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Levels of heavy metals and metallothioneins in liver and muscle tissue of demersal fish species from the Moroccan Atlantic area

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Abstract

The concentration of trace metallic elements (Cu, Fe, Zn, Mn, Cd, Cr and Pb) was determined in two accumulation organs (liver and muscle) of five fish species (Zeus faber, Merluccius merluccius, Pagellus acarne, Diplodus vulgaris and Chelidonichthys lastoviza) which were collected from coastal, median, and wide stations along the southern Moroccan Atlantic coast. The sampling was carried out in 2 campaigns: during the summer 2013 (period of strong upwelling) and in autumn 2013 (period of low upwelling). Statistical tests showed no significant differences in the accumulation of metals over time. The results obtained showed that the TME average concentrations in the liver were respectively 0.08, 4.29, 381.44, 21.26, 172.19, 11.13, 0.29 ppm of fresh weight in the liver and 0.10, 0.70, 20.13, 0.95, 16.96, 0.03, 0.10 ppm in the muscle. The Metallothioneins (MT) were used as exposure biomarker. MT was used as exposure biomarkers of Z.faber to TME. Their grades vary according to the accumulation organ independently of the sampling season. The maximum levels in the consumable part of the fish remain below the tolerable levels for human consumption as recommended by international standards.

Keywords: Metallothioneins, heavy metals, fish, south Atlantic coast, Morocco.

Introduction

More than 40% of the world's population now lives within 100 km of the shoreline and this population continues to grow, increase its dependence and impact on the coastal ecosystem[1]. Although upwelling ancestry systems represent less than 1% of the world's ocean area, they are considered the most productive regions in the world contributing to about 11% of the world's primary ocean production[2] and 20% of the world fish catch. The aquatic supply of aquatic products is a major challenge and most catches come from the continental shelf (depths below 200 m) and upwelling areas (rich in nutrients)[3]. Upwelling zones are also heavily anthropized[4], and under the threat of land-based pollution and global changes that can act by potentiating impacts contaminants and especially metal contaminants through changes in dynamics[5].

At the same time, urbanization and industrial development have a negative effect on the quality of coastal waters with a threat of amplification by global warming posing potential health risks for humans[6].

The Canary Current ecosystem zone, off the Moroccan coast, is considered one of the richest regions in the world for fisheries resources[7]. However, this fishing potential has suffered some degradation. Indeed, the Moroccan coast is threatened by the impacts of anthropogenic pollution as well as the global changes that directly affect water resources but also changes in ocean dynamics and the descent emergence[8].

Most heavy metals emitted into water as products or by-products from industrial activities or directly applied to sources can travel long distances from their main source and eventually end up in the marine environment. In fact, environmental pollutants and metals are part of particular concern because of their potential for toxicity and bioaccumulation in the aquatic compartment[9]. The pollution of aquatic chain by heavy metals is a real concern because of their toxicity, accumulation and bio-magnification by marine organisms[10]. Heavy metals naturally occurring in seawater at very low concentrations, but concentrations increased due to anthropogenic pollutants. Marine pollution by metallic trace elements (TME) can therefore be potentiated by the reinforcement of upwelling and increase the vulnerability of human societies through fisheries.

Bioaccumulation of heavy metals is linked to biotic and abiotic factors such as water temperature, fish habitat, chemical form of metal in water, fish species[11]. TME accumulate in the aquatic food chain, in molluscs and fish, and then pass to the final consumer. The latter are considered biomonitors of aquatic ecosystems to estimate the pollution of heavy metals. Different fish organs are known to accumulate various concentrations of heavy metals when exposed to them[12]. Various studies (surveys on fish and fishery products) have been carried out in different countries. Many of these studies have been developed in areas polluted by heavy metals. Since fish is an integral part of the human diet, it is normal that many studies have been conducted on trace metal contamination of different fish species[12-17]. It is also logical to assume the presence of contaminants in other non-commercial species, although levels of contamination in these non-target and/or discarded species are generally not evaluated[18].

Recently, and in view of the increasing extent of aquatic ecosystems contamination, biomarkers are increasingly being used to understand the effect of chemical pollutants on these ecosystems and in particularly on marine ecosystems. All ready to think that biochemical indices have the ability to reflect the state of the environment better than chemical monitoring. However, these two methods of analysis remain complementary[19]. Assessment of the impact of micro-contaminants on the liver and muscle of the selected species is carried out using the MT biomarker. These are low-molecular-weight cysteine-rich proteins which play an important role in the transport and storage of essential metals but also protect against the toxic effect of essential and non-essential metals by linking free metal ions and making them less available for interaction with sensitive biomolecules[20].

The objective of this study is to determine the heavy metals levels (Cr, Mn, Fe, Cu, Zn, Cd and Pb) in the liver and muscle of five fish species from the southern Moroccan Atlantic area, then evaluate liver and muscle metallothionein concentration as biological response to metals exposition. Also to evaluate spatial trends in the quality of fish habitat during both upwelling periods in July, high season and December with low inflow. Cd, Cr, Cu and Pb are chosen because of their persistence and toxicity; they can accumulate in biota and biomagnify through trophic layers, biomagnification being particularly important for aquatic organisms. While Fe, Zn and Mn are assayed because they are essential elements for fish. This study is of particular importance as the Canary Current eco-region represents a considerable fishery potential for populations in the southern Moroccan Atlantic area[21].

Materials and methods

Sampling: Five species of fish (*Zeus faber, Merluccius merluccius, Pagellus acarne, Diplodus vulgaris* and *Chelidonychthys lastovisa*) were sampled from 27 stations (from Tantan to Dakhla) divided into 9 radials during two surveys on board the N / R Antea. Sampling was conducted during periods of high and low upwelling (July and December 2013) (Figure-1). After sampling, fish liver and muscle are stored at -20°C until they are analyzed in the laboratory.

Field parameters (temperature, salinity, dissolved oxygen and fluorescence) are determined on board using an SBE 911 bathysonde equipped with sensors.

Chemical and biological analysis: The method of Company et al. was used for the freeze-drying step[22] to determine trace metals elements (Cu, Fe, Zn, Mn, Cd, Cr and Pb) in fish. The mineralization was followed by the AOAC method[23]. Finally the metal levels were assayed by ICPMS (Element II, Thermo).

For metallothionein detection, the method of Viarengo et al.[24] was applied. The results are expressed in µg / g of wet weight.

Statistical analysis: Statistical analysis was performed using STATISTICA software. PCA was used to estimate mesological variability[25,26].

In order to compare metallic contamination in fish species, a Mann-Whitney U-test was used to study the presence of a significant difference in TME accumulation by the organs analyzed and by species studied. This test is also used to compare between the two surveys results (Antea1 and Antea2).

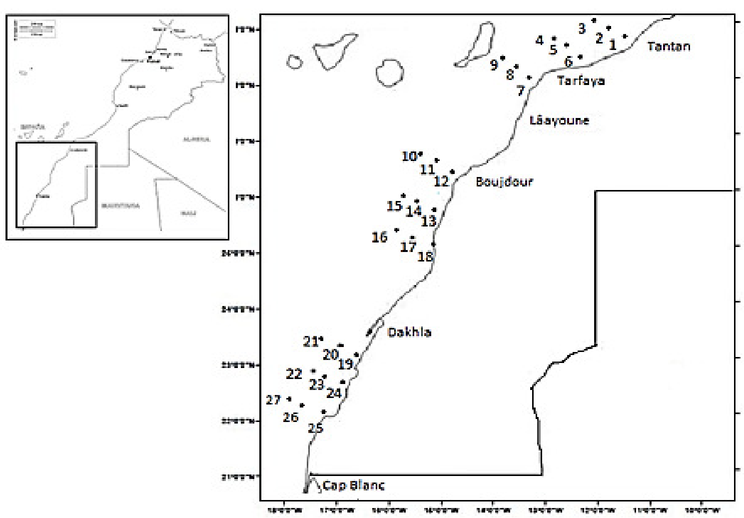


Figure-1: Location of sampling points.

