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Bioaccumulation of heavy metals in commercially important marine fishes (*Palaemon Serratus* and *Solea Vulgaris*) caught in the Mediterranean coast from the North East of Morocco

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ABSTRACT

To conduct a health risk assessment of some heavy metals attributed to consumption of common edible fish species available for consumers. Concentrations of Cd, Pb, Cu, Fe, Zn, Ni and Cr were determined in muscles, gills and livers, of two common edible fish species, namely *Palaemon Serratus* and *Solea Vulgaris*. Concentrations of heavy metals were determined by inductively coupled plasma- atomic emission spectroscopy (ICP-AES) and expressed as mg/kg of wet tissue. Results showed that iron and zinc were the most abundant among all fish tissues under investigation. The data obtained in the present work were compared well with the counterpart data reported internationally. The estimated values of all metals in muscles of fish in this study were below the permissible limits. Generally, risk values for the measured metals do not pose unacceptable risks at mean ingestion rate for muscles. It can be concluded that the investigated metals in edible parts of the examined species have no health problems for consumers.

Keywords: Heavy metals, bioaccumulation, *Palaemon Serratus*, *Solea Vulgaris*, contamination, Mediterranean Sea.

INTRODUCTION

Anthropogenic activities continuously increase the amount of heavy metals in the environment, especially in aquatic ecosystem. Pollution of heavy metals in aquatic ecosystem is growing at an alarming rate and has become an important worldwide problem. [1] Increase in population, urbanization, industrialization and agriculture practices have further aggravated the situation [2,3]. As heavy metals cannot be degraded, they are deposited, assimilated or incorporated in water, sediment and aquatic animals [4] and thus, causing heavy metal pollution in water bodies [1]. Therefore, heavy metals can be bioaccumulated and biomagnified via the food chain and finally assimilated by human consumers resulting in health risks [5]. Consequently, fish are often used as indicators of heavy metals contamination in the aquatic ecosystem because they occupy high trophic levels and are important food source [5]. In addition to its important source of protein, fish typically have rich contents of essential minerals, vitamins and unsaturated fatty acids [6]. However, fish are relatively situated at the top of the aquatic food chain; therefore, they normally can accumulate heavy metals from food, water and sediments [7]. The content of toxic heavy metals in fish can counteract their beneficial effects; several adverse effects of heavy metals to human health have been known for long time [8]. This may include serious threats like renal failure, liver damage, cardiovascular diseases and even death [9]. Therefore, many international monitoring programs have been established in order to assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystem [10]. In the last few decades, the

concentrations of heavy metals in fish have been extensively studied in different parts of the world [11]. Most of these studies concentrated mainly on the heavy metals in the edible part (fish muscles). Nevertheless, other studies reported the distribution of metals in different organs like the liver, kidneys, heart, gonads, bone, digestive tract and brain. According to the literatures, metal bioaccumulation by fish and subsequent distribution in organs is greatly inter-specific. In addition, many factors can influence metal uptake like sex, age, size, reproductive cycle, swimming patterns, feeding behavior and living environment (i.e., geographical location)[12].

In Morocco, there is not enough studies on the heavy metal content in the *Palaemon Serratus* and *Solea Vulgaris*. Consequently, it was necessary to provide with advantage of data on the concentrations of these metals in edible tissues of these species to better assess the risk linked with the consumption of this commodity. 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 *Palaemon Serratus* and *Solea Vulgaris* in the Mediterranean, landed in the port of Nador during the autumn of the year 2014. We have retained this species also as a sentinel organism and bio-indicator of pollution in this area.

MATERIALS AND METHODS

Fish sampling

The *Palaemon Serratus* and *Solea Vulgaris* have been retained as bioindicator in this study to his wide commercial exploitation in the fishing sector in Morocco. The individuals of *Palaemon Serratus* and *Solea Vulgaris* were taken at the level of Mediterranean Sea (port of Nador) (Figure 1).

The studied site extends over a coastline of 153 km [13]. 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 allows releasing a clear 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.

