



## Trace metals content in marine species from the north-western Moroccan Atlantic waters

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### ABSTRACT

Metal concentrations were assessed in seafood species from the north-western Atlantic waters using microwave digestion and atomic absorption spectrometry (AAS). The levels of Fe, Zn, Cd, Pb, Cu, As, Mn, and Ni were, in general, lower than the international safety standards for seafood quality. The health risk assessment of metal ingestion through seafood consumption was estimated using the target hazard quotient (THQ) and the estimated daily intake (EDI). While, the metal pollution index (MPI) was used as a quantitative indicator of the contamination level of marine organisms. The obtained results showed that the THQ and EDI were lower than the unity ( $<1$ ) and the oral reference doses (RfD), respectively. Hence, the consumer is not likely to experience health effects through the ingestion of seafood. The metal pollution index values were relatively higher for cephalopods, compared to decreasing order of mussels, crustaceans, and fish, respectively.

### INTRODUCTION

Heavy metals are of great importance from a toxicological point of view due to their presence in different components of the aquatic environment (water column, sediments, and marine organisms) in addition to their non-biodegradable properties. Another important characteristic is their ability to accumulate in marine organisms from water, food, and sediments and become biomagnified through the food chain (Yuan *et al.*, 2011; Bosch *et al.*, 2016; Cameron *et al.*, 2018; Zhong *et al.*, 2018). Heavy metals in the aquatic environment can reach high levels due to anthropogenic sources, which can lead to an increase in their concentration in marine organisms (Türkmen *et al.*, 2009). Studies shedded light on the biological, physicochemical, and environmental parameters affecting the concentration of metals in fish and fishery products, such as length, organs, sampling locations, pH and temperature (Tapia *et al.*, 2012; Zeitoun & Mehana, 2014; Giri & Singh, 2015; Liu *et al.*, 2015). The presence of some heavy metals in living organisms is essential for the good and stable function of their body as long as they are maintained at a reasonable level (Beldi *et al.*, 2006; Kamaruzzaman *et al.*, 2011; Łuczyńska *et al.*, 2018). Pb, Cd, and As are toxic elements with no role in the metabolic activities of the

organism, while Cu, Zn, Fe, and Mn are considered essential elements (Tuzen, 2009; Kamaruzzaman *et al.*, 2011). Exposure to cadmium (Cd) is responsible for health problems such as prostatic proliferative lesions, lung cancer and kidney failure (Rahman *et al.*, 2012 ; Rahman *et al.*, 2019). Pb exposure can affect the nervous system and cause mental retardation, and skeletal hematopoietic function disorder (Zhong *et al.*, 2018; Ahmed *et al.*, 2019). Although Cu, Zn, Fe, and Mn are essential elements, excessive intake or deficiency in their levels in the organism can lead to detrimental health effects. Nausea, bowel pain, and diarrhea along with fever are caused by excessive exposure to the essential element, Cu (Rajeshkumar & Li, 2018). Whereas, Zn deficiency is associated with appetite loss, growth inhibition, immunological abnormalities, and skin changes (Tuzen, 2009; Gu *et al.*, 2016). Notably, the consumption of seafood is the main source of protein supply for human body. Nevertheless, the intake of contaminated seafood with high levels of metals, exceeding the permitted concentrations of the monitoring agencies, viz. the World Health Organization, and Food and Agriculture Organization may threaten human health. The heavy metal content and the risk associated with the consumption of seafood were documented using different risk factors such as daily intake and the target hazard quotient (Alipour *et al.*, 2014; Chahid *et al.*, 2014; Giri & Singh, 2015; Liu *et al.*, 2015; Gu *et al.*, 2016; Afandi *et al.*, 2018; Baki *et al.*, 2018; Ahmed *et al.*, 2019).

The present study was conducted to measure the concentration of heavy metals (Fe, Zn, Cu, As, Mn, Ni, Pb, Cd) in the edible tissue of different marine organisms from the north-western Moroccan Atlantic Waters and assess the health risks associated with the consumption of seafood.