Results and discussion

Mesological analysis: Station 52 sampled between Boujdor and Dakhla was not considered in the ACP because it concentrates the point cloud of the F1xF2 plane because of its factor weight. This station is marked by high levels of NO_3^-, PO_4^{3-} and SIO_2^-.

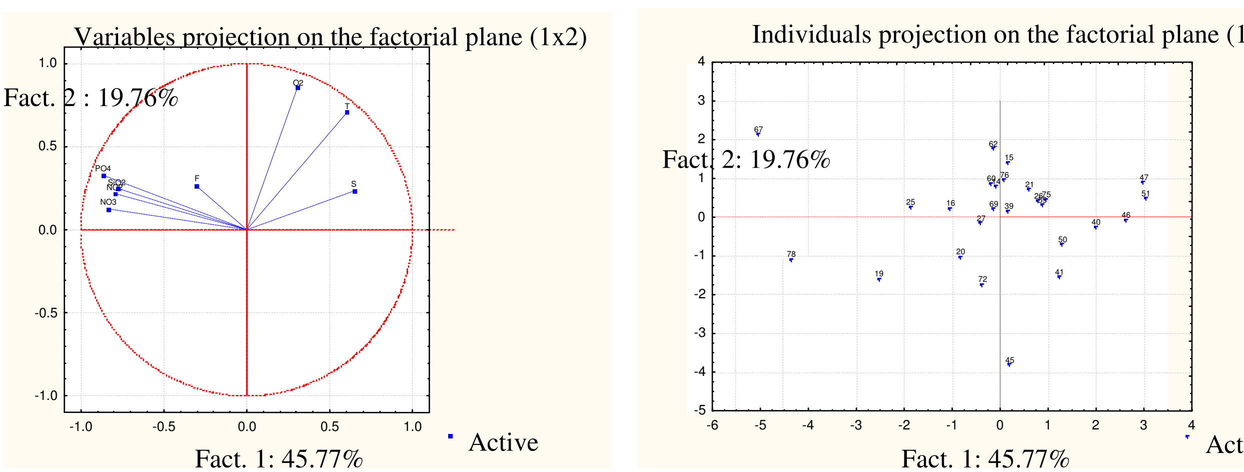
Antea 1 campaign: The correlation circle (Figure-2A) shows that the eight variables taken into account in the ACP contribute to the definition of the factor plane F1 x F2. Temperature (T), salinity (S) and dissolved oxygen (O_2) contribute positively to the formation of the F1 axis. For axis F2, the structuring variables are NO_3, PO_4 and NO_2.

Based on the factorial map F1 x F2 (Figure-2B) stations located at Cap Boujdor (46, 47 and 51) would be marked by thermal and saline factors, stations Tantan (67 and 78) would be relatively marked by the abundance of phytoplankton from which the richness of nutrients. The structuring of these variables around the F2 axis can be explained by the fact that the algal development is dependent on the nutritive factors contained in the environment.

Antea 2 campaign: T, S contributes negatively to the formation of F1 axis, O_2 contributes positively to the formation of this axis. For F2, the structuring variables are F, NO_2, PO_4 positively and NO_2 and NO_3 negatively.

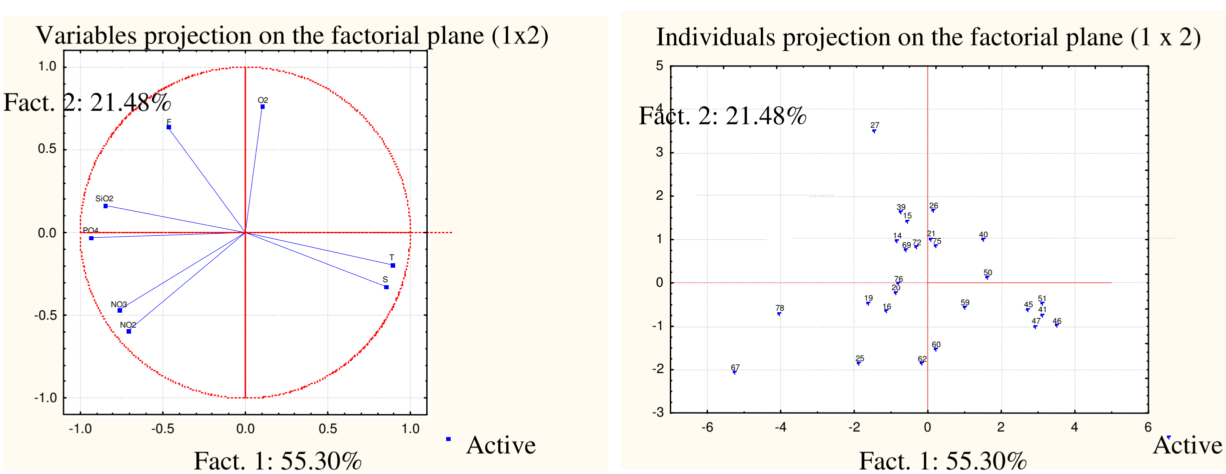
The structuring of these variables around the F2 axis can be explained by the fact that the algal development is dependent on the nutritive factors contained in the environment.

Based on the factorial map F1 x F2 (Figure-3D), it can be seen that the stations (Dakhla Bay) and stations 67 and 78 (at Tantan) would be marked by fluorescence and nutrient factors, which is synonymous with significant phytoplankton activity.



(A) (B)

Figure-2: Representation of Variables (A) and Stations (B) in the F1xF2 Main Plan of the ACP (Antea1 campaign).



(C) (D)

Figure-3: Representation of Variables (C) and Stations (D) in the F1xF2 Main Plan of the ACP (Antea2 campaign).

ETM in fish: The average values and standard deviations of the levels of TME contained in the organs of the different species sampled by radials are shown in Figures-4, 5, 6, 7 and 8 for the two campaigns.

Iron: The maximum value (834.14ppm) is found in the liver of *D. vulagris* at north of Dakhla and the minimum value (0.96 ppm) south of Cintra Bay for the same species. The maximum muscular value (105.58ppm) is observed in *Z.faber* from Dakhla Bay and the minimum (0.01ppm) is observed in *D.vulgaris* from south of Cintra Bay.

Zinc: In the periodic table, Zinc is located in group IIB with two toxic metals; cadmium and mercury. However, Zn is considered relatively non-toxic to humans[12]. It is to the south of Cintra Bay that hepatic concentration is maximal in Zn (1192.11 ppm) in the *C. lastoviza* fish population and minimal (0.26ppm) in *D. vulgaris.* In the Dakhla Bay *Z.faber* muscle, the maximum level is 36.79ppm, while the minimum value is recorded in the *D. vulgaris* population of Cintra Bay (0.02ppm).

Copper: Copper is an essential trace element that exists in small amounts in a variety of cells and tissues and at high concentrations in the liver[12]. The maximum value of this metal (53.54ppm) is observed in fish liver *P.acarne* in Tantan, while the minimum (0.06ppm) is recorded in *D.vulgaris* in the south of Cintra Bay. For muscle, the maximum level (3.3ppm) is found in *Z.faber* at Dakhla Bay and the minimum in *D.vulgaris* and *C.lastoviza.*

Manganese: In the Bay of Dakhla, the maximum values in Mn are recorded both in liver (8.14ppm in *C.lastoviza*) and in muscle (2.18 ppm in *Z.faber*). The minimum values are close to 0 for most species (except *Z.faber*).

