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Assessment of Heavy Metals (Fe, Cu and Ni) Contamination of Seawater and Mussel, Mytilus Galloprovincialis, from Al Hoceima Moroccan Coasts

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Heavy metal concentrations in *Mytilus galloprovincialis*

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ABSTRACT

In the present work, both seawater and mussels (*M. galloprovincialis*) were collected monthly from the mussel farm located along the coastline of Al Hoceima (Morocco) and their heavy metal concentrations (Fe, Cu, Ni) were determined using inductively coupled plasma – optical emission spectrometry (ICP-OES). The trace metals found in the mussel tissues are much higher than those found in the environment. The order of the abundance of metals in the mussels is Fe > Cu > Ni. The pattern of the temporal variation of mussel trace metal levels was similar among stations with higher values during the months of winter season and lower during the months of summer period. Heavy metals uptake is dependent on both environmental and biological factors. Results obtained in this study for Fe, Cu and Ni, indicate that the species under investigation poses no health hazard to consumers because its trace metal contents remain within the permissible range established by various international guideline for safe human consumption. Our study, indicate that bivalve molluscs can be used as sentinel bioindicators in marine environments impacted by heavy metals in this area of the Moroccan coastline.

KEYWORDS Heavy metals, seawater, Moroccan Mediterranean coastline, *Mytilus galloprovincialis*, humain health.

INTRODUCTION

The rapidly increasing population and developing industrial and agricultural activities in mediterranean countries have caused increase in industrial, agricultural and municipal water pollution. The discharge of effluent, treated or not treated, into the sea and rivers has resulted in soil and water pollution. The anthropogenic activities wastewater contains not only organic matter, but also other pollutants such as heavy metals [1]. These trace elements occur in the marine environment to levels that are hazardous for marine biota and can be dangerous for human health by consuming these marine organisms. A number of national and international programs have been established over recent decades to identify efficient and accurate biomonitors of trace metal pollutant availabilities [2, 3]. Extensive surveys have been undertaken in many countries [4, 5]. Authores (Pérez-López et al. [6]; Yuan et al. [7]) reviewed the use of biological indicators to monitor chemical pollutants such as trace metals and concluded that such organisms may have advantages over classical methods of water and sediment analysis. Mussels are widely used as sentinel organisms in marine pollution monitoring programs. In this occurance, mussels have been proposed as biomonitors because of their wide geographical distribution, easy sampling, sedentary nature, filtering habits, resistance to stress, sessile lifestyle and ability to bioaccumulate heavy metals in their tissues to levels many times higher than their surrounding water [8-10]. In the bivalve molluscs, trace metals have been absorbed both from water and from ingested phytoplancton and other suspended particles [2]. Thus, trace metals accumulation in these marine organisms can be affected by environmental (salinity, temperature, intertidal zone, available food, etc) and physiological (age, reproductive cycle, sex, etc.) factors [11, 12]. No research, however, has been carried out so far on seasonal variation in metal accumulation or on biological factors affecting metal accumulation in mussel populations from the mediterranean moroccan coasts.

The goal of this study was to determine the monthly levels of Fe, Cu and Ni in the soft tissue of mussels *Mytilus galloprovincialis* and seawater samples along the Al Hoceima coastline. The results of this study may provide information on the use *Mytilus galloprovincialis* as biological indicators of heavy metals in this part of the Mediterranean coasts. The work studies also the levels of toxic metals in the mussels and if they are within the maximum permissible levels set by the international guidelines.

MATERIAL AND METHODS

1. Area of study, collection of samples and preparation of mussels

The Mediterranean mussel *M. galloprovincialis* and seawater were obtained from the mussel farming installed in the coastal areas of Al Hoceima. Waters and mussel samples were collected monthly in 2018, from Junuary to December, from five sampling sites (A, B, F, J and I) (Fig. 1), to ensure homogeneous distribution of samples. Water was sampled by hand into several bottles according to the analytical specifications and transported at +4°C in a cold box to the laboratory until analysis.