## MATERIALS AND METHODS

### Sample collection and preparation

Fish, cephalopods, mussels, and crustaceans samples were purchased directly from fishermen at the Port of Mehdiya (Kenitra, Morocco). The edible part examined in the present work is the muscle tissue of fish and the whole soft tissue of mussels and cephalopods. The internal organs were removed since they are generally not consumed. The muscle tissue was cleaned using distilled water, frozen for 24 hours, and then freeze-dried until getting a constant weight. A blender was used to ground down into a fine powder and homogenize the freeze-dried samples.

### Determination of heavy metal concentrations

An amount of 0.3g of each sample was weighed and put into separated vessels to which 5ml of suprapur HNO<sub>3</sub> was added along with 1ml of H<sub>2</sub>O<sub>2</sub> and left to digest overnight. Then, a microwave digestion system was used to assist the acid digestion process. The obtained solution was diluted using 50ml of bi-distilled water. The usage of this method is of great interest with respect to both time and recovery values. A blank digest was prepared in the same way to correct the results of the samples.

Cu, Zn, Fe, As, Mn, Ni, Pb and Cd concentrations in the prepared samples were determined using atomic absorption spectrometry (ThermoFischer iCE 3000 series). The limits of detections were as follows: Fe (0.006 mg.L<sup>-1</sup>), Zn (0.002 mg.L<sup>-1</sup>), Cu (0.2 µg.L<sup>-1</sup>), As (1 µg.L<sup>-1</sup>), Ni (1 µg.L<sup>-1</sup>), Cd (0.03 µg.L<sup>-1</sup>), Mn (0.004 µg.L<sup>-1</sup>) and Pb (0.3 µg.L<sup>-1</sup>).

The reference material SRM 1568a supplied by the National Institute of Standards and Technology (NSIT) was used to assess the accuracy of the measurements carried out during the present work. The obtained results coincided with the certified values are listed in Table (1). Recovery rates ranged from 87 to 98 % for all metals investigated.

**Table 1.** Metal concentrations measured in the certified reference material SRM 1568a

Metal	Observed values mg.kg <sup>-1</sup>	SD mg.kg <sup>-1</sup>	Certified values mg.kg <sup>-1</sup>	SD mg.kg <sup>-1</sup>
Cu	2,7	0,066	2,4	0,3
Fe	8	0,027	7,4	0,9
Zn	19,6	0,016	19,4	0,5
Mn	23	0,032	20	1,6
Pb	< LD	–	< 0,010	–
Cd	< LD	–	0,022	0,002

## RESULTS

Measured concentrations of trace metals (mg.kg<sup>-1</sup> fresh weight) in marine organisms collected from the local fish market of Kenitra, Morocco are summarized in Table (2). The mean metal concentrations decreased in the following order **Fe>Zn>Ni>Mn>Cu>As>Pb>Cd** in the fish samples, **Fe>Cu>Zn>Ni>Mn>As>Pb>Cd** in the crustacean sample, **Fe>Mn>Zn>Ni>Cu>Cd>Pb>As** in the mussel sample and **Fe> Cu> Zn> Mn> Ni> Cd> As> Pb** in the cephalopod sample.

The concentration of Pb in muscle tissues of fish, cephalopods, crustaceans and mussels ranged from 0.004 to 0.21 mg.kg<sup>-1</sup>. The highest concentration (0.21 mg.kg<sup>-1</sup>) was recorded in the mussel (*Mytilus galloprovincialis*), while the lowest (0.004 mg.kg<sup>-1</sup>) was detected in the meagre (*Argyrosomus regius*). Pb distribution in marine organisms displayed large inter-specific variability; high concentrations were associated with mussels, followed by crustaceans, cephalopods, and fish. Reported values of Pb concentrations ranged from <0.06 to 8.92 mg.kg<sup>-1</sup> for marine fish and <0.06 to 9.47 mg.kg<sup>-1</sup> for crustaceans (Baki *et al.*, 2018). While, Pb concentration values were 0.013 to 0.114 mg.kg<sup>-1</sup> for marine fish (Chahid *et al.*, 2014), 0.12 mg.kg<sup>-1</sup> for mussels, and 0.008 mg.kg<sup>-1</sup> for crustaceans (Diop *et al.*, 2016). According to the international safety standards, Pb concentration should not exceed 0.3 mg.kg<sup>-1</sup> in fish, 0.5 mg.kg<sup>-1</sup> in crustaceans, 1.5 mg.kg<sup>-1</sup> in mussels, and 1.0 mg.kg<sup>-1</sup> in cephalopods (European Union, 2006). The current values did not exceed the permissible limits.

