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Assessment of heavy metal concentrations in (*Sardinapilchardus*) caught in the Mediterranean coast from the North East of Morocco

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Abstract

The Moroccan coast corresponds to a space of intense economic activities (industrial, agricultural, domestic,...etc), and thus constitutes the receptacle of significant quantities of substances of natural or anthropic origin, many of which possess toxic properties. Heavy metals are pollutants whose harmfulness is related to their persistence and their speciation. They are poorly metabolized, so they can be transferred to the food web and accumulate in living matter. In order to assess the level of pollution by metallic trace elements in the Moroccan coast, we have determined the concentration of (Cd, Pb, Ni, Cu, Fe, Zn, Cr) in the muscles, gills and livers, of *Sardina pilchardus* caught in the Mediterranean coast from the North East of Morocco, landed in the port of Nador during the autumn and spring of the years 2019 and 2020 .Concentrations of heavy metals were determined by inductively coupled plasma- atomic emission spectroscopy (ICP-AES). Except the iron the concentrations of the metal elements studied were generally within the limit allowed by the WHO for human consumption.

Keywords: Heavy metals; Contamination; *Sardina pilchardus;* Mediterranean Sea; Bioaccumulation

1. Introduction

Large amounts of pollutants are released daily into the environment. Among them, heavy metals are considered to be serious pollutants in the aquatic environment [1,2] Contamination by trace metals in the aquatic environment is a worldwide threat due to their abundance, toxicity, persistence and their resultant accumulation in aquatic ecosystems [3,4,5,6,7,8]. The heavy metal pollution has dreadful effects on the environmental equilibrium and a variety of aquatic entities [9,10]. In the list of animal species, detrimental effects of these pollutants, can never be negligible for fishes [11,12]. Fishing is a also general pastime [13,14,15] including in urban areas [16].

However, fish are also sometimes a major contributor to exposure to certain environmental contaminants [17,18]. Metallic trace elements (ETM), such as, cadmium lead and persistent organic pollutants (Dioxins, Polychlorobiphenyls, Polybromodiphenylethers, etc.) represent a group of toxic substances that accumulate in the tissues of fish and transfer along from the food chain to humans [19].

Fish are important organisms of the aquatic ecosystem because they are one of the largest animals inhabiting this environment while at the same time, occupying different levels of the trophic pyramid [20,21]. In addition, fish are crucial for the management of water resources (fishery management) and recreational use [22]. Amateur-caught fishes

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are valuable fishing trophies and are very often used as cuisine, being part of the human diet and a valuable source of protein [23,24]. This phenomenon applies especially to locally occurring reservoirs, where local peoples often use the small reservoirs to obtain fish. Due to the diversity of nutritional requirements, food selectivity, and food availability of various fish species, their nutrition is closely related to their living environment [25,26]. In this regard, heavy metals have the tendency to accumulate in various organs of aquatic organisms, especially in fish which in turn may enter into the human metabolism through consumption which cause serious health problems [27]. Metals such as Zn, Fe, Cu and Mn are essential elements. They may produce toxic effects when their levels exceed certain limits in organisms [28]. Nwaedozie [29] reported that Zn contamination affects the hepatic distribution of other trace metals in fish. Zn, Cu and Mn, which are essential elements, compete for the same site in animals. This will undoubtedly affect tissue metals concentrations as well as certain important physiological processes. Therefore, many consumers regard any presence of these metals in fish as a hazard to health. Fish are integral component of human diet. They need to be carefully screened to ensure that unnecessarily high levels of heavy metals will not be transferred to human population through consumption of contaminated fish [30].

The aim of our study is therefore interested in the evaluation of the content in seven metallic elements (Cd, Pb, Fe, Cu, Zn, Ni and Cr) in the samples of *Sardina pilchardus,* gathered from the Mediterranean coast in the North East of Morocco, landed in the port of Nador during the autumn and spring of the years 2019-2020. We have retained this species because they are mostly consumed by the population and perhaps these species can be considered as a sentinel organism and bio-indicator of pollution in this area.