Chromium: Chromium is an essential element for humans and animals. However, when taken in excessive quantities, the hexavalent form may be toxic[12]. In most prospected stations, concentrations of this metal are minimal in both the liver and muscle for most species; the maximum value (0.42ppm) is found in *M.merluccius* south of Cintra Bay.

Cadmium: Cadmium is a highly toxic element for mammals and fish. It tends to be accumulated in living organisms very rapidly[12]. The maximum (45.29ppm) and minimum (0.04ppm) Cd values in the liver were recorded in the same species (*D.vulgaris)* in the Dakhla and Cintra bays, respectively. Cd levels in muscle are minimal in most stations; the maximum value (0.03 ppm) is recorded at *Z.faber* at Dakhla Bay.

Lead: Lead is one of the most important heavy metals that can be found in the environment, particularly in the aquatic environment[27]. Pb levels are relatively low in both liver and muscle; the maximum hepatic level (0.68 ppm) is observed in *P. acarne* in the south of Cintra Bay and the maximum muscle (0.1 ppm) in *D.vulgaris* north of Dakhla.

Comparison of metal levels by organ: Comparison of data in Tables-1 and 2 on metallic liver and muscle contamination in *Z.faber, M.merluccius, P.acarne, D.vulgaris* and *C.lastoviza* was performed by a nonparametric U Mann-Whitney test. In all species, this test revealed significant differences ($p<0.05$) in Mn, Fe, Cu, Zn and Cd levels between liver and muscle (Table-3). The same is not true for Cr and Pb, for which the U-test is generally not significant whatever the season.

Table-1: Descriptive statistics of TME in liver and muscle during the Antea1 campaign (Mean ± SD).

	Z.faber		M.merluccius		P.acarne		D.vulgaris		C.lastovisa	
	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
Cr	0.07± 0.06	0.06±0.05	0.21±0.2	0.24±0.41	0.13±0.13	0±0	0.12±0.09	0.05±0.03	0.06±0.03	0±0
Mn	2.55±1.2	0.72±0.73	5.86±2.8	0.34±0.59	5.59±1.37	0.01±0	4.21±2.47	0.57±0.37	4.28±2.59	0±0
Fe	192.01±233.87	24.23±39.98	152.79±73.02	4.47±7.58	306.84±74	0.31±0.07	458.11±311.43	13.33±10.67	371.66±123.07	0.44±0.26
Cu	7.05±6.4	0.86±1.2	14.97±5.12	0.44±0.74	38.49±9.47	0.04±0.01	29.09±18.45	0.6±0.37	22.78±13.19	0.02±0.01
Zn	66.1±55.03	18.19±9.38	54.32±19.63	10.43±18	134.79±17.93	0.13±0.02	132.92±79.12	12.69±7.16	459.59±431.35	0.46±0.43
Cd	0.6±0.77	0.02±0.01	1.51±2.27	0.01±0.02	17.99±14.56	0.01±0.01	20.64±17	0.02±0.01	6.95±6.21	0.01±0.01
Pb	0.04±0.05	0.06±0.1	0.12±0.13	0±0	0.31±0.24	0±0	0.29±0.17	0.10±0.03	0.04±0.04	0±0

Table-2: Descriptive statistics of heavy metals in liver and muscle during the Antea2 campaign (Mean ± SD).

	Z.faber		M.merluccius		P.acarne		D.vulgaris	
	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
Cr	0.04± 0.01	0.06±0.02	0.1±0.02	0.12±0.07	0.08±0.05	0.15±0.05	0.14±0.09	0.1±0
Mn	2.72±0.63	0.37±0.03	4.23±1.52	0.54±0.08	6.58±1.48	1.34±1.35	4.8±1.51	0.81±0.27
Fe	127.77±20.07	8.49±3.33	250.51±221.83	19.04±5.12	434.65±265.66	107.72±179.53	599.87±261.57	26.61±12.54
Cu	6.05±2.57	0.54±0.16	13.1±6.58	1.23±0.21	105.24±74.53	6.36±6.51	40.64±28.82	1.4±0.33
Zn	54±21.17	12.97±1.07	56.49±28.1	15.72±1.58	224.99±82.81	45.38±62.5	145.73±60.45	89.31±15.39
Cd	0.39±0.21	0.02±0.01	9.611±16.36	0.02±0.01	29.94±18.06	0.02±0.02	4.57±2.52	0.01±0.02
Pb	0.03±0.02	0.06±0.05	0.13±0.11	0.12±0.02	0.95±1.3	0.03±0.01	0.05±0.04	0.05±0.04