Ni concentrations in marine organisms ranged from 0.21 to 33.4 mg.kg<sup>-1</sup>. The highest concentration (33.4 mg.kg<sup>-1</sup>) was observed in the common Pandora (*Pagellus erythrinus*) and the lowest (0.21 mg.kg<sup>-1</sup>) in the thin-lipped grey mullet (*Liza ramada*). The high levels of Ni (0.21 – 33.4 mg.kg<sup>-1</sup>, with a mean value of 4.12 mg.kg<sup>-1</sup>) were found in fish species in comparison to cephalopods (2 mg.kg<sup>-1</sup>), crustaceans (1.5 mg.kg<sup>-1</sup>) and mussels (0.9 mg.kg<sup>-1</sup>). Nickel concentrations were reported in the range of 0.008 - 0.07 mg.kg<sup>-1</sup> (**Makedonski *et al.*, 2017**). On the other hand, they ranged from 0.14 – 0.61 mg.kg<sup>-1</sup> in fish, from 0.24 – 0.46 mg.kg<sup>-1</sup> in crustaceans (Gu *et al.*, 2016), and from 0.14 - 0.33 mg.kg<sup>-1</sup> with respect to dry weight in fish species (**Afandi *et al.*, 2018**). The maximum concentration of nickel according to Food and Drug Administration is set at 70-80 mg.kg<sup>-1</sup> (**USFDA, 1993b**). Consequently, the safety limit was not surpassed in all marine organisms analyzed in the present work.

As concentrations in marine organisms ranged from 0.00015 to 0.5 mg.kg<sup>-1</sup>. Shrimp (*Aristeus antennatus*) displayed the highest arsenic concentration (0.48 mg.kg<sup>-1</sup>) in comparison with cephalopods (0.12 mg.kg<sup>-1</sup>), fish (0.00015-0.17 mg.kg<sup>-1</sup>, with a mean value of 0.05 mg.kg<sup>-1</sup>) and mussels (0.012 mg.kg<sup>-1</sup>). Among fish species, the blue shark (*Prionace glauca*) and the greater weever (*Trachinus draco*), respectively, displayed the highest (0.17 mg.kg<sup>-1</sup>) and the lowest (0.00015 mg.kg<sup>-1</sup>) arsenic concentrations. The reported values of arsenic concentrations ranged <0.08 mg.kg<sup>-1</sup> for marine fish and <0.08 to 0.283 mg.kg<sup>-1</sup> for crustaceans (**Baki *et al.*, 2018**). Whereas, they ranged from 0.38 to 1.1 mg.kg<sup>-1</sup> for fish species (**Makedonski *et al.*, 2017**). According to the Chinese Food Codex, the maximum permitted As levels in fish, crustaceans and mussels are 0.1, 0.5, and 0.5 mg.kg<sup>-1</sup>, respectively (MPHC, 2012). Hence, safety limits were not exceeded in the present work, except for some fish species. It should be noted that the toxicity of the organic form of arsenic is low and can be negligible. Therefore, in the present work, the arsenic content in seafood is considered in its toxic inorganic form, estimated as 3% of the total arsenic (**EFSA, 2009**).

The concentration of Fe in muscle tissues of fish, cephalopods, crustaceans, and mussels ranged from 1.4 to 174.9 mg.kg<sup>-1</sup>. The highest concentration (174.9 mg.kg<sup>-1</sup>) was measured in the common pandora (*Pagellus erythrinus*), while the lowest (1.4 mg.kg<sup>-1</sup>) was detected in the meagre (*Argyrosomus regius*). Mussels (*Mytilus galloprovincialis*) and fish (*Pagellus erythrinus*) displayed high values of Fe in comparison to cephalopods (*Sepia officinalis*) and crustaceans (*Aristeus antennatus*). Fe was found to be the most abundant metal in all marine organisms. The concentrations of Fe were reported in the range of 1.2 – 165 and 9.5 – 360 mg.kg<sup>-1</sup> in fish and crustaceans (**Baki *et al.*, 2018**). Whereas in different mollusk species, Fe concentrations were 50 – 76.8 mg.kg<sup>-1</sup> (**Ragi *et al.*, 2017**), and 37.07-68.53 mg.kg<sup>-1</sup> dry weight in fish species (**Afandi *et al.*, 2018**). According to **WHO (1989)**, the safety limit was set to 100 mg.kg<sup>-1</sup>. Our values were lower than the safety limit, except for both the common Pandora (*Pagellus erythrinus*) and the mussel (*Mytilus galloprovincialis*).

