revealed significant differences (p<0.05) of Cu, Zn and Cd levels between the liver and the muscle. In addition, Cu Zn and Cd, Hake concentrate more than John Dory both in the liver and in the muscle.

Table-7: Comparison of TME levels in the liver and muscle of the two species (Zeus faber vs Merluccius merluccius) combined.

Test t for Independent Samples (ETM_Ave_140417_Nansen12)											
	Average	Average	Value t	dl	p	N Active	N Active	SD	SD	Ratio F	p
	Group 1	Group 2				Group 1	Group 2	Group 1	Group 2	variances	variances
Cr_ZF vs. Cr_MF	0.007	0.018	-1.716	32.000	0.096	17.000	17.000	0.008	0.025	9.556	0.000
Mn_ZF vs. Mn_MF	0.479	0.690	-1.301	32.000	0.203	17.000	17.000	0.445	0.497	1.246	0.665
Fe_ZF vs. Fe_MF	18.594	34.158	-1.681	32.000	0.103	17.000	17.000	15.716	34.799	4.903	0.003
Cu_ZF vs. Cu_MF	1.278	3.265	-2.424	32.000	**0.021**	17.000	17.000	1.044	3.216	9.494	0.000
Zn_ZF vs. Zn_MF	9.626	24.194	-2.066	32.000	**0.047**	17.000	17.000	6.032	28.436	22.225	0.000
Cd_ZF vs. Cd_MF	0.057	0.748	-1.919	32.000	0.064	17.000	17.000	0.045	1.484	1093.0 50	0.000
Pb_ZF vs. Pb_MF	0.005	0.031	-1.422	32.000	0.165	17.000	17.000	0.008	0.075	90.191	0.000
Cr_ZM vs. Cr_MM	0.013	0.019	-1.449	32.000	0.157	17.000	17.000	0.015	0.009	2.672	0.058
Mn_ZM vs. Mn_MM	0.089	0.152	-1.801	32.000	0.081	17.000	17.000	0.107	0.097	1.208	0.710
Fe_ZM vs. Fe_MM	1.815	2.733	-1.491	32.000	0.146	17.000	17.000	2.138	1.369	2.440	0.084
Cu_ZM vs. Cu_MM	0.101	0.224	-4.834	32.000	**0.000**	17.000	17.000	0.088	0.057	2.348	0.098
Zn_ZM vs. Zn_MM	2.307	4.449	-3.456	32.000	**0.002**	17.000	17.000	1.838	1.775	1.073	0.890
Cd_ZM vs. Cd_MM	0.002	0.004	-2.187	32.000	**0.036**	17.000	17.000	0.002	0.003	1.919	0.203
Pb_ZM vs. Pb_MM	0.003	0.003	0.218	32.000	0.829	17.000	17.000	0.010	0.008	1.482	0.440

Note: Variables treated as independent samples.

From the ecotoxicological point of view, the processes of bioaccumulation of metals in organisms and organs are influenced directly and indirectly by many interactive factors: First, the conditions of fish exposure to direct and trophic pathways (metal concentrations in water); transport by the circulatory system and distribution to the various tissue compartments and abiotic factors of aquatic biotopes and their natural or anthropogenic variations. The latter act simultaneously on the bioavailability of metals, on their transfer and bioaccumulation capacities, in relation to adaptive responses to the main physiological functions[35,36].

Cd, Pb, Cr, Cu, Mn, Fe and Zn are metals in the liver and the muscle of *Z.faber* (Figure-4, 5, 6 and 7) and *M.merluccius* (Figure-8, 9, 10 and 11). Mean concentrations are expressed in ppm of wet weight. The comparison of TME between stations reveals significant variations. For the two species studied, the level of toxic metals (Cd and Pb) in the tissues is relatively low. These fish samples were taken from the southern Atlantic coast of Morocco, which is mainly influenced by cold surface waters that are nutrient-rich sedimentary particles due to the circulation of water.

The level of these trace metals in the tissues of fish can have several sources (water, sediment, food ...). On the western Atlantic coast, Bruland and Franks (1983)[37] showed that the origin of cadmium was the upwelling surface. Dissolved cadmium is also correlated with phosphate concentrations in seawater. Thus, the increase in phosphate content in seawater is caused by rock leaching and fertilizer Substrates phosphate bases.

Another study, carried out by Romeo et al. (1999)[32] on the western Atlantic coast, showed that high concentrations of Cd are attributed to natural origins. However, more can be learned about anthropogenic activity in this region or the presence of source of chemical contamination and in particular metallic contamination. Morocco is the second largest producer of phosphate in the world. During the production of phosphoric acid, particles containing a significant abundance of croutons as heavy metals are produced and released in the Atlantic Ocean[38].