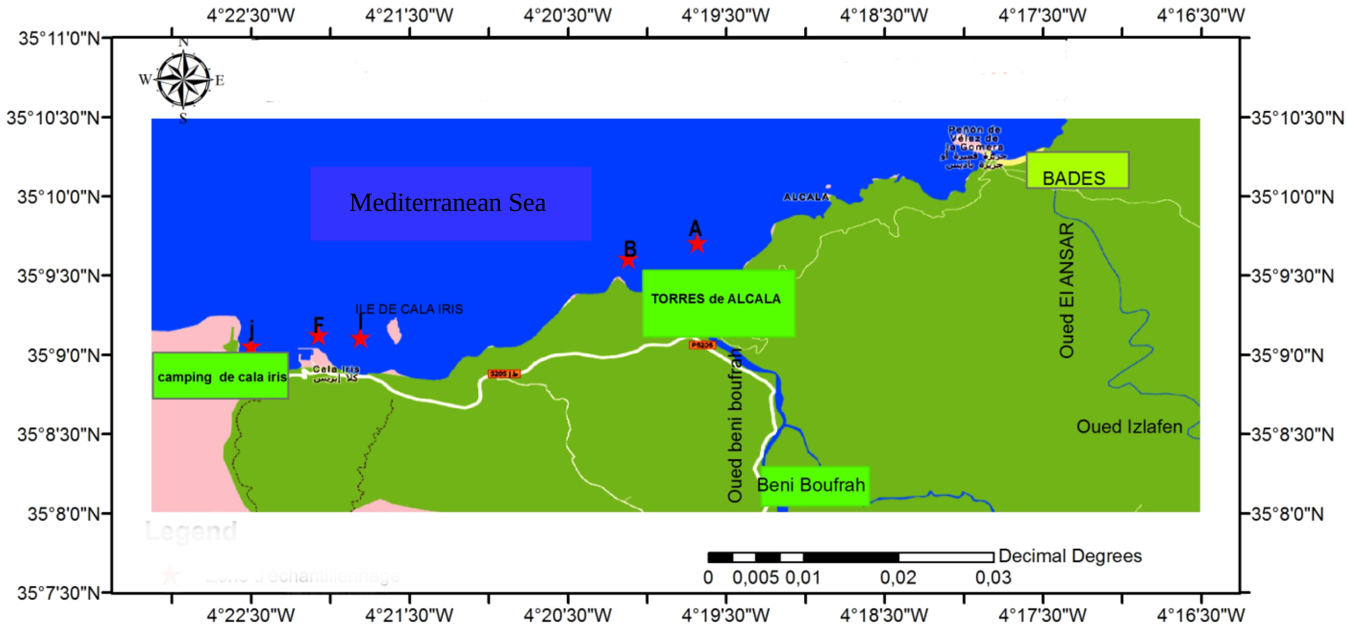

Figure 1: Geographical location of mussel sampling sites along the mussel farming from Al Hoceima coastline (Moroccan Mediterranean).

Approximate 50 mussels (*Mytilus galloprovincialis*) from the sampling sites were collected. The mean value ± standard deviation (SD) of the total width, length and whole weight of samples was 39.3 ± 0.6 mm, 66.1 ± 1.1 mm and 29.1 ± 1.3 g, respectively. The specimens were immediately washed at each site with seawater to eliminate encrusted organisms, stored in bags, kept in a cooler box with ice and transported to the laboratory. For a 48-hour period, the mussels were kept alive in clean seawater to purge their digestive systems, in order to be able to measure only the heavy metals biologically deposited in their tissues [13, 12].

The whole soft tissues of mussels were removed from the shells using a steel knife, then thoroughly rinsed with distilled water to remove the extraneous impurities. Whole tissues were dried at 60 °C for 48 h to obtain the dry weight, and were ground in a mortar for homogenization. The ground powder of mussel tissues were grouped in five pools monthly (each pool corresponds to five individuals of similar size from each sites) for chemical analyses of metals.

2. Metals analysis

The metal contents (Fe, Cu and Ni) in the whole soft tissues of mussels and water samples were analyzed by inductively coupled plasma – optical emission spectrometry (ICP-OES 720-ES), (Agilent Technologies, USA) in the Scientific Instrumentation Service at the Estación Experimental del Zaidín, Consejo Superior de Invesigaciones Cientificas (EEZ-CSIC), Granada, Spain.