Cu and Zn concentrations in marine organisms ranged from 0.1 to 17.9 mg.kg<sup>-1</sup> and 1.3 to 12.8 mg.kg<sup>-1</sup>, respectively. The highest Cu and Zn concentrations were measured in the cephalopod (*Sepia officinalis*) (17.9 and 12.8 mg.kg<sup>-1</sup>, respectively) and the lowest was recorded in the common sole (*Solea vulgaris*) (0.11 mg.kg<sup>-1</sup>) and the blue shark

(*Prionace glauca*) ( $1.35 \text{ mg.kg}^{-1}$ ), respectively. Zn and Cu concentrations in the literature ranged from  $38.8$  to  $93.4 \text{ mg.kg}^{-1}$  and  $0.65 - 2.78 \text{ mg.kg}^{-1}$ , respectively in fish species (Tuzen, 2009), and  $20.78-37.49$  and  $2.13-6.17 \text{ mg.kg}^{-1}$  dry weight in fish species (Afandi *et al.*, 2018). The safety limits of Zn and Cu according to FAO (1983) ( $100$  and  $10 \text{ mg.kg}^{-1}$ ) and WHO (1989) ( $100$  and  $30 \text{ mg.kg}^{-1}$ ) are higher than measured concentrations in the present work. Zn and Cu are both considered essential metals, playing important roles in the metabolic processes of the organisms, but chronic exposure may induce adverse health problems. Hg and Ag are considered the most toxic heavy metals that pose risk to the life of marine organisms, followed by Cu mainly accumulated by marine organisms through food rather than seawater (Kamal *et al.*, 2015).

Cd concentrations ranged from  $0.0002$  to  $0.087 \text{ mg.kg}^{-1}$  in fish,  $0.5 \text{ mg.kg}^{-1}$  in cephalopods,  $0.625 \text{ mg.kg}^{-1}$  in mussels and  $0.041 \text{ mg.kg}^{-1}$  in crustaceans. Based on the obtained results, high concentrations of Cd were associated with mussels followed by cephalopods, crustaceans and fish. Cadmium levels in the literature have been reported in the range of  $0.01-2.12 \text{ mg.kg}^{-1}$  in mussels and  $0.01-0.25 \text{ mg.kg}^{-1}$  in crustaceans (Diop *et al.*, 2016). Whereas, ranges were  $0.029-0.525 \text{ mg.kg}^{-1}$  and  $0.002 - 0.480 \text{ mg.kg}^{-1}$  in crustaceans and fish (Giri & Singh, 2015). In fish samples, they were recorded in ranges from  $0.005-0.036 \text{ mg.kg}^{-1}$  (Chahid *et al.*, 2014). The safety standards set for Cd in fish ( $0.05 \text{ mg.kg}^{-1}$ ), crustaceans ( $0.5 \text{ mg.kg}^{-1}$ ), mussels ( $1 \text{ mg.kg}^{-1}$ ), and cephalopods ( $1 \text{ mg.kg}^{-1}$ ) were higher than the obtained concentrations. It should be noted that, different safety limits were set for *Scomber* species ( $0.1 \text{ mg.kg}^{-1}$ ) along with *Xiphias gladius*, *Engraulis* species and *Sardina pilchardus* ( $0.25 \text{ mg.kg}^{-1}$ ) (European Union, 2014).