The demersal species *M.merluccius* reacts differently to microcontaminants. Indeed, and whatever the organ of accumulation, the Hake accumulates more TME in comparison with *Z.faber*. Similarly, a study of three fish species from the southern Atlantic coast of Morocco, *D.vulgaris*, *S.pilchardus* and *P.acarne* showed that differences in metallic levels between species may be related to habitat, Or the mobility of fish[28]. Moreover, the difference in the concentrations recorded does not depend on the size or weight of the individuals analyzed. Indeed, these observations are similar to those of Henry et al. (2004) at the level of the French coast, which showed any species studied, there is no correlation between fish size and weight and metal content[21].

The highest levels of TME (both essential and non-essential) are recorded particularly at the Cape (particularly Cape Barbas, Laayoune and Tarfaya) for John Dory and the choice of these organs is explained by the fact that the liver is an organ that has a main role of detoxification and the muscle is usually analyzed because it is the part consumed by men[39]. These results are in agreement with those of Usero et al. (2004), who is reported value for *Liza aurata* is 6.07 mg/kg fresh weight compared to 7.81 for *Solea vulgaris*[31]. A high level of Zn compared with other elements is typical of fish muscle[40].

Other studies carried out on the same hake species showed higher levels of Cr, Cd and Pb (0.125 mg / kg, 0.35 mg / kg, 0.064 mg / kg respectively)[19,26]. As in the case of Fernandes et al. (2008)[7], the metal concentrations observed in the muscle culture of another species (sea bass) was below the FAO maximum recommended limits for fish species Commercial (Cu: 30 mg / kg and Zn: 30 mg / kg)[34]. The results of this work are in line with those of a study carried out on the same species in the Mediterranean Sea, where the levels of Cd and Pb are respectively 0.03 mg / kg; 0.02 mg / kg for *M.merluccius* and 0.01 mg / kg; 0.03 for *Z.faber*[33].The European Community has proposed limit values for the maximum tolerated level for concentrations of metals in fish flesh intended for human consumption. For non-essential metals, the standards are expressed in ppm of fresh weight, which are respectively 0.05 ppm for Cd and 0.3 ppm for Pb. According to the guidelines, the results obtained remain below the standards[17].

MT in fish: Spatial evolution: The spatial evolution of MT (Figure-10) shows a significant variation between the prospected stations. Thus, the maximum concentration in the liver is found at station 9 for *Z.faber* (216.41 ppm) and station 11 for *M.merluccius* (204.67 ppm). It should also be noted that MT levels remain relatively high in stations 4, 6 and 11 for John Dory and the same observation can be made for hake in stations 2, 6 and 13. The minimum values for MT in the liver are obtained in station 1 for *Z.faber* (32.67 ppm) and in station 8 for *M.merluccius* (27.98 ppm).

In the muscle, the maximum recorded value (33.87 ppm) is recorded in station 2 for *Z.faber* and station 5 for *M.merluccius* (28.70 ppm). It should be noted that in the John Dory fished in stations 1 and 6, the MT are close to the maximum. The same is true for hake fished in stations 4, 7 and 14. The minimum is determined in stations 15 and 17 for *Z.faber* and in station 1 for *M.merluccius* (0.88 ppm).

Comparison of MT levels: The comparison of the variances by the Pearson T-test revealed significant differences ($p<0.05$) for MT levels between the liver and muscle (Table-8 and Figure-11). For both species, the liver synthesizes more than 8.5 times the MT compared to the muscle. On the other hand, this statistical test revealed no significant difference between MT in the liver of *Z.faber* and *M.merluccius*. The same is true for the muscle of these two species (Table 8).This organ is considered as the intersection of endogenous and exogenous metabolism. The metals of bioaccumulation in the organs are fixed by metalloproteins and excreted by the body to prevent the toxicity of the latter[42]. These results are consistent with those of Fernandes et al. (2008) and Beg et al. (2015)[7,14].