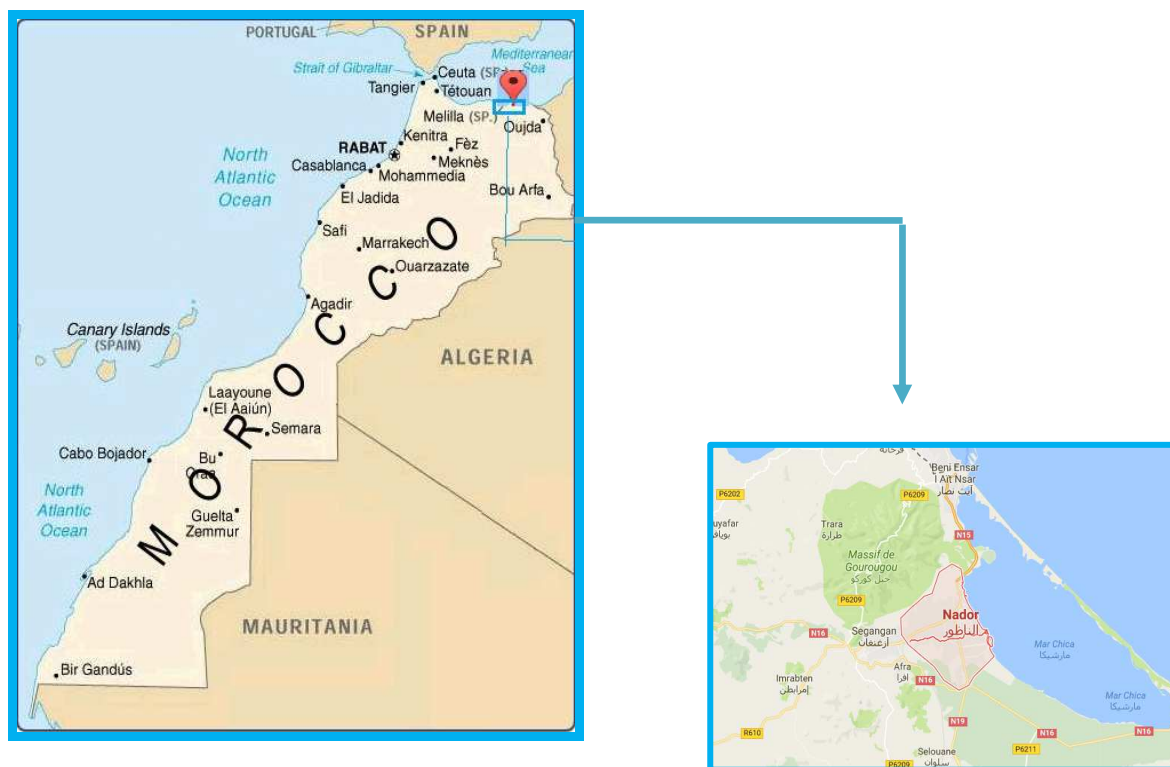


Figure 1: Location of the studied site (Google maps)

Preparation and treatment of samples

The samples of *Palaemon Serratus* and *Solea Vulgaris* 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 precautions described in the manual Aminot [14].

In the laboratory, the samples of *Palaemon Serratus* and *Solea Vulgaris* were dissected. On each individuals of sole, three parts: The liver, the gills and the muscle, were taken separately and then dried at 80 °C to constant weight. Then they are finely ground 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 [15]: Calcination at 550 °C during 4 hours followed by an acid attack at ambient temperature overnight, followed by digestion at 60 °C for 2 hours. Then we have preceded to membrane filtration "Millipore" 0.45 microns of porosity. The filtrate obtained is completed with ultrapure water to a volume of 25 mL, and then stored in piluliers in polypropylene at 4 °C until analysis. The metallic elements studied were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

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. White 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 (MRC : oyster tissue)	
	Target	% Recouv
Cadmium	4.2 ± 0.4	107
Copper	66 ± 4	97
Zinc	830 ± 57	95
Iron	921 ± 59	89

SD-M-2 / TM (MRC: marine sediment); NIST 1566 (MRC: oyster tissue); (Mg/g dry weight).

Statistical Analysis

All data generated were analyzed statistically by calculating the mean and standard deviation of the measured parameters. The coefficient of variation and bio concentration factors of the metals were also calculated.

RESULTS AND DISCUSSION

The Accumulation of heavy metals (Cd, Pb, Fe, Cu, Zn, Ni and Cr) in the samples of gills, liver and the muscle of the *Solea Vulgaris* and the samples of muscle of the *Palaemon Serratus* in the Mediterranean during the autumn of the year 2014, analyzed according to the techniques previously described are represented in the table and figures below. The results are expressed in milligrams of the element studied per gram of dry matter (mg/kg DM). Thus, each result represents the average of three trials realized in the same experimental conditions.