Approximately 0.3 g of dry weight of the mussel soft tissues was digested by Start D Microwave Digestion System (Milestone), equipped with a rotor MPR SK-12T (Milestone), using a 10 ml of solution of 1:4 (H_2O_2 (30%) : HNO_3 (65%)), at 190 °C, during 1 h 30 min as described by Belivermiş et al. [14]. An external calibration was performed for quantification, which utilizes different multielement solutions to cover the targeted analyte range [2].

3. Statistical analysis

The data were expressed as means and standard deviations, and treated using one-way analysis of variance (ANOVA), followed by Tukey's test, accepting $P < 0.05$ classified as statistically significant.

RESULTS AND DISCUSSION

1. Trace metals in the seawater of Al Hoceima coastline

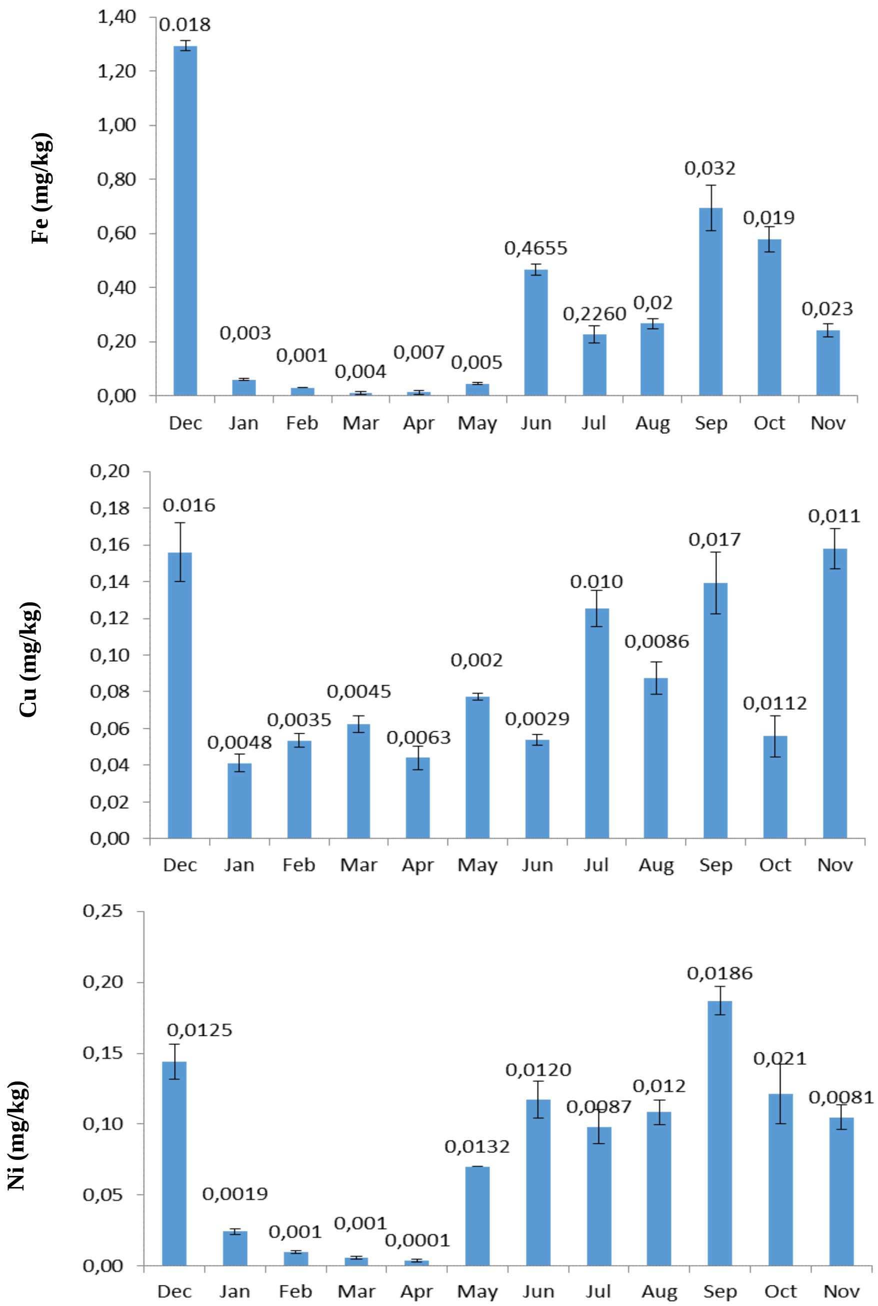

Figure 2: Monthly variations of trace metals (Fe, Cu and Ni) in seawater from Al Hoceima coastline (Moroccan Mediterranean), during the sampling period (displayed as mean ± standard deviation).

Table 1. Comparison of heavy metal concentrations as mg/kg in seawater from Al Hoceima coastline with the permissible limits recommended by the FAO.

	Cu	Fe	Ni
Present Study	0.0905	0.3545	0.1018
FAO [15]	0.5	---	0.5
FAO/WHO [16]		5.0	

The average monthly trace metals of seawater from Al Hoceima coastline areas, during the sampling period of 2018 are shown in Figure 2. The pattern of accumulation in seawater was Fe > Ni> Cu. Iron (Fe) in sea water present a peak in December (1.2930 ± 0.018 mg/kg). The lowest value of Ni content was also observed in April (0.0037 ± 0.0001 mg/kg) and the maximum values during September (0.1869 ± 0.0186 mg/kg). The highest Cu concentration was recorded in autumn with a peak in November (0.1579 ± 0.011 mg/kg), and the minimum value was observed in January (0.04111 ± 0.0029 mg/kg). Trace metal concentrations in seawater of Al Hoceima coastal regions were quite similar to values referred to in other regions of the world coastal waters [17, 6]. The pattern may be the result of local anthropogenic activities, due to industrial, agricultural and domestic sources. In this study, trace metal levels were found to be lower than the recommended limits

established by various international guidelines (Table 1) [16, 15]. These measurements are important to show the metal concentrations in the water and to make a correlation with those in the mussels.
The heavy metals found in the mussel tissues (Fig. 3, see below) are much higher than those found in their marine environment. Similar results are found in previous studies [6, 18]. Mussels absorb trace metals both from water and from ingested phytoplancton and other suspended particles [2, 19]. Because of their tendency to concentrate different pollutants from their environment, marine bivalves have shown useful as sentinel organisms, since the levels of heavy metal contaminations in the molluscs are directly proportional to the level of their availability in the environment [6, 20].