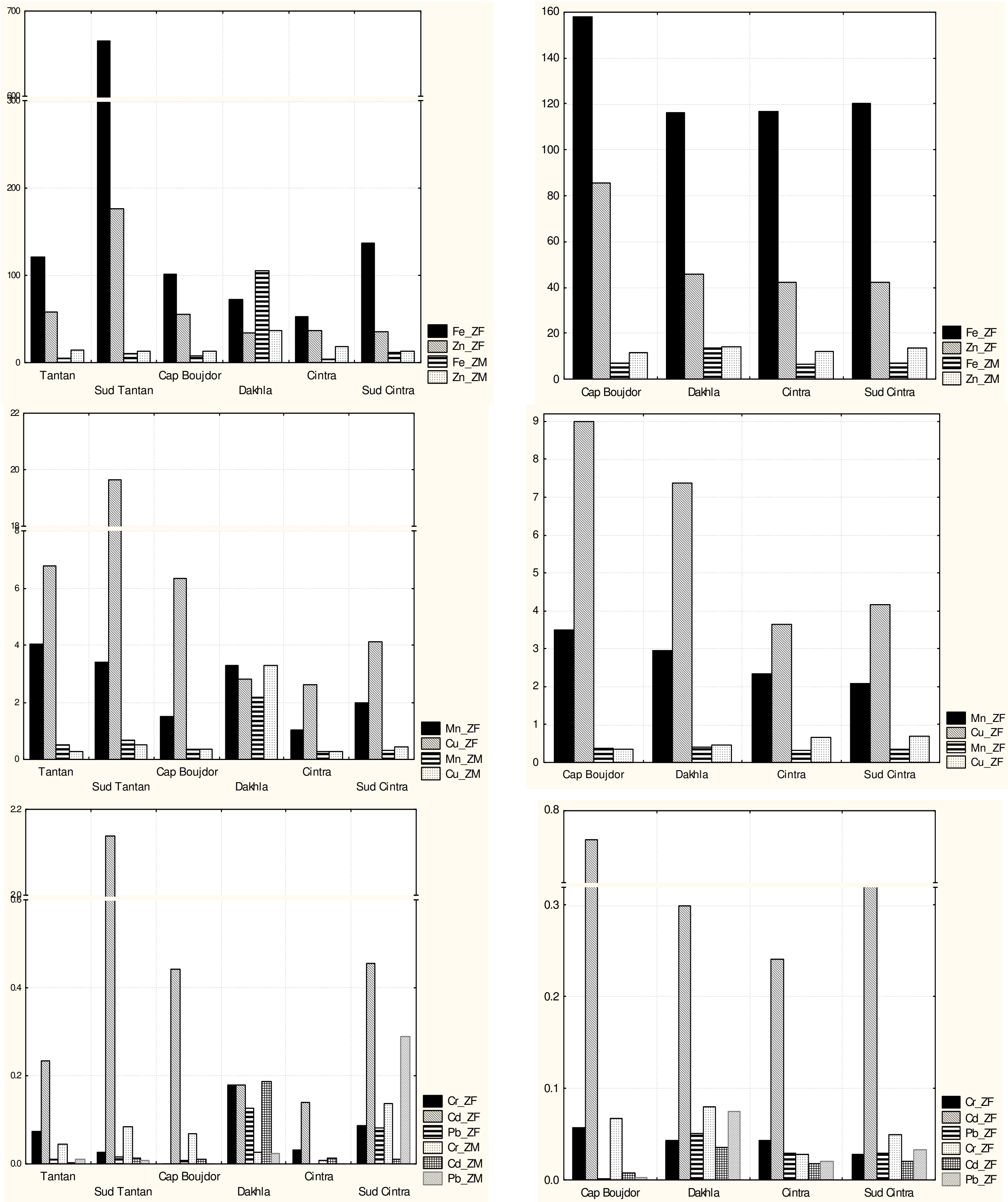


Figure-4: Trace metals in muscle and liver of *Zeus faber* during Antea 1 (A) and Antea2 (B) (ppm / ww).

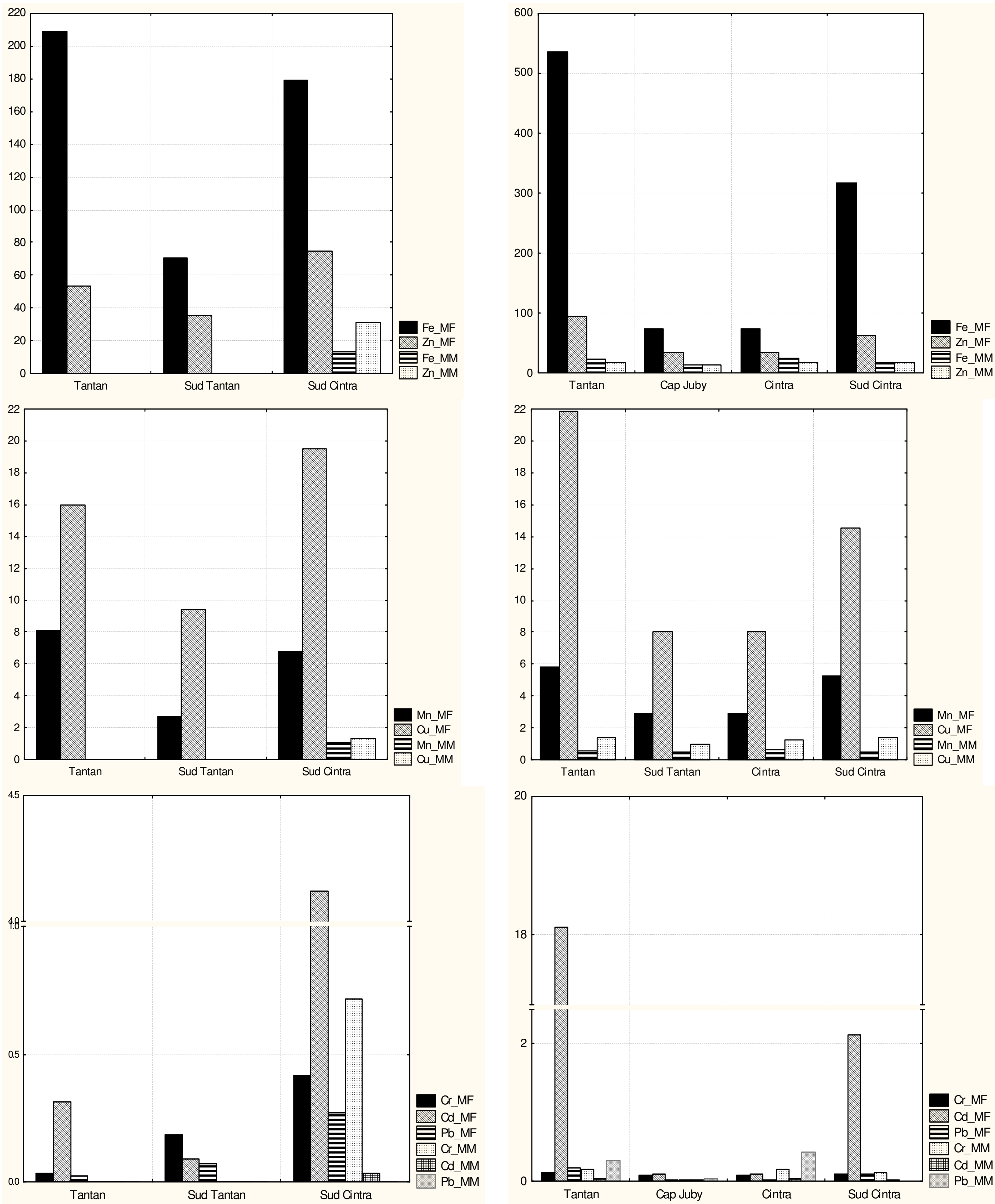


Figure-5: Trace metals in muscle and liver of *M.merluccius* during Antea 1 (A) and Antea2 (B) (ppm / ww).