Table 2: Heavy metal concentration in the tissues of the fish species (*Palaemon Serratus* and *Solea Vulgaris* in mg/kg of the dry matter

	C (Cd) × 10 ⁻³	C (Pb) × 10 ⁻³	C (Zn) × 10 ⁻³	C (Cu) × 10 ⁻³	C (Fe) × 10 ⁻³	C (Ni) × 10 ⁻³	C (Cr) × 10 ⁻³
<i>Palaemon Serratus</i> Muscle	0.71 ± 0.001	98.80±0.05	329.39 ±0.06	46.62±0.02	354.52 ± 0.22	1.56 ± 0.0001	0.97±0.0007
<i>Solea Vulgaris</i> Gills	4.45 ± 0.001	62.03±0.03	116.11±0.05	89.32±0.08	596.37±0.48	235.24±0,08	33.65±0.05
Liver	13.32±0.01	72.58±0.05	206 ± 0.11	24.09 ±0.01	98.85	43.05± 0.04	14.68 ±0.01
Muscle	2.48±0.0007	60.65±0.05	223.70±0.13	14 ± 0.008	2.69 ± 0.0008	1.94±0.0008	2.30±0.0008
CV%	85.54	10.02	31.70	96.26	0.62±0.001	46.22	35.11
Maximum limit WHO/FEPA (mg/kg)	0.3 mg/kg	0.3mg/kg	30 mg/kg	3 mg/kg	0.5 mg/kg	0.6	1

Palaemon Serratus

Cadmium (Cd), Lead (Pb), Zinc (Zn), Copper (Cu), Chromium (Cr), Iron, (Fe) and Nickel (Ni) were analyzed and the levels of the heavy metals concentration were measured in the muscle of the *Palaemon Serratus*. The results of the analysis are presented in Table 2 and further illustrated in Figure 2. Data on heavy metal concentration in the muscle of *Palaemon Serratus* showed that heavy metals were accumulated in this species as follows: Iron (Fe), Zinc (Zn) , Lead (Pb), Copper (Cu), Nickel (Ni), Chromium (Cr) and Cadmium (Cd)

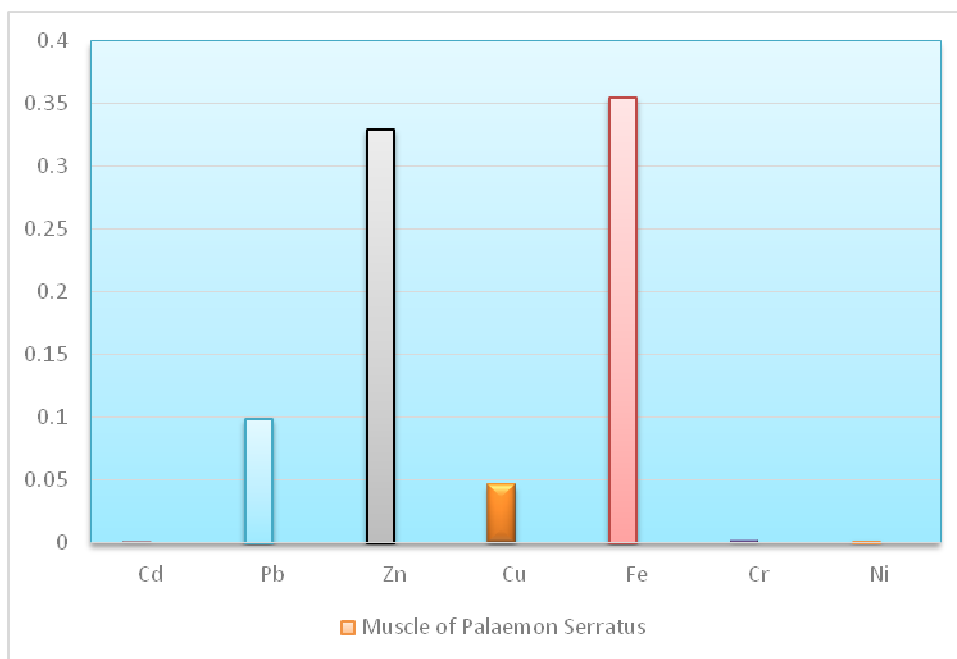


Figure 2: Distribution of Iron (Fe), Zinc (Zn), Lead (Pb), Copper (Cu), Cadmium (Cd), Chromium (Cr) and Nickel (Ni) in mg/kg in the muscle of *Palaemon Serratus* from the Mediterranean Sea