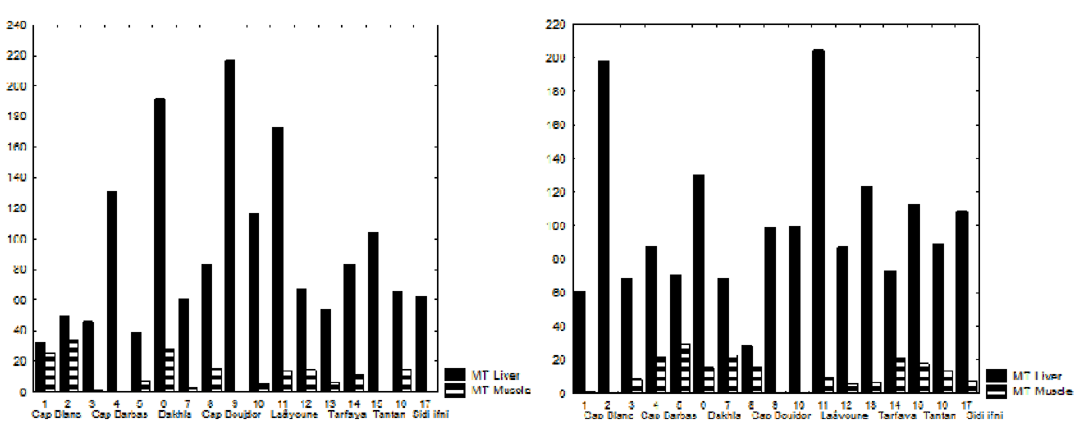

Figure-10: Spatial MT evolution in liver and muscle of *Z.faber* (A) et *M.merluccius* (B) (ppm/ww).

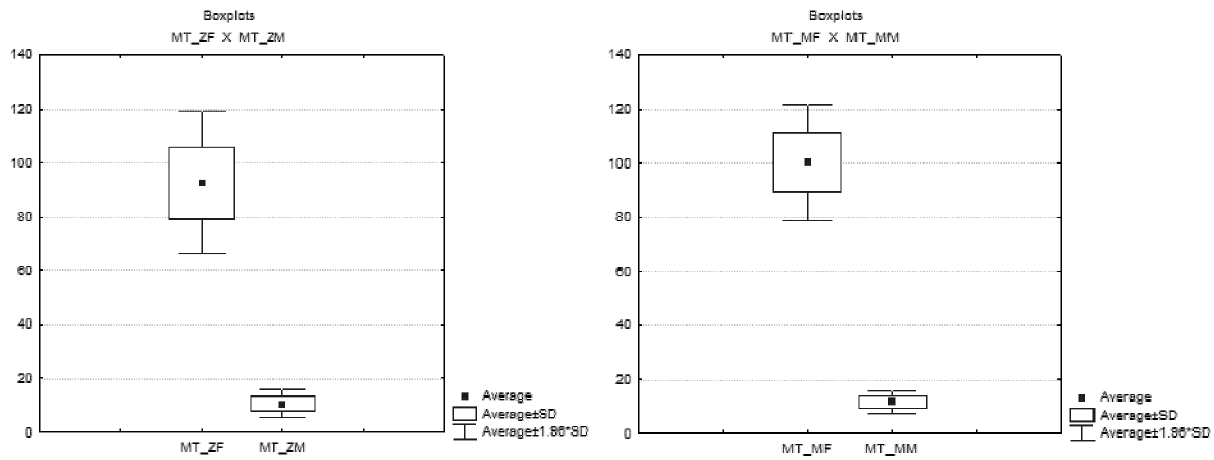

Figure-11: Comparison between MT levels in *Z.faber* (C) and *M.merluccius* (D) liver and muscle (ppm/ww).

Table 8: Test T for the comparison of MT in liver and muscle of two fish species.

Test t for independent samples (MT_Ave_140417_Nansen12)											
	Average	Average	value t	dl	p	N Active	N Active	SD	SD	Ratio F	p
	Group 1	Group 2				Group 1	Group 2	Group 1	Group 2	Variances	Variances
MT_ZF vs. MT_ZM	92.815	10.631	6.015	32.000	**0.000**	17.000	17.000	55.360	10.445	28.092	0.000
MT_MF vs. MT_MM	100.391	11.687	7.907	32.000	**0.000**	17.000	17.000	45.451	8.591	27.988	0.000
MT_ZF vs. MT_MF	92.820	100.390	-0.436	32.000	0.666	17.000	17.000	55.360	45.450	1.000	0.439
MT_ZM vs. MT_MM	10.630	11.690	-0.322	32.000	0.750	17.000	17.000	10.440	8.590	1.000	0.443

Note: Variables treated as independent samples.