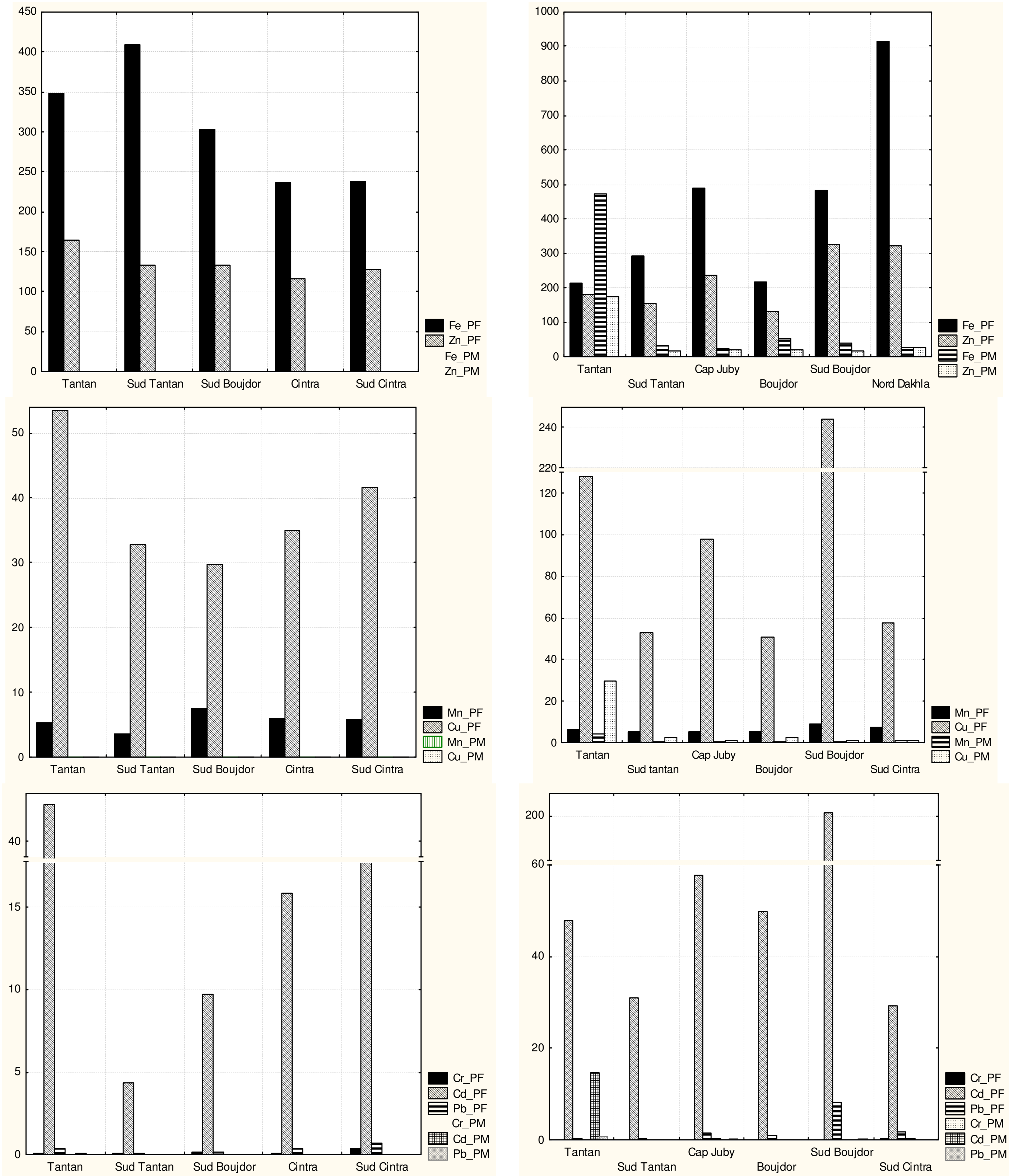


Figure-6: Trace metals in muscle and liver of *P.acarne* during Antea 1 (A) and Antea2 (B) (ppm / ww).

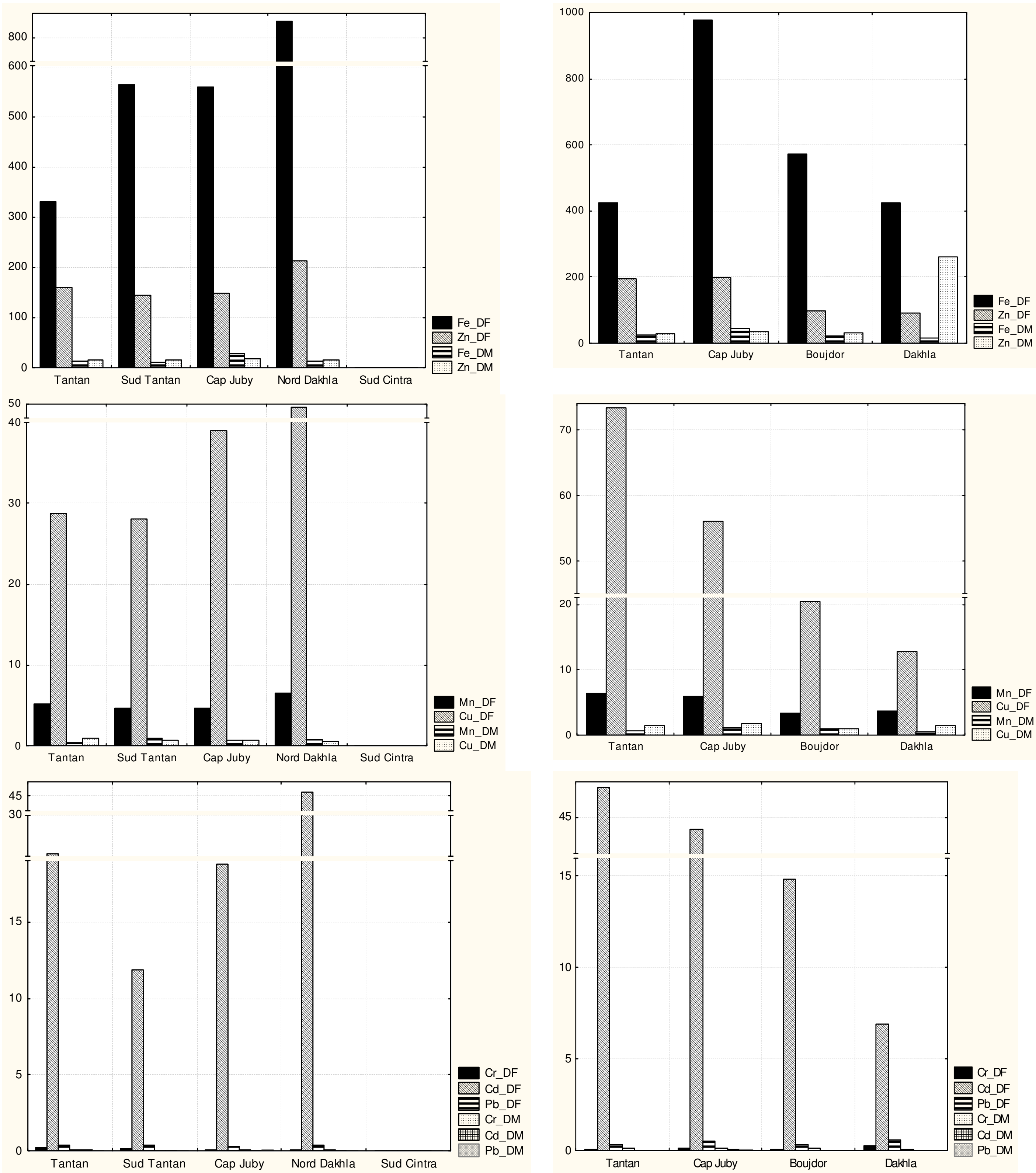


Figure-7: Trace metals in muscle and liver of *D.vulgaris* during Antea 1 (A) and Antea2 (B) (ppm / ww).