Figure 1 Location of the studied site (Google maps)

2. Material and methods

2.1. Fish sampling

The samples of *Sardina pilchardus,* were selected as bioindicator; because these specie is widely consumed by Moroccans. The individuals of these specie were taken at the level of Mediterranean Sea (port of Nador) (Figure 1). The studied site extends over a coastline of 153 km [31]. This site is influenced by freshwater flowing watershed of wadis especially in period of floods. Inter alia, the province of Nador accounts 170 industrial units. The ventilation by branch reveals a predominance of the chemical and para-chemical industry with 58 units, followed by the agrifood industry, mechanical, metallurgical industry, textile, leather and electrical industry, which generate industrial waste loaded with chemical products.

2.2. Preparation and treatment of samples

The samples of *Sardina pilchardus,* were obtained by the anglers from when they landed. Three individuals per sample were stored in plastic bags and conserved at -20 °C. To avoid any contamination by the environment or the sampling equipment, the procedures of sampling were performed according to the procedure described in the Aminot manual [32]. In the laboratory, the samples of *Sardina pilchardus*, were dissected. We studied the three parts of these species: The liver, the gills and the muscle, were taken separately and then dried at 80 °C to constant weight. Then they were finely grounded using an agate mortar to avoid any external contamination by heavy metals. A quantity varying between

0.5 g and 1 g of dry weight of the biological material was used for analysis of the following metals: lead, copper, zinc, iron, cadmium, chromium, and nickel. The mineralization was realized in two steps [33]: Calcination at 550 °C for 4h followed by an acid treatment at ambient temperature overnight, followed by digestion at 60 °C for 2 h. Then the samples were subjected to membrane. filtration "Millipore" 0.45 microns of porosity. The filtrate obtained is diluted with ultrapure water, and then the samples are stored in polypropylene bottles at 4 °C until the analysis. The metallic elements studied were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

2.3. Quality control of the results

To take account of the matrix effect that can sometimes induce important analytical errors, reference materials (SRM: NIST 1566b) were used for the calibration of the measures. These samples were treated in the same conditions as our samples. Control samples were used in parallels. The results of the recovery percentages of the four metallic elements in the reference materials used are presented in Table 1.

Table 1 Recovery of various heavy metals of certified reference materials (CRMs)

NIST 1566 (CRM : oyster tissue)		
	Target	% Recovery
Cadmium	4.2 ± 0.4	107
Copper	66 ± 4	97
Zinc	830 ± 57	95
Iron	921 ± 59	89
Lead	0.35 ± 0.13	102.86
Chromium	0.77±1.18	96.97
Nickel	2.50±0.19	85.20

SD-M-2 / TM (CRM: marine sediment); NIST 1566 (CRM: oyster tissue); (mg/g dry weight)

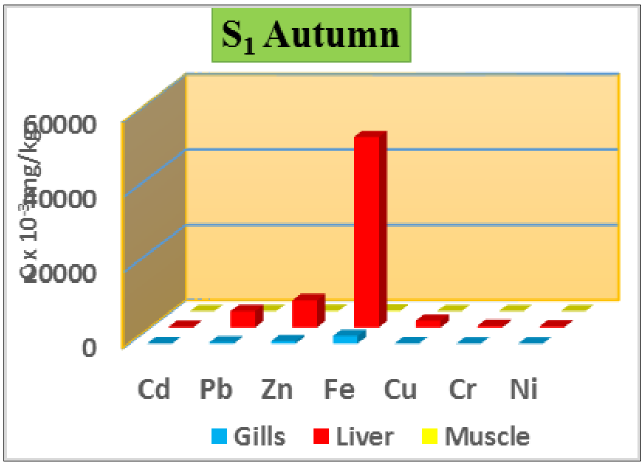
2.4. Statistical analyzes

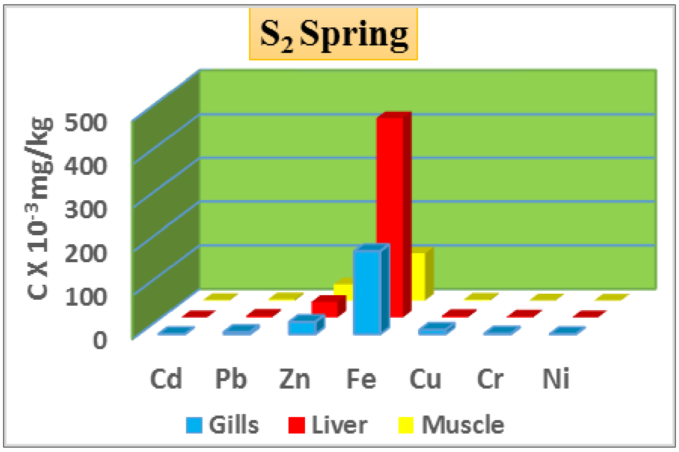
The processing of all data by Principal Component Analysis (PCA) was made by the software XL-STAT 2018.