The results of this study indicated that concentrations of Iron and Zinc had the highest concentrations in the muscle of *Palaemon Serratus*, such as their average concentration was 0.354 and 0.329 mg/kg respectively. Lead and Copper had the lowest concentrations (0.098 and 0.046 mg/kg), Cadmium, Chromium and Nickel had very negligible concentrations. thus the comparison of the concentrations registered in these species studied shows that the concentrations of trace elements (Fe, Zn, Cu) are higher compared to those of toxic elements (Cr, Ni and Cd). The contamination of aquatic systems with a wide range of pollutants has become a matter of concern over the last few decades [16]. Aquatic animals, part of the food chain, can be contaminated by heavy metals [17]. The toxicity and capacity of heavy metal accumulation in the biota are serious problems for human health and ecosystem safety. We found that the concentrations of Fe are higher than other metals (Zn, Pb, Cu, Ni, Cd, and Cr) in *Palaemon Serratus*, which these results are similar to [18]. Different aquatic organisms from other marine environments of the world have also been exposed to heavy metals. [19,20]. Variations in heavy metal concentrations among the samples could have been dependent on factors such as size categories, ecological zones, and trophic levels [21]. The concentrations of heavy metal may be dependent on species, feeding habits, the bioconcentration capacity of each species, or ecological zone [22]. In many studies, increasing amounts of heavy metal accumulation in aquatic organisms have been linked to urbanization, agriculture, oil activities, and anthropogenic sources. The lowest Fe, Zn and Pb, concentrations can be due to the presence of major industries such as SONASID and SOFRENOR in the Nador city.

Comparing the present data with guidelines and limits (Table 2), it can be seen that most of metal concentrations found in the tissues of aquatic animals proved to be below the tolerance levels for human consumption. According to many researchers, some shellfishes by virtue of their mobile nature are not fair indicator of aquatic contamination, but their regular consumption by human beings makes it necessary to monitor their different organs, particularly the muscles. The present study is therefore important not only from the safety point of view of human health, but also from the quality point of view as many of these shellfish species have high export value.

Solea Vulgaris

This paper presents the assessment outcome of Cd, Cr, Cu, Fe, Ni, Pb and Zn levels in muscle, gills and liver, of the *Solea Vulgaris* from the Mediterranean Sea. Concentrations of heavy metals under investigation are presented in Figure 3. There were differences in the concentrations of the studied metals between different organs. Fe, Zn and Pb were the most abundant in all the examined organs. In the liver samples, Fe > Zn > Pb > Cu > Cd > Ni > Cr; in the gills, Fe > Zn > Cu > Pb > Ni > Cr > Cd; in the muscles samples, Zn > Pb > Fe > Cu > Cd > Ni > Cr.