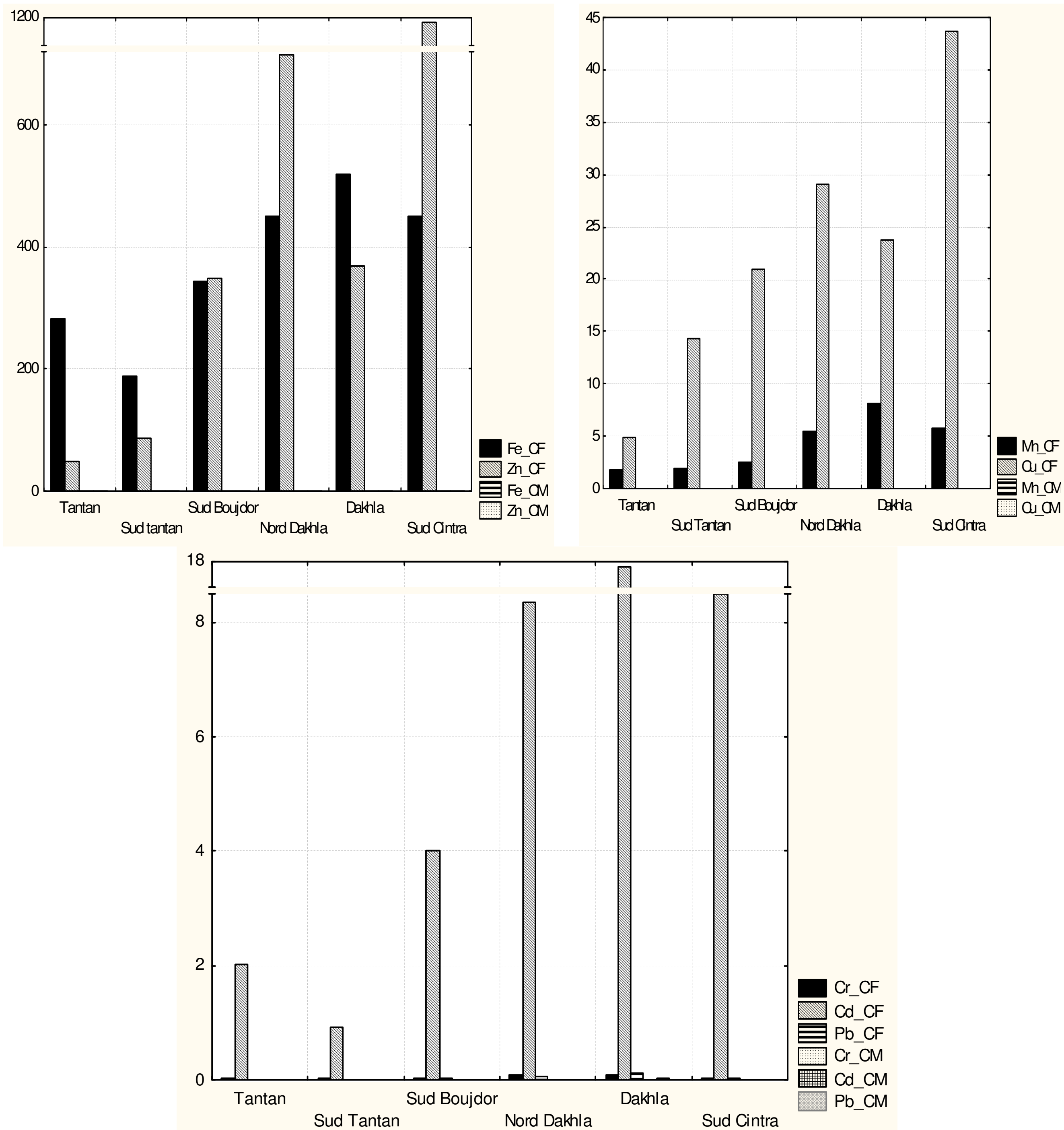


Figure-8: Trace metals in muscle and liver of *Chelidonichthys lastoviza* during Antea1 (ppm / ww).

MT in fish: The metallothioneins level was determined in *Z.faber* liver and muscle during both campaigns (Antea1 and Antea2). The results obtained are shown in Tables-6, 7 and Figure-9. During the first campaign, the MT levels varied between 220.36 and 156.18 ppm for liver and 97.25 and 69.54 ppm for muscle; the hepatic mean of MT is 194.75±22.69 ppm and the muscular one is 84.89±9.86 ppm. During the second campaign, these levels varied between 213.01 and 78.01 ppm for liver and 98.87 and 78.02 ppm for muscle; the hepatic mean of MT is 162.09±44.91 ppm and the muscular one is 85.97±7.69 ppm. The U Mann Whitney test (Table-4) revealed that there is no significant difference between results of 1st and 2nd campaign. In the other hand, this test revealed significant differences ($p < 0.05$) for MT levels according to organs for the specie studied (Table-5).

Table-3: Test U applied to metallic liver and muscle contamination results in *Z.faber, M.merluccius, P.acarne, D.vulgaris* and *C.lastoviza.*

Test U de Mann-Whitney Zeus faber (Par var. Organe) Antea 1										
Tests significatifs marqués à p <.05000										
	SommeRgs	SommeRgs	U	Z	niv. p	Z	niv. p	N Actif	N Actif	2*(1-p)
Cr_ZF	39.000	39.000	18.000	0.000	1.000	0.000	1.000	6.000	6.000	1.063
Mn_ZF	54.000	24.000	3.000	2.402	0.016	2.402	0.016	6.000	6.000	0.015
Fe_ZF	54.000	24.000	3.000	2.402	0.016	2.402	0.016	6.000	6.000	0.015
Cu_ZF	55.000	23.000	2.000	2.562	0.010	2.562	0.010	6.000	6.000	0.009
Zn_ZF	55.000	23.000	2.000	2.562	0.010	2.562	0.010	6.000	6.000	0.009
Cd_ZF	55.000	23.000	2.000	2.562	0.010	2.562	0.010	6.000	6.000	0.009
Pb_ZF	41.000	37.000	16.000	0.320	0.749	0.321	0.748	6.000	6.000	0.818
Test U de Mann-Whitney Merluccius merluccius (Par var. Organe) Antea 1										
Tests significatifs marqués à p <.05000										
	SommeRgs	SommeRgs	U	Z	niv. p	Z	niv. p	N Actif	N Actif	2*(1-p)
Cr_MM	12.000	9.000	3.000	0.655	0.513	0.655	0.513	3.000	3.000	0.700
Mn_MM	15.000	6.000	0.000	1.964	0.050	1.964	0.050	3.000	3.000	0.100
Fe_MM	15.000	6.000	0.000	1.964	0.050	1.964	0.050	3.000	3.000	0.100
Cu_MM	15.000	6.000	0.000	1.964	0.050	1.964	0.050	3.000	3.000	0.100
Zn_MM	15.000	6.000	0.000	1.964	0.050	1.964	0.050	3.000	3.000	0.100
Cd_MM	15.000	6.000	0.000	1.964	0.050	1.964	0.050	3.000	3.000	0.100
Pb_MM	15.000	6.000	0.000	1.964	0.050	1.964	0.050	3.000	3.000	0.100
Test U de Mann-Whitney Pagellus acarne (Par var. Organe) Antea 1										
Tests significatifs marqués à p <.05000										
	SommeRgs	SommeRgs	U	Z	niv. p	Z	niv. p	N Actif	N Actif	2*(1-p)
Cr_PA	40.0000	15.0000	0.0000	2.6112	0.0090	2.6112	0.0090	5.0000	5.0000	0.0079
Mn_PA	40.0000	15.0000	0.0000	2.6112	0.0090	2.6112	0.0090	5.0000	5.0000	0.0079
Fe_PA	40.0000	15.0000	0.0000	2.6112	0.0090	2.6112	0.0090	5.0000	5.0000	0.0079
Cu_PA	40.0000	15.0000	0.0000	2.6112	0.0090	2.6112	0.0090	5.0000	5.0000	0.0079
Zn_PA	40.0000	15.0000	0.0000	2.6112	0.0090	2.6112	0.0090	5.0000	5.0000	0.0079
Cd_PA	40.0000	15.0000	0.0000	2.6112	0.0090	2.6112	0.0090	5.0000	5.0000	0.0079
Pb_PA	40.0000	15.0000	0.0000	2.6112	0.0090	2.6112	0.0090	5.0000	5.0000	0.0079
Test U de Mann-Whitney Diplodus vulgaris (Par var. Organe) Antea 1										
Tests significatifs marqués à p <.05000										
	SommeRgs	SommeRgs	U	Z	niv. p	Z	niv. p	N Actif	N Actif	2*(1-p)
Cr_DV	35.000	20.000	5.000	1.567	0.117	1.567	0.117	5.000	5.000	0.151
Mn_DV	36.000	19.000	4.000	1.776	0.076	1.776	0.076	5.000	5.000	0.095
Fe_DV	36.000	19.000	4.000	1.776	0.076	1.776	0.076	5.000	5.000	0.095
Cu_DV	36.000	19.000	4.000	1.776	0.076	1.776	0.076	5.000	5.000	0.095
Zn_DV	36.000	19.000	4.000	1.776	0.076	1.776	0.076	5.000	5.000	0.095
Cd_DV	39.000	16.000	1.000	2.402	0.016	2.402	0.016	5.000	5.000	0.016
Pb_DV	36.000	19.000	4.000	1.776	0.076	1.776	0.076	5.000	5.000	0.095