Mn concentrations in marine organisms ranged from  $0.5$  to  $20.4 \text{ mg.kg}^{-1}$ . Mussels (*Mytilus galloprovincialis*) displayed the highest Mn concentration ( $20.4 \text{ mg.kg}^{-1}$ ), followed by cephalopods ( $4 \text{ mg.kg}^{-1}$ ), fish ( $3.3 \text{ mg.kg}^{-1}$ ) and crustaceans ( $1.2 \text{ mg.kg}^{-1}$ ). Mn contents in literature have been reported in the ranges of  $2.76 - 9.1 \text{ mg.kg}^{-1}$  in fish samples (Tuzen, 2009) and  $0.025 - 0.42$ , and  $3.233 \text{ mg.kg}^{-1}$  in fish and molluscs (Copat *et al.*, 2018). Furthermore, Mn contents ranged from  $0.51 - 2 \text{ mg.kg}^{-1}$  dry weight in fish species (Afandi *et al.*, 2018). The Safety limit set for Mn by the Turkish Food Codex ( $20 \text{ mg.kg}^{-1}$ ) (TFC, 2002) was lower than the measured Mn concentrations in the present work, except for the mussel (*Mytilus galloprovincialis*) ( $20.4 \text{ mg.kg}^{-1}$ ).



**Table 2.** Metal concentrations (mg.kg-1 fresh weight) in seafood samples

<i>Sample</i>	<i>Fe</i>	<i>Zn</i>	<i>Cd</i>	<i>Pb</i>	<i>Cu</i>	<i>As</i>	<i>Mn</i>	<i>Ni</i>
1- Sardine ( <i>Sardina pilchardus</i> )	6,5±0.03	8±0.01	0,003±0.00013	0,036±0.00011	0,6±0.00002	0,057±0.00004	0,54±0.00004	0,25±0.00006
2- Rubberlip grunt ( <i>Plectorhinchus mediterraneus</i> )	8,6±0.01	11,5±0.0 <sub>1</sub>	0,01±0.00003	0,1±0.00025	0,22±0.00002	0,0004±0.00038	1,53±0.0001	0,39±0.00018
3- Meagre ( <i>Argyrosomus regius</i> )	1,4±0.03	6,04±0.0 <sub>2</sub>	0,0002±0.0001	0,0037±0.00013	0,24±0.00001	0,087±0.00029	2,52±0.00005	0,35±0.00002
4- Grey Mullet ( <i>Liza ramada</i> )	15,3±0.01	7,8±0.02	0,014±0.00004	0,032±0.00015	0,8±0	0,010±0.00031	0,63±0.00007	0,21±0.00008
5- Whiting ( <i>Merlangius merlangus</i> )	16,7±0.01	1,7±0.07	0,007±0.00007	0,038±0.00009	0,17±0.00005	0,018±0.00012	1,59±0.00011	0,99±0.00008
6- Greater weever ( <i>Trachinus draco</i> )	51±0	6,1±0.02	0,009±0.00005	0,019±0.00027	0,14±0.00007	0,00015±0.00009	9,48±0.00023	6,51±0.00024
7- Common pandora ( <i>Pagellus erythrinus</i> )	174.9±0.02	5,7±0.02	0,014±0.00003	0,051±0.00012	0,69±0.00007	0,12±0.00048	7,44±0.0001	33,45±0.00013
8- Brown meagre ( <i>Sciaena Umbra</i> )	43±0	4,8±0.03	0,010±0.00005	0,026±0.00014	0,91±0.00004	0,145±0.0001	3,12±0.00008	4,38±0.00011
9- Surmullet ( <i>Mullus surmuletus</i> )	14±0.01	4,3±0.02	0,008±0.00006	0,034±0.00021	0,59±0.00011	0,0002±0.00008	5,92±0.00016	1,122±0.00011
10- Spotted seabass ( <i>Dicentrarchus punctatus</i> )	85±0.02	2,3±0.02	0,087±0.00002	0,085±0.00003	0,18±0.00005	0,0011±0.000005	5,34±0.00021	11,47±0.00013
11- Common sole ( <i>Solea Vulgaris</i> )	34±0.01	2,3±0.05	0,030±0.00001	0,048±0.00018	0,11±0.00001	0,0024±0.0001	7,28±0.00006	2,87±0.001
12- Pouting ( <i>Trisopterus luscus</i> )	38.1±0.01	2,1±0.09	0,032±0.00002	0,029±0.00011	0,2±0.00013	0,097±0.00007	5,77±0.00011	3,71±0.00021