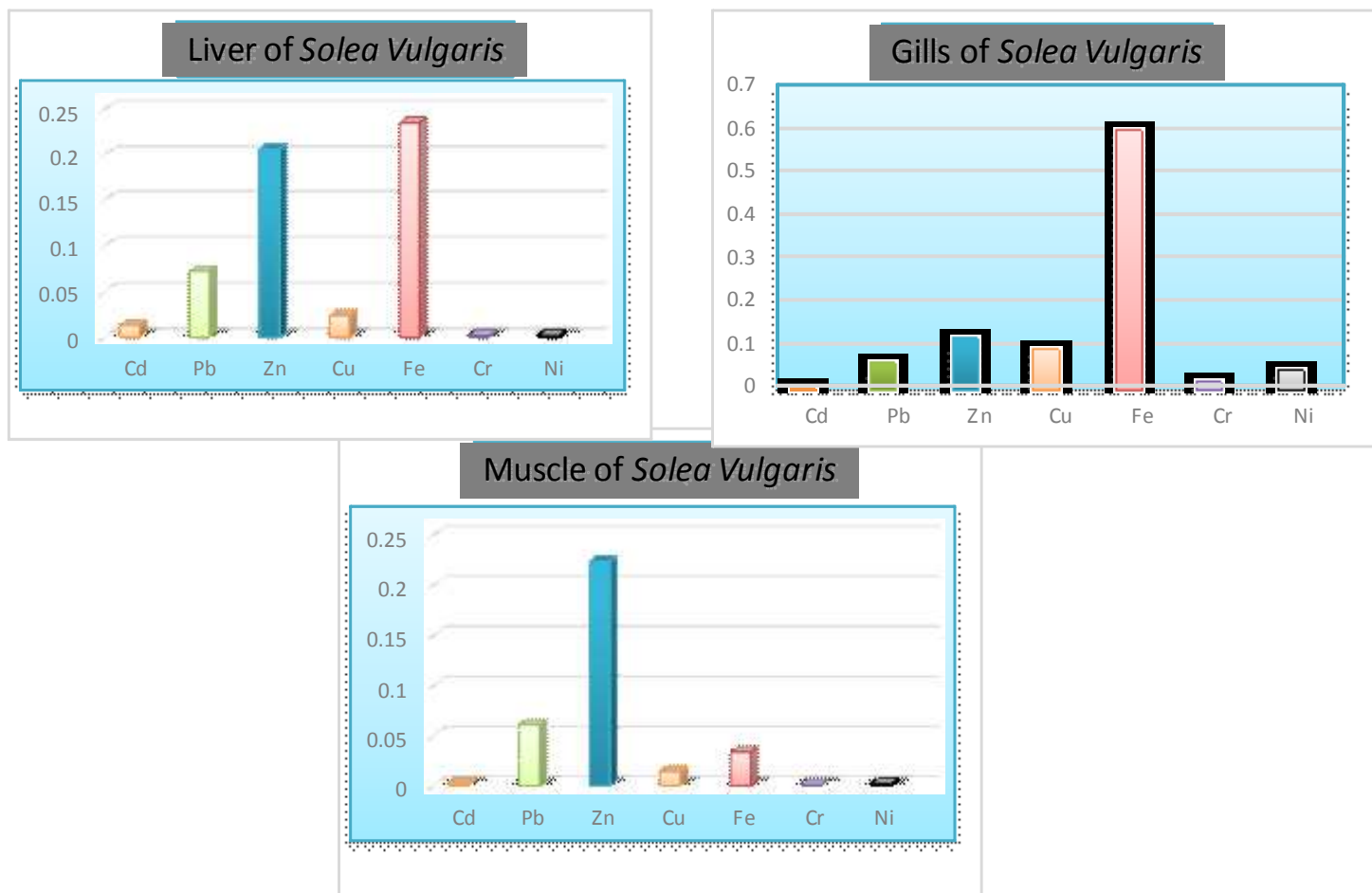


Figure 3: Distribution of Iron (Fe), Zinc (Zn), Lead (Pb), Copper (Cu), Cadmium (Cd), Chromium (Cr) and Nickel (Ni) in mg/kg in the muscle, gills and liver of *Solea Vulgaris* from the Mediterranean Sea

- *The contents of Cd:*

Cd is widely known to be a highly toxic non-essential heavy element. It doesn't have an essential role in biological process in living organisms. Thus, even at its low concentration, Cd could be harmful to living organisms [23]. The distribution pattern of Cd in the present study was the decreasing order of liver > gills > muscle. The liver seemed to be the organ, which accumulates the highest level of Cd. This is in agreement with WHO-IPCS Environmental Criteria of Cd [24], which reported that Cd is stored in the body in various tissues. The main site of accumulation of Cd in aquatic organisms is the kidney and liver [25]. Mean levels of Cd in the muscles was relatively lower than the standard set by the WHO 0.3 mg/kg and the permissible limits suggested by Food and Agriculture Organization (FAO)(0.5 µg/g) [26]. Cd levels in the present study were generally in similar ranges with the literature, where it has been reported as 0.02-0.24 µg/g for muscles of fish from the Black Sea coasts [27]; 0.3-0.12 µg/g for muscles and 0.02-0.35 µg/g for livers of fish from the Tuzla Lagoon, Mediterranean Sea region [28]; 0.13-0.47 µg/g for livers of fish from the Marmara, Aegean and Mediterranean Seas [29]; 0.09-0.27, 0.30-1.49, 0.27-0.66 µg/g and 0.03-0.52, 0.07-0.41, 0.07-0.10 µg/g for muscle, liver and gill tissues respectively for fish from the Egyptian Mediterranean Sea [30,31].