The analysis of the coefficient of variation to compare the means of the levels of heavy metals and to determine the significant differences between the organs of the different species studied, as well as the means and the standard deviations were made by the software SPSS 17.0.

3. Results and discussion

3.1. Seasonal variations in metal contents in *Sardina pilchardus*





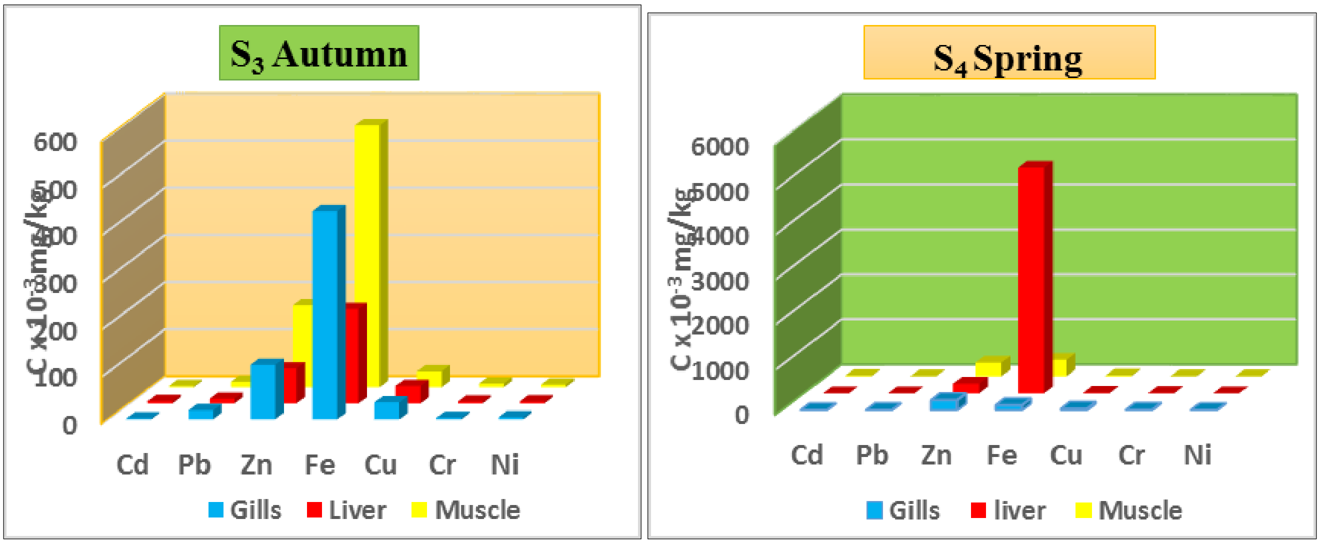


Figure 2 Seasonal variations in metal levels in the various organs studied in *Sardina pilchardus* in mg/kg

The accumulation of heavy metals (Cd, Pb, Zn, Fe, Cu, Cr and Ni) in the different organs (Gills, liver and muscle) of *Sardina pilchardus* of the Nador Sea in the four seasons studied between 2019 and 2020, analyzed according to the techniques described above are represented in the table and the figures below. They are expressed in milligrams of the test element per kilogram of dry matter (mg/kg DM).