2. Seasonal trends of Zn, Cd and Cr in soft tissues of Mytilus galloprovincialis

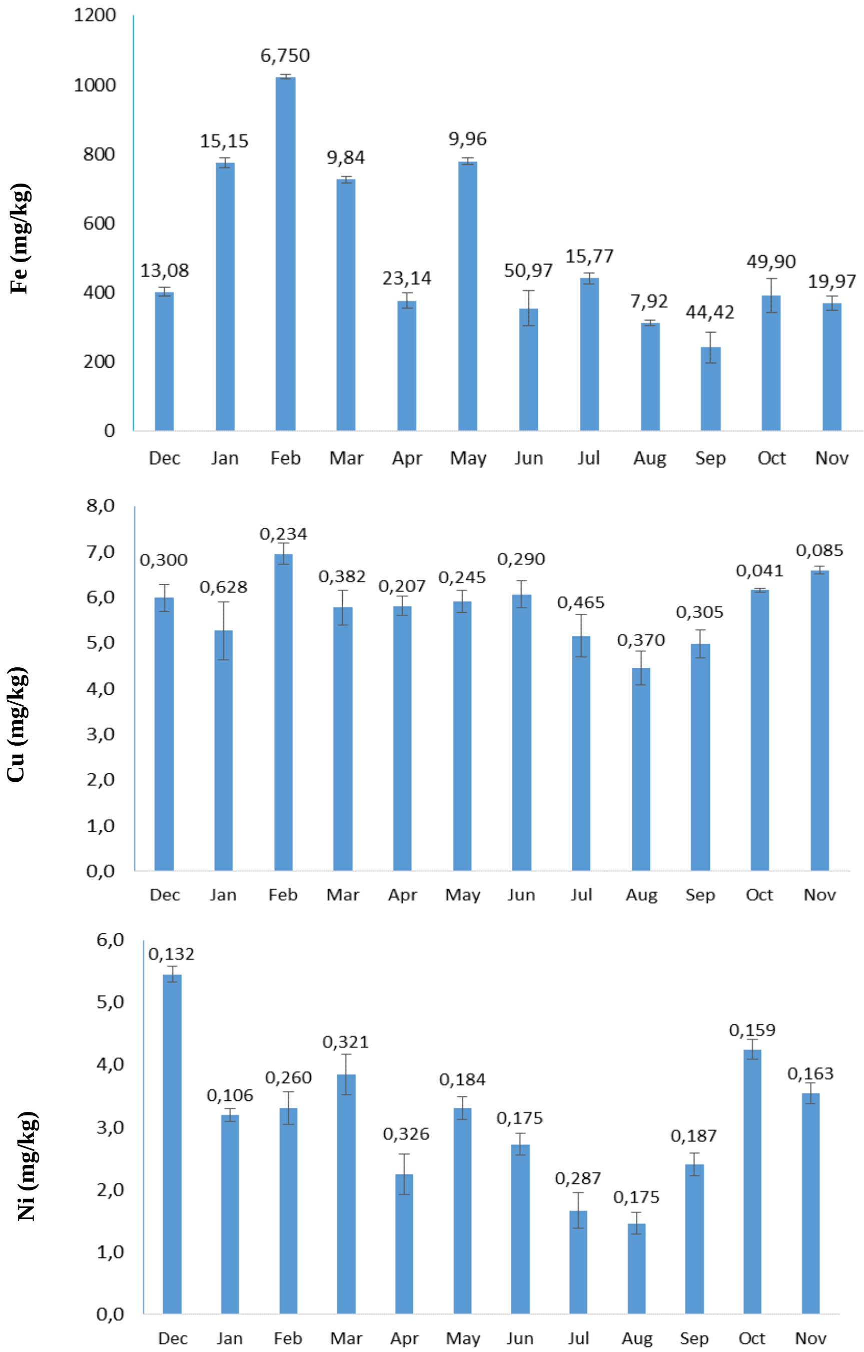

Figure 3: Monthly changes in soft tissue metal (Fe, Cu and Ni) contents (mg/kg dry weight) in *Mytilus galloprovincialis* from Al Hoceima coastline (Morocco), (mean ± standard deviation).

Monthly mean concentrations of each metal in the soft tissue of *M. galloprovincialis* are presented graphically (Fig. 3). Taking into account the highest concentration of metals in any of the months, the orders of decreasing concentration in soft tissues of mussels were Fe > Cu > Ni.

Average metal (Fe, Cu and Ni) concentrations in the whole soft tissues of mussels were (542.57 ± 47.15, 7.193 ± 0.142 and 3.502 ± 0.251 mg/kg, for Fe, Cu and Ni; respectively).

The Fe levels in soft tissues of mussels present higher values in winter period with a peak in February (1022.83 ± 6.750 mg/kg), the minimum and similar values were observed in summer-autumn seasons. The highest Cu content over the period analyzed occurred in February (6.955 ± 0.234 mg/kg) and the lowest in August (4.455 ± 0.370 mg/kg). Elevated concentrations of Ni occurred in winter period with a peak in December (4.545 ± 0.132 mg/kg) and the lowest value was observed in summer season for the month of August (1.457 ± 0.175 mg/kg).