Table 9: Hepatic MT levels (µg/g ww) in fish species from different coastal areas

Area	Specie	MT	Reference
Moroccan atlantic coast	Z.faber	92.82 ± 55.36	Present study
	M.merluccius	100.39 ± 45.56	
Kuwaits marine area	A.latus	184 ± 42.7	14
	C.arel	1060 ± 127	
Northern Iberian shelf	L.boscii	7920 ± 692	7
	T.luscus	1479 ± 129	

The distinction between the natural variability of biochemical indices of responses to pollutants exposure is a difficult task, especially when it is a biological matrix as complex as fish, hence the importance of biomarkers which are widely used in the determination of the marine state environment. Indeed, Fernandes and al. (2008)[7] shows, in a study based on demersal fish species (*Trisopterus luscus* and *Lepidorhombus Boscii*) the importance of using the latter as sentinel organisms in biomonitoring programs to assess possible pollution.

The variation of the biological response in our study is expressed by metallothioneins levels in the liver and muscle of the selected species. Metallothioneins is present in the tissues of all vertebrates. In normal metabolism, it is assumed that the main role of this protein is the homeostatic regulation of the intracellular availability of Cu and/or Zn. The metallothioneins is induced by non essential metals and fix them, in addition to the two essential metals, namely Cu and Zn[41]. The concentrations and levels of the samples are shown in figures 12 and 13. The most important values correspond to the cape (Cape Blanc, Cape Barbas and Cape Boujdor). These are also the same areas which have a high Cd and Cr content. They have the same inducing effect of MT. This could be explained by the metal affinity to the MT ion. Further analyzes were carried out to assess the level of accumulation of the two organs at each species level and the T-test (Table 10) shows that there is a significant difference between the levels of MT in the liver by comparing the muscle independently of the species. Compared to the muscle, the liver has a high level of metallothioneins.

In a similar study, quantification of levels of metallothioneins induction in three organs (liver, gills and kidneys) has shown that the maximum production of these proteins is recorded in the liver[42, 43].

Others studies show that high MT levels in fish liver from contaminated sites compared to those found at reference sites indicate that fish from contaminated sites were affected by Cu and/or Zn and/or Cd that contaminate the environment[44].

Conclusion

The southern Moroccan Atlantic zone is exposed to mainly metallic pollution because of the importance of industrial and anthropogenic activities on the coast and whose effluents which are spread out in the ocean. Several metal pollution monitoring studies are based on measurement of the level in the whole organism or in the respective organs. However, measuring the metal content does not give information on its effect at the subcellular level. The concentrations of trace elements (Fe, Zn, Cu and Mn) are higher than those of the toxic elements (Cr, Pb and Cd). This is typically related to their toxicity. The level of metals also depends on the accumulation organ. TME behave differently in the liver compared to muscle. The metal content in fish liver was considerably higher than in muscle. T-test carried out on independent samples show that there is a significant difference between the levels of the metals in the liver in comparison with the muscle for the following elements: Cd, Mn, Fe, Zn and Cu and for the two species studied. We found high enrichment factors in the liver for Fe and Cu and less for Zn, Cd and Pb. In addition, the Pearson test revealed that for the Cd, the liver concentrated 29 times more muscle for *Z.faber* and 193 times for *M.merluccius*. A comparison of the species analyzed showed that *M.meluccius* is the fish that accumulates the most metals in the liver. For the muscle, the accumulation would be with the same order of importance for the two species. The most important TME (both essential and non-essential) are recorded at the Cape (particularly Cape Barbas, Lâayoune and Tarfaya) for *Z.faber* and at Cape Blanc, Dakhla, Laâyoune and Tarfaya for *M.merluccius*.

Metallothioneins were used in this study as biomarkers because of their specificity and ability to bind metal ions). However, in order to apply MT levels as a metallic exposure biomarker using fish species as a biological matrix, the influence of biotic and abiotic factors on MT synthesis in response to exposure to metal contaminants should be taken in consideration. The use of MT as an index of fish exposure to pollutants is difficult to establish with environmental samples. This is likely because fish are exposed to a mixture of contaminants and the interaction between these contaminants and MT can make correlation between pollutants levels and the increase of MT rather uncertain. Moreover, the use of MT only as a biomarker could not be sufficient to control the quality of the environment. Biomarker batteries should be capable of assessing the toxic effects of as many environmental contaminants as possible.

The results also imply a better understanding on the toxicological effects of this specific toxicant for ascertaining safe levels in the aquatic environment to protect the aquatic habitants. This parameter can be used as potential biomarker of TME toxicity to the fish in the field of environmental biomonitoring.

Presented results can be used as a base for comparison with possible future changes in the The southern Moroccan Atlantic ecosystems regarding trace metal contamination and methalothionein in fish.

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