Table 2 Seasonal variations in metal levels in the various organs studied in *Sardina pilchardus* of the Mediterranean Sea in mg/kg

ML	***S. Pichardus***	**S1 Autumn**	**S2 Spring**	**S3 Autumn**	**S4 Spring**
C(Cd) × 10-3	Gills	4.88±0.003	0.37±0.0001	2.09±0.001	3.88±0.0004
	Liver	7.74±0.004	0.60±0.0004	5.15±0.003	3.24±0.0009
	Muscle	1.83±0.001	1.63±0.002	2.69±0.001	13.60±0.01
	CV%	61.36	77.42	48.98	84.05
C(Pb) × 10-3	Gills	88.86±0.05	5±0.008	19.44±0.01	4.58±0.003
	Liver	89.44±0.1	3.93±0.006	10.27±0.008	3.24±0.0009
	Muscle	125.29±0.07	3.18±0.004	11.80±0.01	4.70±0.002
	CV%	20.62	22.65	35.50	19.42
C(Zn) × 10-3	Gills	8667.6±6.29	28.21±0.01	115.67±0.02	223.17±0.14
	Liver	1653.9±0.65	35.74±0.006	73.87±0.07	214.49±0.15
	Muscle	2957.8±2.55	37.77±0.0005	174.18±0.03	302.91±0.003
	CV%	84.27	13.10	41.55	19.74
C(Fe) × 10-3	Gills	1951.9±1.42	190.82±0.3	441.74±0.01	113.37±0.05
	Liver	1942.9±1.72	456.42±0.3	199.99±0.05	5007.27±0.7
	Muscle	1404.5±0.64	108.83±0.007	556.37±0.08	367.56±0.2
	CV%	17.74	72.09	45.55	150.59
C(Cu) × 10-3	Gills	39.08±0.03	10.03±0.003	36.31±0.0005	35.10±0.05
	Liver	58.28±0.07	3.49±0.001	36.75±0.004	13.38±0.01

	Muscle	38.30±0.01	2.51±0.001	34.26±0.004	18.74±0.01
	CV%	25.02	76.51	3.71	50.49
C(Cr) × 10-3	Gills	6.22±0.0006	1.42±0.001	3.44±0.0008	11.38±0.01
	Liver	13.43±0.005	2.04±0.001	4.11±0.002	9.85±0.01
	Muscle	9.95±0.008	0.58±0.0006	7.77±0.009	4.44±0.0004
	CV%	36.54	54.41	45.64	42.61
C(Ni) × 10-3	Gills	17.77±0.01	0.32±0.0002	4.59±0.003	4.22±0.003
	Liver	8.36±0.005	0.4±0.0001	4.31±0.001	2.16±0.001
	Muscle	2.19±0.0009	0.41±0.0002	5.61±0.003	3.30±0.0008
	CV%	83.11	13.09	14.14	31.98

3.1.1. Cadmium (Cd)

Cadmium concentrations in the various organs studied in *Sardina pilchardus* are generally low, such that the highest value is of the order of 0.0136 mg/kg obtained in the muscle of *Sardina Pilchardus* during the second spring season studied, while the lowest content is of the order of 0.00037 mg/kg obtained in the gills of the species studied during the first spring season. Metal concentrations in *Sardina Pilchardus* during the two spring seasons were lower than those in the two autumn seasons. Cd concentrations in the species studied during all seasons are well below the Word Health Organization maximum allowable limit of 0.3 mg/kg.

3.1.2. Lead (Pb)

The average lead levels recorded in the various organs of *Sardina Pilchardus* show that the highest value is of the order of 0.125 mg/kg obtained in the muscle of *Sardina Pilchardus* during the first autumn season. While the lowest level is in the order of 0.0031 mg/kg obtained in the muscle during the first spring season. Pb concentrations in the species studied during all seasons are well below the WHO maximum allowable limit of 0.3 mg/kg.

3.1.3. Zinc (Zn)

Overall, the accumulation of Zinc in *Sardina Pilchardus* shows a significant difference between the first autumn season and the other seasons studied such that the highest concentration is observed in the gills of the species studied in the first autumn period of 8.667 mg/kg and the lowest concentration is 0.0282 mg/kg recorded in the gills during the first spring season.

3.1.4. Iron (Fe)

Fe levels in the species studied show that the highest value is of the order of 5.007 mg/kg obtained in the liver in the second spring season, While the lowest value is of the order of 0.108 mg/kg obtained in the muscle of *Sardina Pilchardus* caught in the first spring season. The results show that the iron values in *S. Pilchardus* exceed the WHO standard of 0.5 mg/kg by a value of 5.007 mg/kg in the liver during the second spring season.