13- swordfish ( <i>Xiphias Gladius</i> )	14.4±0.01	6,6±0.05	0,051±0.00002	0,05±0.00009	0,26±0.00003	0,053±0.00006	0,51±0.00003	0,71±0.00009
14- Blue shark ( <i>Prionace glauca</i> )	13.7±0.01	1,3±0.08	0,008±0.00001	0,038±0.00017	2,94±0.00002	0,17±0.0002	0,78±0.00004	0,62±0.00008
15- Atlantic mackerel ( <i>Sciomer-scombrus</i> )	35.2±0	5,3±0.02	0,019±0.00006	0,087±0.00006	1,39±0	0,03±0.00003	1,03±0.00008	1,31±0.00017
16- Atlantic horse mackerel ( <i>Trachurus trachurus</i> )	23.4±0.01	1,7±0.04	0,007±0.00005	0,055±0.00008	0,22±0.00006	0,0008±0.00013	0,67±0.00003	0,74±0.00017
17- Anchovy ( <i>Engraulis encrasicolus</i> )	26.4±0.02	2,7±0.07	0,011±0.00004	0,043±0.00008	0,72±0.00011	0,096±0.00009	1,36±0.00007	0,91±0.00001
18- red shrimp ( <i>Aristeus antennatus</i> )	28.7±0.01	4,3±0.02	0,041±0.00004	0,077±0.00022	6,29±0.00003	0,48±0.00006	1,18±0.00006	1,54±0.0002
19- Mussel ( <i>Mytilus galloprovincialis</i> )	174±0	12,1±0.0 1	0,625±0.00004	0,21±0.00009	0,74±0.0002	0,012±0.00024	20,4±0.00005	0,89±0.00019
20- Sepia ( <i>Sepia officinalis</i> )	20.5±0.01	12,8±0.0 2	0,503±0.00007	0,058±0.00019	17,92±0.0000 1	0,12±0.00012	3,97±0.00007	2,01±0.00005





## DISCUSSION

Heavy metal concentrations vary widely in marine organisms, which can be attributed to different biological and environmental conditions. Hence, the feeding habit (carnivore, omnivore, herbivore, filter feeder fish & bottom feeder fish) is a key factor in driving metals accumulation in marine organisms (Liu *et al.*, 2015). For instance, omnivore species were relatively weak accumulators of metals, compared to herbivores (Giri & Singh, 2015), which contradicts with the current results. Remarkably, the omnivore species *Pagellus erythrinus* displayed higher metals content than the herbivores *Liza ramada*. This could be attributed, at least partially, to the metabolic activity and size, since higher metabolic activity is associated with small organisms, while lower metabolic activity is associated with large organisms (Monikh *et al.*, 2013). Measured levels of contaminants in fish species, molluscs, and crustaceans are not uniform and depend on the bioavailability of metals in the aquatic environment and the feeding habits and habitats of these species (Baki *et al.*, 2018; Bonsignore *et al.*, 2018; Suami *et al.*, 2019). The current study confirms the same results.

### - The estimated daily intake of metals

The estimated daily intake of metals was calculated using the following equation (Giri & Singh, 2015; Ahmed *et al.*, 2019; Sofoulaki *et al.*, 2019):

$$EDI(\text{mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}) = \frac{(C \times AvC)}{Bw} \quad (1)$$

Where, C is the metal concentration in the edible part of marine organisms ( $\text{mg} \cdot \text{kg}^{-1}$ ); AvC is the average consumption of seafood per day ( $\text{g} \cdot \text{d}^{-1}$ ), and Bw is the adult body weight of the general population (kg). The average consumption of seafood used in the computation of the estimated daily intake of metals is  $37.3 \text{ g} \cdot \text{d}^{-1}$  for fish,  $2.7 \text{ g} \cdot \text{d}^{-1}$  for mussels and crustaceans, and  $1.4 \text{ g} \cdot \text{d}^{-1}$  for cephalopods. The adult body weight of the general population assumed is 70 kg.