In the present study, the seasonal fluctuations of soft tissue metal concentrations in mussels may be driven by various environmental and biological factors. Changes in trace metal contents (Fe, Cu and Ni) found in *M. galloprovincialis*, with higher values in mussels collected in the winter and lower contents were observed in soft tissues of mussels in the summer, have long been considered [21, 5, 22, 23]. The influence of seasonal changes in metal bioaccumulation in mussels can be explained in terms of the characteristics of climate change in the Mediterranean area (warm, dry in summers and wet, rainy in winters). Of the processes affecting metal dynamics in mussels, variability in physicochemical conditions of the water and sediments. The period of winter is known as a rainy season. During this period, rivers collect water through branched drainage canals from large inland areas and discharge it to the sea, often with great quantities of terrestrial inputs from the studied region. The coastal marine environment of Al Hoceima region receive a large amount of suspended matter and systematically large charge of particle-associated elements of various kinds including heavy metals, that can originate from natural sources (processes of chemical weathering and geochemical activity) and from local pollution sources (agriculture, municipal wastewater and industrial activity). In addition, the increase of metal (Fe, Cu and Ni) concentrations in mussel tissues during the winter period, may have been associated with faster metal uptake and accumulation during intense food uptake of the mussels in this period [24]. Trophic transfer of metals is increasingly recognized as an important pathway for metal accumulation in marine bivalves [25]. In Other studies, the seasonal changes in trace metal contents in marine biota have been exclusively attributed to variations in the levels of these elements in water [3, 19].

Another factor which affect the metal contents in mussels is the salinity gradient. Authors (Blust et al. [26]; Blackmore and Wang [27]) indicated that trace metal bioavailability decreases with increasing salinity, according to the concentrations of free metal ion species in the exposure water. Authors (Azizi et al. [19]; Cinnirella et al. [28]) found that in the Mediterranean coats, the concentration of traces metals increase and reach its maximum in the winter when the salinity levels decrease and reach its lowest level.

The seasonal trends of trace metal concentrations in mussels can also be explained in terms of changes in physiological status (reproductive stage, sex, age, growth, etc). Among physiological processes, reproduction is one of the most energy demanding and can result in energetic trade-offs and modulation of physiological performance [5]. Phillips [29] reported that seasonal fluctuations in heavy metal concentrations in mussel are mainly due to dry weight fluctuations against a background of fairly stable metal content throughout a year. The effect of weight changes on metal levels has been also established by authors (Sokolowski et al. [30]) in marine bivalves. Furthermore, many authors (Widdows [31]; Dokmeci, [12]) have suggested that tissue metal concentrations in mussels vary considerably according to reproductive stage. Azizi et al. [19] found that up to 40% of tissues were lost during the spawning period.

Table 2. Comparison of heavy metal concentrations as µg/kg dry weight in mussels, *Mytilus galloprovincialis*, from the diverse coastal locations with the tolerance levels.

Location	Cu	Ni	Fe	Références
Cala Iris Sea	7.193	3.502	542.57	Présent work
Saronikos gulf, Greece	20.0	4.9	593	Strogyloudi et al. [5]
Western Scheldt estuary (Netherlands)	27.4	16.2	572	Mubiana et al. [32]
Chacopata- Bocaripo Lagoon (Venezuela)	9.00	8.47		Pinto et al. [33]
Apulian Coast (Italy)	8.17			Spada et al. [2]
ElJadida, Moroccan coast	142.2	94.3		Maanan [34]
Galicia and Cantabria, Spain	10.09			Besada et al. [11]
Brown Bay, Argentina	2.00		144.69	Giarratano and Amin [21]
Island of Sylt, Germany	8.272	2.007		Helmholz et al. [35]
Baltic Sea (norway)	12.10	9.60	1130	Pempkowiak et al. [36]
Norwegian Sea	7.57	3.49	475	Pempkowiak et al. [36]
Moroccan Atlantic coast	43.1			Maanan [13]
Andalucia coast (Spain)	11.9			Benedicto et al. [37]
Galicia and Gulf of Biscay (NW Spain)	14.06			Besada et al. [38]
Croatian Coasts (Adriatic Sea)	10.53			Kljaković-Gaspić et al. [39]
Montenegro Coasts (Adriatic Sea)	8.26			Joksimovic et al. [40]
Baleari Islands (Western Mediterranean)	4.76			Deudero et al. [41]
Guideline	50-150			USEPA [42]
Guideline	70			FAO [43]
Guideline		80		USFDA [44]
Guideline	20			TKB [45]
Guideline	20			UNEP [46]

The comparison of the some heavy metal concentrations obtained in the mussel samples collected from the Al Hoceima coastal region with the data from different areas and with the tolerance levels in mussels given by the international standards was given in Table 2.