Test U de Mann-Whitney Chelidonichthys lastoviza (Par var. Organe) Antea 1									
Tests significatifs marqués à p <.05000									
	SommeRgs	SommeRgs	U	Z	niv. p	Z	niv. p	N Actif	N Actif	2*(1-p)
Cr_CL	57.000	21.000	0.000	2.882	0.004	2.882	0.004	6.000	6.000	0.002
Mn_CL	57.000	21.000	0.000	2.882	0.004	2.882	0.004	6.000	6.000	0.002
Fe_CL	57.000	21.000	0.000	2.882	0.004	2.882	0.004	6.000	6.000	0.002
Cu_CL	57.000	21.000	0.000	2.882	0.004	2.882	0.004	6.000	6.000	0.002
Zn_CL	57.000	21.000	0.000	2.882	0.004	2.882	0.004	6.000	6.000	0.002
Cd_CL	57.000	21.000	0.000	2.882	0.004	2.882	0.004	6.000	6.000	0.002
Pb_CL	57.000	21.000	0.000	2.882	0.004	2.882	0.004	6.000	6.000	0.002

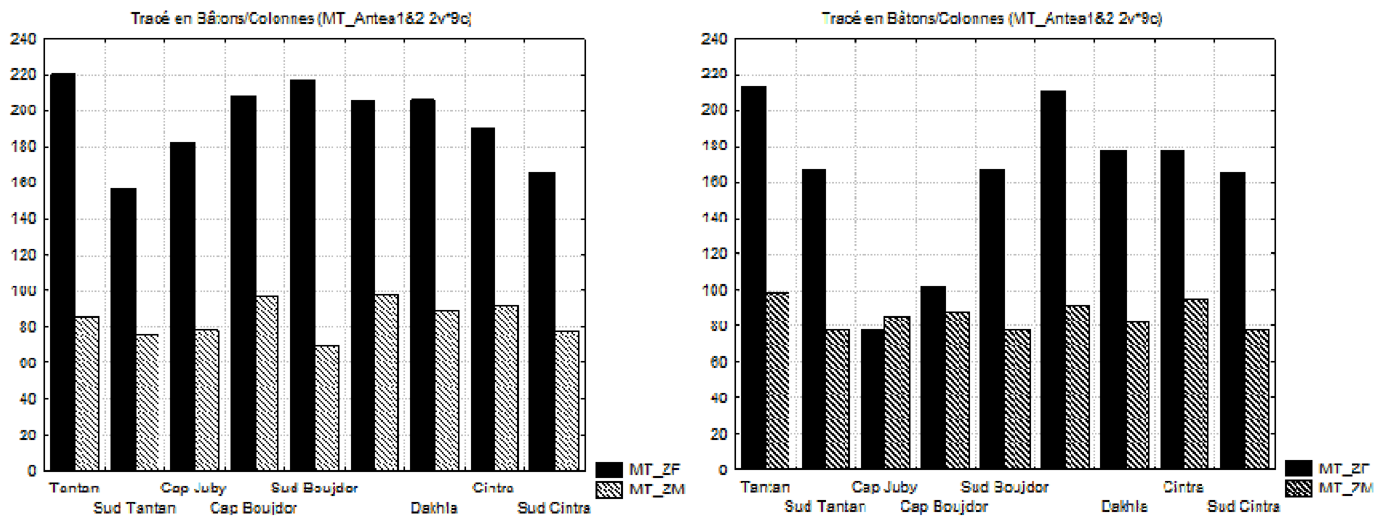


Figure-9: Comparison of MT levels in *Zeus faber* liver and muscle (µg/g) during Antea1 (A) and Antea2 (B).

Table-4: Test U for comparison between MT levels in the Antea1 and Antea2 campaigns.

Test U de Mann-Whitney (MT_Antea1&2) Par var. Campagne										
Tests significatifs marqués à p <.05000										
	SommeRgs	SommeRgs	U	Z	niv. p	Z	niv. p	N Actif	N Actif	2*(1-p)
MT_ZF	84.00000	87.00000	39.00000	-0.132453	0.894626	-0.133003	0.894191	9	9	0.931427
MT_ZM	82.00000	89.00000	37.00000	-0.309058	0.757278	-0.309058	0.757278	9	9	0.796174

Table-5: Test U applied to the results of MT levels in the liver and muscle of *Z.faber*.

Test U de Mann-Whitney (MT_Antea1&2) Par var. Organe										
Tests significatifs marqués à p <.05000										
	SommeRgs	SommeRgs	U	Z	niv. p	Z	niv. p	N Actif	N Actif	2*(1-p)
ZF_Antea1	126.0000	45.00000	0.00	3.576237	0.000349	3.576237	0.000349	9	9	0.000041
ZF_Antea2	117.5000	53.50000	8.500000	2.825669	0.004718	2.827128	0.004697	9	9	0.002756

The main effort of environmental agencies is to assess the “quality” of an ecosystem by evaluating the chemical contaminants present in different matrices. However, the only way to establish the possible effects of toxic contaminants is to associate the chemical data with the results of ecotoxicological and ecological studies[28]. In this study, the assessment of the levels of contamination of trace metals in the different species of fish of the southern Moroccan Atlantic coast showed the presence of differences in contamination between the species and the organs considered, as well as very significant variations between the elements of the metals analyzed.

Intra-specific variations of metals accumulation: The liver and muscle are chosen as target organs to assess the accumulation of metals. The selection criteria for the different organs are based on the fact that muscle is the edible part of the fish, whereas the liver is the organ of accumulation par excellence after the gills that are in contact with the external environment and therefore more exposed to the contaminants. Indeed, the liver is very active in the absorption and storage of heavy metals[29]. In addition, it is often recommended as an environmental indicator of water pollution that any other fish organ[30]. In our study, fish species analyzed during the two high and low inflow campaigns always exhibited the lowest concentration of metals in the muscles. These results corroborate those obtained by Henry et al.[14], El Morhit et al.[15], Abeshi et al.[29], Talba et al.[31], Diop et al.[32] and Bat et al.[33]. According to Roomiani et al.[34] and Zhao et al.[35] the accumulation of essential metals (Cu, Fe and Zn) in the liver is probably related to their roles in metabolism.

Elevated levels of zinc and copper in liver tissues are generally related to natural binding proteins such as metallothioneins (MT) which act as a reservoir of metal essential to satisfy enzymatic and other metabolic requirements. Similarly, iron tends to accumulate in hepatic tissues due to the physiological role of the liver in the production of blood cells and the synthesis of hemoglobin[16].

Significant values of lead in fish are not correlated with dietary habits) but may have a terrestrial origin or be the result of a geomorphological area structure. Cr and Pb are elevated in the liver respectively to the muscle; this can be attributed to the affinity or strong coordination of metallothionein proteins to these elements[29]. High levels of Pb and Zn could also be attributed to the presence of industrial activities in the region[36]. The Cd model differs between those species where the highest concentrations were observed in *P. acarne.* On the other hand, the liver also showed high levels of toxic metals such as Cd; this finding could be explained by the Cd's ability to displace the essential element associated with TME in hepatic tissues.

Inter-specific variations of metals accumulation: The results of this study have shown that fishes from the southern Atlantic coast of Morocco have wide interspecific variations in the accumulation of TME by the liver or muscle. The results of the 1st campaign (Table-6) showed that *Z.faber* accumulates the most metals (in particular Zn, Cu, Mn, Pb and Fe) in the muscle. Whereas for the liver, the maxima depend on the species under consideration. The data in this table also show that it is south of the bay of Cintra and in the Dakhla regions in particular where the maxima of contamination by the TME have been observed. The order of accumulation of TME during the 1st season by species is shown in Table-6. The results of Table-7 of the second season (Antea2) revealed that Axillary seabream, which predominantly feed on crustaceans and molluscs, appears to be the most accumulating species in both liver and in the muscle. This fish population has maxima in Mn, Cu, Zn, Cd and Pb in the liver south of Boujdor, while that of Tantan has maxima in Mn, Fe, Cd and Pb in the muscle. The order of accumulation of TME during the 2nd season by species is shown in Table-9.