3.1.5. Copper (Cu)

The copper contents in the various organs studied of *S. Pilchardus* are generally low such that the highest value is of the order of 0.0582 mg/kg obtained in the liver of *S. Pilchardus* caught during the first autumn season. The lowest concentration is in the range of 0.0005 mg/kg obtained in the muscle of the species studied during the first spring season.Cu levels in *S. Pilchardus* are below the WHO standard of 30 mg/kg.

3.1.6. Chromium (Cr)

The mean chromium levels recorded in the various organs of *S. Pilchardus* show that the highest value is of the order of 0.013 mg/kg obtained in the liver of *S. Pilchardus* during the first autumn season. The lowest concentration is in the order of 0.0013 mg/kg in the gills and muscle of the species studied during the first spring season. Cr levels in *S. Pilchardus* are below the WHO standard of 0.6 mg/kg.

3.1.7. Nickel (Ni)

Nickel levels in the various organs studied in *S. Pilchardus* are generally low, such that the highest value is of the order of 0.0177 mg/kg obtained in the gills of *S. Pilchardus* during the first autumn season studied, whereas the lowest content is of the order of 0.0003 mg/kg obtained in the gills of the species studied during the first spring season. In addition, Ni levels in the species studied during all seasons are well below the maximum permissible limit set by the WHO, which is in the order of 1 mg/kg.

Based on these results, we can establish the organ accumulation orders of the *S. Pilchardus* species for each metal studied as follows:

Table 3 Organ accumulation order of the species *Sardina pilchardus*for the metal elements studied

Metal elements	**Order of accumulation**
Cd	Muscle> Liver>Gills
Fe	Liver > Gills > Muscle
Cr	Liver > Muscle > Gills
Ni, Cu	Gills > Liver > Muscle
Zn	Gills > Muscle > Liver
Pb	Muscle > Branches > Liver

Similarly, we can establish for each organ an accumulation order (Table 4) for the metallic elements studied in this species

Table 4 The order of accumulation of the metallic elements studied in the various organs of *Sardina Pilchardus*

Organs	**Metal elements**
Gills	Zn> Fe>Cu>Pb >Ni> Cr> Cd
Liver	Fe>Zn>Cu>Pb>Cr>Cd>Ni
Muscle	Zn>Fe>Pb>Cu>Cr>Cd>Ni

3.2. Principal Component Analysis (PCA) of Metal Contamination at *Sardina pilchardus*

This PCA is carried out on a matrix of data consisting of (3 organs: Gills, liver and Muscle X 4 campaigns: S_1: autumn 2019, S_2: Spring 2019, S_3; Autumn 2020, S_4: Spring 2020) in which the 7 variables (Cd, Pb, Zn, Cu, Fe, Cr, Ni) were measured. The proper values of the two components F1 and F2 and their contribution to total inertia are shown in Table 5 and Figure 3

Table 5 Distribution of the inertia of the ETM between the two axes F1XF2 at *Sardina pilchardus*

	F1	**F2**
Proper value	3.428	1.168
Variability (%)	48.978	16.679
Cumulative %	48.978	65.657