- *The contents of Pb:*

Pb is a non-essential metal and a toxic element, which can affect fish in high doses, lead to a decrease in survival, growth rates, development and metabolism and to the increased mucus formation. Pb may have many adverse health effects including neurotoxicity and nephrotoxicity [32]. This is due to the fact that Pb and Ca are similar in deposition and in mobilization from bones as previously reported by Moore and Ramamoorthy [33]. The distribution pattern of Pb in the present study was the decreasing order of liver > gills > muscle. In this study, lead levels were below the recommended limits of 0.3 mg/kg for fish food. Lead levels in the literature have been reported in the range of 0.22-0.85 µg/g for fish from the middle Black Sea [34]; 0.33-0.93 µg/g for fish from the Black and Aegean Seas for muscles [35]; 1.41-3.92 µg/g, 0.38-5.20 µg/g, 0.83-3.71 µg/g for fish livers [28,29]. The average concentration of Pb in the muscles measured in this study was considerable lower than the Turkish food codex (0.2 µg/g), the European commission (0.3 µg/g) and the Saudi Arabia Standards Organization (2 µg/g) [36].

- *The contents of Zn:*

Zn is one of the most important trace elements for normal growth and development of humans. Its deficiency results from inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in Zn metabolism [37]. Moreover, Zn is also considered as highly bioavailable in the aquatic environment and thus may exhibit higher accumulation in various tissues [7]. The distribution pattern of Zn in the present study was the decreasing order of muscle > liver > gills. Average concentrations of Zn in the muscle of *Solea Vulgaris* measured was within the permissible limits suggested by FAO (30 µg/g) [26], Saudi Arabian allowable limits (50 µg/g). These amounts of Zn in the muscle cannot cause harm to the fish themselves as well as consumers. Zn concentrations in the samples were compared well to earlier reports on the fish species from the Black Sea coasts with 9.5-22.9 µg/g for muscles [27], 9.83-195 µg/g for livers of fish from Turkish seas [38], 21.7-99.8 µg/g for livers of fish from Tuzla Lagoon, Mediterranean sea region [28], 26.2-43.5 µg/g for livers of fish from Marmara, Aegean and Mediterranean seas [29], 16.1-31.4, 30.5-67.7 and 27.3-76.2 µg/g for muscles, gills and livers of fish from Mediterranean sea [39].

- *The contents of Cu:*

Copper is an essential metal in fish and is regulated in the muscle tissue with high molecular weight proteins (metallothionein-like) [40]. The distribution pattern of Cu in the present study was the decreasing order of gills > liver > muscle. In this study, copper levels were below the recommended limits of WHO/FEPA for copper 3 mg/kg. The concentrations of Cu in these samples were far below this value; therefore, regular consumptions of fish with such low amounts of Cu could not lead to any serious health risk as far as Cu is concerned. Cu levels in the present study are in good agreement with previous recorded values of fish species where the values ranged from 0.35 to 12.00 µg/g for livers of fish from the Tuzla Lagoon [28]; 0.32 to 6.48, 5.29 to 14.90 µg/g for fish from Marmara, Aegean and Mediterranean Seas for muscles and livers, respectively [29]; 0.74 to 2.24 for fish from the Iskenderun Bay for muscles [41]; 0.70 to 27, 3.10 to 323 µg/g for fish from the Lake Budi [42]; 7.32 to 13.22, 9.13 to 10.83, 12.97 to 14.92, 68.84 to 184.85 µg/g for muscles, skins, gills, livers, respectively, for fish from Lake Qaroun [43].

- *The contents of Fe:*

Iron is an essential element of most life forms and normal human physiology. In humans, it is an essential component of protein involved in oxygen transports from the lungs to the tissue [44]. Fe is also essential for the regulation of cell growth and differentiation [45]. The distribution pattern of Fe in the present study was the decreasing order of gills > liver > muscle. In this study, the observed mean value of Fe in the gills of *Solea Vulgaris* is a bit beyond the WHO/FEPA recommended limits of 0.5 mg/kg with a value of 0.596 mg/kg. The highest content is obtained in the gills because the gills are considered as organs of accumulation of all metallic elements. This is the first organ of accumulation that is in direct contact with water. The content which is the lowest obtained in the flesh is significant because the muscle is an organ of metabolization. However, an essential heavy metal, Fe has the tendency to become toxic to living organisms, even when exposure is low. Iron concentrations in the present study were generally in agreement with the literature where iron levels mainly ranged between 0.6 and 0.7 mg/L accumulated in the soft part of the sea urchin *Paracentrotus lividus* of the Mediterranean littoral of Saida [46].