Table-6: Summaries of TME maxima by species, organs and stations surveyed (Antea1 campaign).

Organ	Liver			Muscle		
Metal	Max	Specie	Station	Max	Specie	Station
Cr	0.42	M.merluccius	Sud Cintra	0.72	M.merluccius	Sud Cintra
Mn	8.14	C.lastovisa	Dakhla	2.18	Z.faber	Dakhla
Fe	834.14	D.vulgaris	Nord Dakhla	105.58	Z.faber	Dakhla
Cu	53.54	P.acarne	Tantan	3.3	Z.faber	Dakhla
Zn	1192.11	C.lastovisa	Sud Cintra	36.79	Z.faber	Dakhla
Cd	45.29	D.vulgaris	Nord Dakhla	0.03	D.vulgaris	Tantan
Pb	0.68	P.acarne	Sud Cintra	0.12	Z.faber	Sud Cintra

It would appear that levels of TME in different species are due to their food and trophic rank; hence the need to monitor metal contamination along the trophic chain to which the studied fish belong.

Seasonal variations of metals accumulation: The results of the U Mann-Whitney test carried out by comparing the Antea1 and Antea2 campaigns showed no significant difference at p = 0.05 between the first campaign carried out during the upwelling period and the second campaign (period of low upwelling) with the exception of *P. acarne* where there is a significant increase in TME levels in the muscle of this species (Table-10).

Assessment of biological levels due to metal accumulation: The induction of metallothioneins MT in the liver is the main form of storage and detoxification of metals in fish. Increased concentrations of metals in the liver may represent the level of metal storage in the organ[37]. It is known that MT whose role is the control of metals within cells are proteins present in the majority of vertebrates. It is assumed that they are induced by both types of essential and toxic metals. MT regulates the trace metal metabolism in fish during the sexual maturation in females and during stress. The most important MT value could be explained by her metal affinity[38]. In our study, the liver has a high level of metallothioneins compared to the muscle. Those levels are below the results find by others authors for different species (Table-11). Perhaps because our stations are less polluted by heavy metals.

Assessment of fish consumption risks for health: In order to assess the risk to human health arising from fish consumption, we compared metal levels in muscle with the maximum allowable human consumption limits (MAL) established by several organizations (Table-12). Thus, the levels of essential and non-essential metals recorded for all the species studied do not exceed the tolerable doses recommended by FAO, WHO and the European Commission (EC).

Conclusion

Present study provides information on the Cr, Mn, Fe, Zn, Cu, Cd and Pb concentration in different species of fish from the southern Moroccan Atlantic coast.

Metal concentrations are consistent with previous studies in different marine areas of the world. The results showed that, on the one hand, the accumulation of the metal varied between the organs; the liver concentrates the metals more than the muscle. On the other hand, the level of contamination varies according to the species studied; John Dory and white Seabream have shown high levels of accumulation relative to other species. No significant difference in results for metallic contamination was observed between the period of strong (Antea1) and low upwelling (Antea2).

Because of the high specificity of metals, MT were chosen to evaluate biologically the effects of trace metals in this study but also in biomonitoring networks. Having information on heavy metals in fish is important for biodiversity, quality habitat and human health. Risk analysis of heavy metals in fish edible parts showed levels below the limits recommended by international legislation.

Table-7: Order of accumulation of TME in the liver and muscle according to species (campaign: Antea1).

Organ	Metal	Order of accumulation
Liver	Cr	MM > PA > DV > ZF > CL
	Mn	CL > MM > PA > DV > ZF
	Fe	DV > ZF > CL > PA > MM
	Cu	PA > DV > CL > ZF > MM
	Zn	CL > DV > ZF > PA > MM
	Cd	DV > PA > CL > MM > ZF
	Pb	PA > DV > MM > ZF > CL
Muscle	Cr	MM > ZF > DV > CL > PA
	Mn	ZF > MM > DV > PA > CL
	Fe	ZF > DV > MM > CL > PA
	Cu	ZF > MM > DV > PA > CL
	Zn	ZF > MM > DV > CL > PA
	Cd	ZF > DV > PA > MM > CL
	Pb	ZF > DV > MM > CL = PA

Table-08: Summaries of TME maxima by species, organs and stations surveyed (Antea2 campaign).

Organ	Liver			Muscle		
Metal	Max	Specie	Station	Max	Specie	Station
Cr	0.28	D.vulgaris	Dakhla	0.21	P.acarne	Sud Cintra
Mn	9.17	P.acarne	Sud Boujdor	4.05	P.acarne	Tantan
Fe	978.24	D.vulgaris	Cap Juby	473.62	P.acarne	Tantan
Cu	243.95	P.acarne	Sud Boujdor	29.81	P.acarne	Cap Boujdor
Zn	324.21	P.acarne	Sud Boujdor	262.36	D.vulgaris	Dakhla
Cd	200.72	P.acarne	Sud Boujdor	0.03	P.acarne	Tantan
Pb	8.23	P.acarne	Sud Boujdor	0.05	P.acarne	Tantan

Table-09: Order of accumulation of MES in the liver and muscle according to species (campaign: Antea2).

Organ	Metal	Order of accumulation
Liver	Cr	DV > PA > MM > ZF
	Mn	PA > DV > MM > ZF
	Fe	DV > PA > MM > ZF
	Cu	PA > DV > MM > ZF
	Zn	PA > DV > MM > ZF
	Cd	PA > DV > MM > ZF
	Pb	PA > DV > MM > ZF
Muscle	Cr	PA > MM > DV > ZF
	Mn	PA > DV > MM > ZF
	Fe	PA > DV > MM > ZF
	Cu	PA > DV > MM > ZF
	Zn	DV > PA > MM > ZF
	Cd	PA > DV > MM = ZF
	Pb	PA > MM > ZF > DV

Table-10: Maxima comparison in *P. acarne* muscle according to U Mann-Whitney test.

Metal	Cr	Mn	Fe	Cu	Zn	Cd	Pb
Antea 1	0.00	0.01	0.41	0.05	0.16	0.03	0.05
Antea 2	0.21	4.05	473.62	29.81	172.78	0.03	0.12

Table-11: Comparison between hepatic MT concentrations in fish from some coastal areas.

Area	Specie	MT (µg/g ww)	Reference
Moroccan atlantic coast	Zeus faber	194.75 ± 22.63	Present study
Kuwaits marine area	Acanthopagrus latus	2800	19
	Cynoglossus arel	550	
Tunisia	Sparus.aurata	379.8 ± 109.95	39
		399.53 ± 36.66	
Northern Iberian shelf	Dicentrachus labrax	3802 ± 598	20
Bermuda area	Haemdon scium	196 ± 145	38
	H. aurolinea	347 ± 25	

Table-12: Maximum Allowable Limit (MAL) of trace metals in fish muscle (ppm/ww) according to international standards.

International Standards	Metal						Reference
	Cu	Zn	Pb	Cd	Fe	Mn	
FAO	30	40	0.5	0.5			40
MAFF (UK)			2	0.2			41
FDA			0.5	2			42
WHO	30	100	2	1	100	1	43
EC			0.2	0.05			44

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