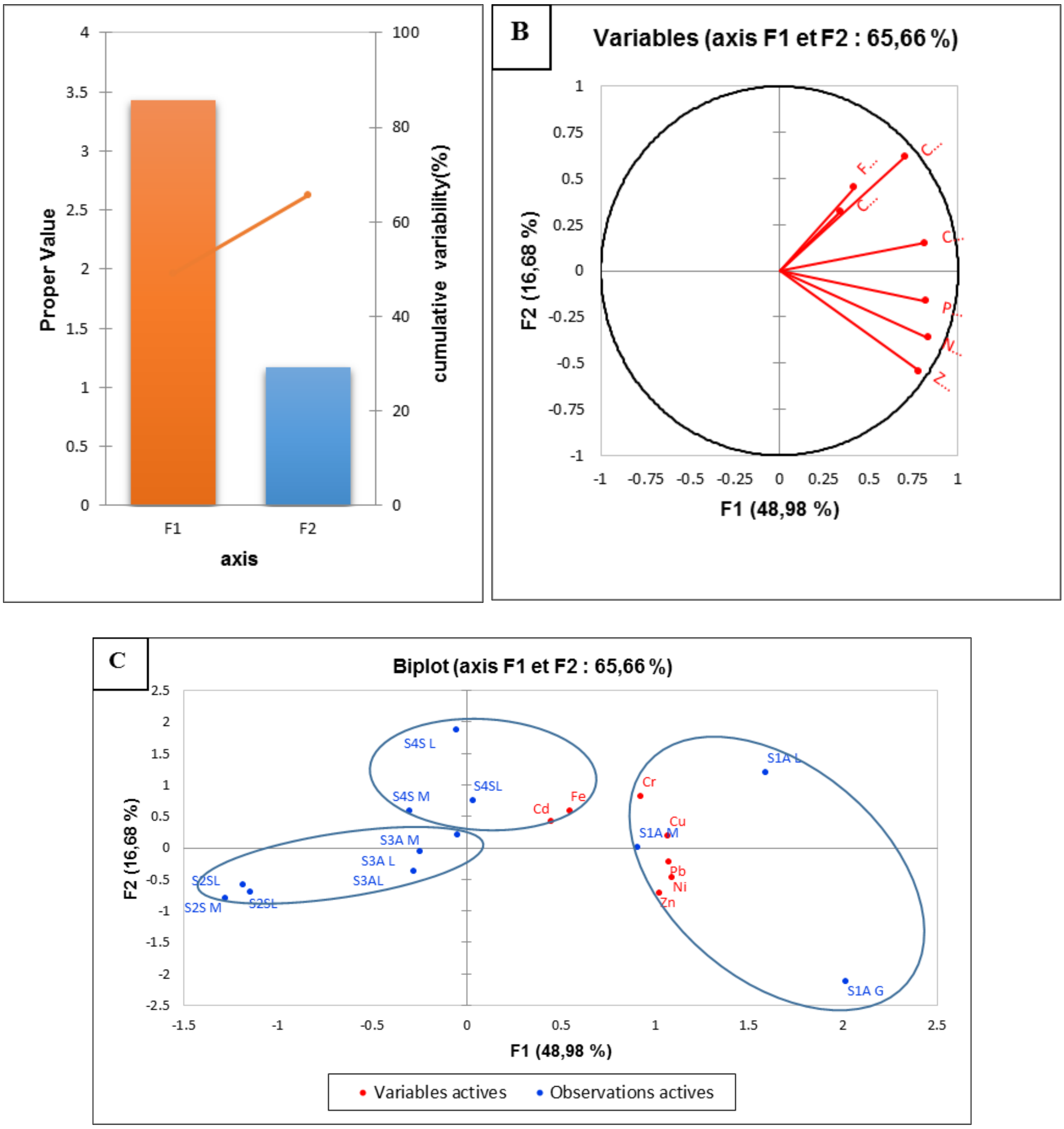


Figure 3 Graphical representation of the PCA analysis of the TA-M: A: Distribution of inertia between the two axes (F1XF2). -B: circle of correlations of the 7 variables in the factorial plane F1 xF2 of the PCA. C: Factorial map of seasons and organs

The two axes taken into consideration to describe the correlations between the variables related to spatial structures alone hold 65.66% of the total information with respectively 48.98% for axis 1 and 16.68% for axis 2.

In order to establish two-to-two links between the different metals in *Sardina pilchardus* tissues, correlation coefficients were calculated. Table 6 shows the values of the correlation coefficients founded.

Examination of the matrix of correlation between variables (Table 6) reveals the presence of: A first set of variables, consisting of descriptors well correlated with each other, is:

- {Pb/Zn (r=0.706), Pb/Cu (r=0.641)}. (Significant correlations)
- {Zn/Ni (r = 0.855)}. (Very significant correlation)
- {Cu/Cr (r = 0.699), Cu/Ni (r = 0.625)}. (Significant correlations)

Table 6 Correlation matrix (Pearson (n)) between metallic element variables at *Sardina pilchardus*

Variables	**Cd**	**Pb**	**Zn**	**Fe**	**Cu**	**Cr**	**Or**
Cd	1						
Pb	0.064	1					
Zn	0.096	0.706	1				
Fe	0.037	0.249	0.263	1			
Cu	0.311	0.641	0.389	0.079	1		
Cr	0.259	0.506	0.203	0.504	0.699	1	
Or	0.299	0.516	0.855	0.211	0.625	0.329	1

Values in bold are significantly different from 0 at a significance level alpha=0.05

In the factorial plane F1XF2 (FIG.3), the 1st component (axis F1) contributes with 48.98% inertia, partially describes the contamination of *Sardina pilchardus* with Ni (0.833), Pb(0.818), Cu(0.816), Zn(0.780), Cr(0.707), Cd(0.341).With an inertia of 16.67% the second component (axis F2) describes the enrichment of *Sardina pilchardus* to Fe (0.452).