The intake of metals through the consumption of seafood was assessed in the present work using equation 1, and the obtained results are summarized in Table (3). According to the USEPA, the oral reference dose is defined as “an estimate (with uncertainty spanning perhaps an order of magnitude) of daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime” (USEPA, 2012). The corresponding values of RfD for analyzed metals are  $0.7 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$  for Fe,  $0.02 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$  for Ni,  $0.04 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$  for Cu,  $0.3 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$  for Zn,  $0.001 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$  for Cd,  $0.0003 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$  for In-As,  $0.14 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$  for Mn (USEPA, 2017). For Pb, the values were  $0.00357 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$  (USEPA, 2014). The daily intake of metals through the consumption of fish, mussels, cephalopods, and crustaceans ranged from  $8 \times 10^{-8}$  to  $0.09 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$ ,  $4.85 \times 10^{-7}$  to  $0.007 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$ ,  $1.13 \times 10^{-6}$  to  $0.0004 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$  and  $1.6 \times 10^{-6}$  to  $0.001 \text{ mg} \cdot \text{kg}^{-1} \text{ bw} \cdot \text{d}^{-1}$ , respectively. The obtained results were found to be lower than the Oral Reference Dose (RfD). Therefore, the consumption of seafood

collected from the local market of Kenitra will not pose any risk on the consumer's health. It should be noted that, the values of EDI under or higher than the reference oral dose do not conclude that consumption of seafood is risk-free (USEPA, 2008; Baki *et al.*, 2018). According to the New York health department, EDI values 1-5 and 5-10 times the RfD are considered low and moderate, and values 10 times the RfD are considered risky (NYSDOH, 2007).

Reported values of the target hazard quotient were lower than unity, indicating that there is no non-carcinogenic risk on the consumer's health (THQs<1) (Alipour *et al.*, 2014; Gu *et al.*, 2016). Among different metals measured, the target hazard quotient of Cd was higher than unity, suggesting that the intake of assessed species at high rates would put the consumer's health at risk (Baki *et al.*, 2018).

Variations in the intake of metals through seafood consumption depend on the intake of seafood and the measured concentration of metals. In the study of Alamdar *et al.* (2017), the authors computed the intake of metals through the consumption of fish for the general public (10 g.d<sup>-1</sup>) and fishing communities (100 g.d<sup>-1</sup>). The obtained results were ten times higher for those living in fishing communities, compared to the general public. Hence, the importance of the amount of seafood consumed daily should be considered.

#### - The target hazard quotient

The target hazard quotient was estimated in the present work for metals having an oral reference dose using the following equation (Giri & Singh, 2015; Liu *et al.*, 2015):

$$THQ = EDI / RfD \quad (2)$$

Where, EDI is the estimated daily intake of metals (mg.kg<sup>-1</sup> bw.d<sup>-1</sup>), and RfD is the oral reference dose of a given metal (mg.kg<sup>-1</sup> bw.d<sup>-1</sup>).

The values of the target hazard quotients (THQs), estimated due to the exposure to metals through the intake of seafood are listed in Table (4). The THQs of Cd, As, Fe, Pb, Cu, Zn, Ni, and Mn estimated for fish were generally higher than THQs estimated, respectively, for crustaceans, mussels and cephalopods.

The THQ values under the limit set to unity indicate that there are no non-carcinogenic effects due to the consumption of seafood on the health of the consumer. While, values exceeding the limit mean would likely cause bad health effects on the consumer (USEPA, 1989; Saha & Zaman, 2013). Therefore, the consumption of seafood collected from the local fish market of Kenitra will not affect the consumer's health. According to (USEPA, 1989), the THQ do not present any dose-response relationship and do not directly measure risk.

Reported values of the target hazard quotient were lower than unity, indicating that there is no non-carcinogenic risk on the consumer's health (THQs<1) (Alipour *et al.*, 2014; Gu *et al.*, 2016). Among different metals measured, the target hazard quotient of Cd was

higher than unity, suggesting that the intake of assessed species at high rates will put the consumer's health at risk (Baki *et al.*, 2018).

#### - Metal pollution index

The metal pollution index was assessed in the present work using the following equation (Ali & Khan, 2018; Ahmed *et al.*, 2019):

$$MPI = (C_i \times \dots \times C_n)^{1/n} \quad (3)$$

Where,  $C_i$  is the concentration of metal in seafood, and  $n$  is the number of metals analyzed.