The trace metal concentrations in mussels, expressed in mg/kg dry weight, for Fe, Cu, and Ni are: 542.57, 7.193 and 3.502, respectively. Copper is an essential mineral for human health and at the same time can be toxic, depending upon the amounts ingested [47]. In this sens, a severe deficiency of copper has been associated with bone malformation during development [48], and as a contributory factor to osteoporosis in adults [49], it has also been associated with cardiovascular risk and altered immune responses [50]. However, high uptakes of copper may cause liver and kidney damage and even death [51]. Nickel is a hard metal found in soil as well as foods. In small quantities nickel is essential, but when the uptake is too high it can be a danger to human health. This compound can cause a variety of adverse effects on human health such as dermatitis, chronic bronchitis, cardiovascular and kidney diseases, and cancer of the lung and nasal sinus [52]. Iron is also an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport. However, in excess quantities, Iron can also be poisonous and cause serious threats to human health such as conjunctivitis, choroiditis, retinitis, and also participate in development of a siderosis [53]. Based on a comparison of the permissible limits set by various international organizations, Cu levels in this study were lower than the Food and Agriculture Organization permissible limit which is 70 mg/kg [43], and the United Nations Environment Programme [46] and the Turkish [45] permissible limit which is 20 mg/kg. On the other hand, according to the United States Environmental Protection Agency [42], the maximum permissible Cu levels are 50-150 mg/kg for molluscs. The Ni contents in the present study were lower than the United States Food and Drug Adminstration permissible limit wich is 80 mg/kg [44]. However, for Fe, there is no information about maximum permissible iron concentrations in fish/molluscs tissues recommended by various organizations (Table 2).

In general, metal levels of the present study were in agreement with the results of previous mussel studies in diverse coastal locations. Copper (Cu) levels obtained in this study were lower than those reported for ElJadida, Moroccan coast [34], Western Scheldt estuary of Netherlands [32], Saronikos gulf of Greece [5], Galicia and Gulf of Biscay of Spain [38] and Croatian Coasts of Adriatic Sea [39], but higher than those reported for Baleari Islands of Western Mediterranean [41] and for Brown Bay of Argentina [21]. On the other hand, our findings for Cu levels were in agreement with ones reported for Norwegian Sea [36]. For Ni, the concentrations found in *M. galloprovincialis* in the present work, were in the range of the literature which are 2.01-94.3 mg/kg, in tissues of *Mytilus galloprovincialis* from Island of Sylt, Germany [35] and El Jadida, Moroccan coast [34]; respectively (Table 2). Iron (Fe) levels found in tissues of mussels from Cala Iris Sea, were in the range of the bibliographic works, which are 144.69-1130 mg/kg, in soft tissues of mussel from Brown Bay of Argentina [21] and Baltic Sea of Norway [36]; respectively (Table 2).

CONCLUSION

Our studies reveal interesting results concerning the bioaccumulation of Fe, Cu and Ni in *Mytilus galloprovincialis* from the Moroccan Mediterranean coasts during the season period. Trace metal levels in soft tissues of mussels present higher values in winter period because of the Mediterranean climate change, river flow discharges chemical pollutant from local pollution sources (industrial, agricultural and domestic activities), and also of the physiological status of the bivalve (age, weight and reproductive cycle). The results obtained in this study were compared with those reported in earlier studies and concluded that, the mussel from Al Hoceima coastline was in general not considered a metal polluted molluscus according to the international guidelines (FAO, USFDA, TKB, USEPA, UNEP). So, the Fe, Cu and Ni, under investigation in this work, pose no health hazard to consumers. The heavy metals found in soft tissues of mussel are much higher than those found in their marine habitats. We concluded that the marine biota can be used as bioindicator for monitoring of coastal water pollution in the region of Morocco.

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