From left to right, the F1 axis thus reflects an increasing gradient of contamination of *Sardina pilchardus* in Ni, Pb, Cu, Zn, Cr and Cd. From the bottom upwards, the F2 axis thus reflects an increasing gradient of contamination of *Sardina pilchardus* by Fe.

The examination of Figure.3 shows the variability between the organs of *Sardina pilchardus* during the four seasons studied. In fact, the typological structure revealed by the F1XF2 plan shows the individualization of three zones according to their degree of contamination:

- ✓ The first zone is formed by the first autumn season (S_1Autumn Gills, S_1A Liver, S_1A Muscle), which is characterized by lead, zinc, chromium, copper and nickel contamination
- ✓ The second zone is formed by the fourth spring season (S_4Spring gills, S_4 Spring Liver, S_4 Spring Gills), which is characterized by iron and cadmium contamination.
- ✓ The third zone is formed by the second spring season and the third autumn season (S_2.S.G, S_2.S.L, S_2.S.M, S_3.A.G, S_3.A.L, S_3.A.M), which is characterized by very low contamination of metallic elements studied.

3.3. Comparison of our results with the literature:

The study of heavy metal contamination in the various organs of fishery products has been the subject of several studies.

The table below shows the heavy metal contents obtained by some authors in some species of the Mediterranean Sea

Table 7 Metal contents obtained by some authors in some fish species

	Metals **Cd Pb Zn Cu Fe Cr Ni**	**Authors**
Paracentrotuslividus	*0.1 20 130 4.2 132.2*	*Demnati [34]*
Sardinapilchardus	0.1 0.2 34 5.17 117.3 0.05	El Morhit [35]
Diplodus Vulgaris	0.19 0.23 52 3.31 86.62 0.09	
Sardinellabrasiliensis	0.009 0.3 2.9	Medeiros [36]
Caranx sp. (Shalateen)	8.37 246.15 5.9 376.37	El-Moselhy [37]
Paracentrotuslividus	0.004 0.07 0.08 0.72	Fahssi [38]

By comparing our results with the results of other authors who have worked on other species or the same species as *Sardina pilchardus* of the Mediterranean Sea we can deduce that our results are much lower than those found by some authors such as [37,39] and similar to those found by [35,36].

We can conclude that heavy metal levels in *Sardina pilchardus* studied are below the World Health Organization and food codex standards for Cd, Cu, Zn, Ni and Cr, but this does not prevent traces of these in the fish studied. In addition, there is a high level of iron contamination in all the species studied and this may be due to industrial and agricultural discharges from the city of Nador which are loaded with iron and which are discharged directly into the sea, even though the city of Nador now has a sewage treatment plant but the latter does not take into account the elimination of heavy metals in these stages of treatment of these effluents.

For seasonal variations, we noted that most high levels are recorded during the spring season. This may be due to weather influences. Some authors [40], have hypothesized that the bioaccumulation of heavy metals changes over time as a function of the seasons and also as a function of the organisms studied. Physical factors such as temperature, salinity, dissolved oxygen, currents and water turnover, which play a role in the retention of toxic substances by organisms, must also be taken into account [41].

4. Conclusion

The values reported in this study can serve as baseline data to monitor future anthropogenic activities along the coast, these results show that the concentrations of Cd, Pb, Zn, Cu, Ni, Cr in the samples of *Sardinapilchardus*are below the norm except for Fe in the samples which exceeds a little the standard set by the WHO. However, it should be noted that the concept of bioaccumulation result of several mechanisms, acting simultaneously or with a time lag. Bioaccumulation, for the same chemical products can vary considerably depending on the species, stage of development of individuals, habitat characteristics (pH, salinity and temperature), sex, diet, properties of the contaminant. Even if the levels of bioaccumulation are not yet very critical, other monitoring programs should be conducted. Moreover, the results of this work can also be used to understand the chemical quality of fishes and to evaluate the possible risk associated with their consumptions.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors agree no conflict of interest.

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