Table (3) summarizes the metal pollution index values, computed for seafood collected from the local fish market of Kenitra. Based on the results obtained, the metal pollution index estimated for fish species ranged from 0.1 to 1.4, with a mean value of 0.5. The highest value was recorded in the common pandora (*Pagellus erythrinus*); whereas, the lowest value was detected in the meagre (*Argyrosomus regius*). The MPI estimated for the crustaceans, mussels, and cephalopods was 1.1, 1.6, and 1.8, respectively. The higher the MPI, the higher the pollution level for a given species. Hence, the metal contamination is important for cephalopods, compared to mussels, crustaceans and fish. The metal pollution index values have been reported in the range of 0.46- 0.76 and 0.65- 0.89 in sardines and anchovy (with a respective mean value of 0.6 and 0.75), respectively (Sofoulaki *et al.*, 2019). While, its values ranged from 3.65 to 4.70 in demersal and pelagic fish species, respectively (Ahmed *et al.*, 2019). Obtained results in the present work were lower than the previously reported values.

**Table 3.** Estimated daily intake of metals through seafood consumption and metal pollution index values

<i>Estimated Daily Intake of seafood (mg.kg<sup>-1</sup> bw.d<sup>-1</sup>)</i>									
	<b>Fe</b>	<b>Zn</b>	<b>Cd</b>	<b>Pb</b>	<b>Cu</b>	<b>As</b>	<b>Mn</b>	<b>Ni</b>	<b>MPI</b>
<i>Fish (Mean values)</i>	0.0007 – 0.09	0.0007 – 0.006	9×10 <sup>-08</sup> – 5×10 <sup>-05</sup>	2×10 <sup>-06</sup> – 5.3×10 <sup>-05</sup>	6×10 <sup>-05</sup> – 0.002	8×10 <sup>-08</sup> – 9.2×10 <sup>-05</sup>	0.0003 – 0.005	0.0001 – 0.02	0.1 – 1.4
<i>Crustaceans</i>	0.0188	0.0025	1.01E-05	2.42E-05	0.0003	2.78E-05	0.0017	0.0022	0.5
<i>Mussels</i>	0.001	0.0002	1.6×10 <sup>-06</sup>	3×10 <sup>-06</sup>	0.0002	1.9×10 <sup>-05</sup>	4.6×10 <sup>-05</sup>	6×10 <sup>-05</sup>	1.1
<i>Cephalopods</i>	0.007	0.0005	2.44×10 <sup>-05</sup>	8.2×10 <sup>-06</sup>	0.00003	4.85×10 <sup>-07</sup>	0.0008	3.47×10 <sup>-05</sup>	1.6
	0.0004	0.0003	9.8×10 <sup>-06</sup>	1.13×10 <sup>-06</sup>	0.0004	2.28×10 <sup>-06</sup>	7.77×10 <sup>-05</sup>	3.93×10 <sup>-05</sup>	1.8

**Table 4.** Estimated target hazard quotient of heavy metals through seafood intake

<i>Target Hazard quotient</i>								
	<b>Fe</b>	<b>Zn</b>	<b>Cd</b>	<b>Pb</b>	<b>Cu</b>	<b>As</b>	<b>Mn</b>	<b>Ni</b>
<i>Fish (mean value)</i>	0.001 – 0.13	0.002 – 0.02	0.0001 – 0.05	0.0005 – 0.015	0.0015 – 0.04	0.0003 – 0.3	0.002 – 0.4	0.006 – 0.9
<i>Crustaceans</i>	0.027	0.084	0.01	0.0068	0.0081	0.093	0.012	0.11
<i>Mussels</i>	0.0016	0.0006	0.0016	0.0008	0.006	0.06	0.0003	0.003
<i>Cephalopods</i>	0.001	0.002	0.02	0.002	0.0007	0.0016	0.006	0.002
	0.0006	0.0008	0.0098	0.0003	0.009	0.008	0.0006	0.002

## CONCLUSION

Quality assessment of a variety of seafood species from the north-western Moroccan Atlantic Waters, with respect to metals content showed that, the concentrations of Pb, Zn, Cu, Ni, Mn, Fe, Cd, and As were generally below the safety limits set by several international organizations. Substantial variability of metals content was noted among species groups; high concentrations were found in bivalves (mussels), followed by cephalopods, crustaceans and fish. This simple classification will significantly simplify any further studies on toxicity assessment or marine organisms and their use as bio-indicators of the quality of waters in the region. The estimated target hazard quotient and daily intake, associated with sea product consumption did not exceed the safety standards based on the data obtained. It is necessary to emphasize the importance of careful interpretation of the calculated parameters, which were derived from a single monitoring campaign.

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