- *The contents of Ni:*

The Nickel concentration in the *Solea Vulgaris* studied don't present a significant difference such that the highest value is in the range of 0.043 mg/kg obtained in the gills, whereas the lowest concentration is in the range of 0.0023 mg/kg obtained in the muscle. The distribution pattern of Ni in the present study was the decreasing order of gills > liver > muscle. The nickel concentrations in this study are very low and they aren't dangerous neither for the species nor for human health, the results found are similar to those found by Babatunde et al. [47], who found 0 mg/kg in the liver and muscles of some fish and Eralagere et al. [48] which found 0.039 to 1.44 mg/kg in *Oreochromis mossambicus* Jannapura lake of India.

- *The contents of Cr:*

Cr is an essential trace metal. The biologically usable form of chromium plays an essential role in glucose metabolism. It has been estimated that the average human requires nearly 1 µg/day [49]. The distribution pattern of Cr in the present study was the decreasing order of gills > liver > muscle. The present Cr concentrations were comparable to those reported from Masoud et al. [50] with 0.23-1.26, 1.02-5.71 and 0.39-3.22 µg/g for fish from Alexandria coastal waters, Egypt, for muscle, liver and gill tissues, respectively. The maximum guideline, 12-13 µg/g, stipulated by the United States Food and Drug Administration was higher than the concentrations of Cr measured in all the fish samples used in this study [51]. The present study revealed that the concentrations of Cr in the gills, liver and muscle were not consistently higher than the WHO/FEPA recommended limits.

Bio-Concentration Factors of Heavy Metals:

The bio-concentration factors (BCF) of the heavy metals obtained in fish samples were as recorded below, using equation:

$$BCF = \frac{C_{\text{organism}}}{C_{\text{water}}}$$

Where:

- C organism = Concentration of metal in the organism
- C water = Concentration of metal in water

Table 3: The bio-concentration factors of heavy metals in fish samples

	Cd	Pb	Zn	Cu	Fe	Ni	Cr
<i>Palaemon serratus</i>							
Muscle	0.03	1.30	4.47	6.60	1.10	0.46	0.04
<i>Solea Vulgaris</i>							
Gills	1.06	0.14	0.42	0.20	0.69	0.16	0.02
Liver	3.17	0.14	0.57	0.57	0.24	0.16	0.03
Muscle	0.59	0.11	0.62	0.33	0.03	0.13	0.01
Mean	1.6	0.13	1.19	0.88	0.94	0.15	0.02

From the results of bio-concentration factor obtained in Table 3, it was observed that in the samples of *Palaemon serratus* copper and zinc had the highest value respectively 6.60 and 4.47, as well as Pb, Fe, with BCF values of 1.30 ;1.10 respectively, only the Cr,Ni and Cr are the values lower than the 1.00 recommended limits of WHO/FEPA. For samples of *Solea Vulgaris* the highest bio-concentration factor was observed is 1.6 and 1.19 for cadmium and zinc, however Pb, Cu, Fe, Ni and Cr, with BCF values of 0.13 ;0.88 ;0.94 ;0.15 and 0.02 are the values lower than the recommended limits of WHO/FEPA. This shows that the samples of *Palaemon serratus* and *Solea Vulgaris* have a strong tendency to bioaccumulate certain heavy metals like Zn, Cu, Fe, Pb for *Palaemon serratus* and Cd, Zn for *Solea Vulgaris*. 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, etc. . . . [52].

BCF values in this study are significantly lower than those obtained by Katemo Manda [53] who reported BCF 101 to 143 for the Cd, from 28 to 53 for Pb and 157-346 for As in the muscles of two species of tilapia (*Oreochromis* and *Tilapia macrochi rendalli*).

CONCLUSION

In view of the importance of fish to diet of man, it is necessary that biological monitoring of the water and fish meant for consumption should be done regularly to ensure continuous safety of the seafood. Safe disposal of domestic sewage and industrial effluents should be practiced and where possible, recycled to avoid these metals and other contaminants from going into the environment. Laws enacted to protect our environment should be enforced. 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, Fe, Ni, Cr in the samples of *Palaemon serratus* are below the norm except for Fe in the samples of *Solea Vulgaris* which exceeds a little the standard set by the WHO, while values of BCF show that the samples of *Palaemon serratus* and *Solea Vulgaris* have a strong tendency to bioaccumulate certain heavy metals like Zn,Cu,Fe,Pb for *Palaemon serratus* and Cd,Zn for *Solea Vulgaris*. 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.

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