

Bioaccumulation and health risk assessment of trace elements in *Mytilus galloprovincialis* as sea food in the Al Hoceima coasts (Morocco)

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Abstract. The monthly variations in metal concentration of Cadmium (Cd), Zinc (Zn) and Chromium (Cr) were determined in tissues of *Mytilus galloprovincialis* and seawater obtained from the mussel farm installed along the Al Hoceima coasts, during the sampling period of 2018. The heavy metals encountered in the bivalves were higher than those observed in their environment with a decreasing order of Zn > Cr > Cd. The results also showed a decreasing trend for the metals studied during the summer sampling campaign. The maximum values were obtained during the wet periods. The monthly variations in trace metals observed in the mussel tissues were due to environmental parameters and the physiological state of the bivalves. Our results revealed that the average concentrations of pollutants found in *M. galloprovincialis* were below the limits allowed by the Food and Agriculture Organization of the United Nations (FAO) which are 2.0, 1000 and 1.0 mg/kg, for Cd, Zn and Cr, respectively in molluscan shellfish for human consumption. Therefore, the trace elements present in the tissues of Al Hoceima shore mussels do not represent a risk to human health associated with their consumption, even during the period of their maximum bioaccumulation. Our work highlights the potential use of *Mytilus galloprovincialis* in the Biomonitoring of metallic pollutants in this region of the northern Moroccan coasts.

Keywords: *Mytilus galloprovincialis*; Al Hoceima coastline; bioaccumulation of trace metals; environmental parameters; human health.

1 Introduction

The progress of any nation in the world is based on the industrial technology. In parallel with technology development, a boom in industrial and agricultural activities has led to an increase in the production and use of chemicals [1,2]. Nevertheless, one of the disadvantages of all of these activities is the input into the surrounding environment of by-products and untreated chemicals [3]. These chemical products can represent a major source of pollution, affecting the marine coastal environment [4]. The industrial, domestic and agricultural sewage discharges containing the toxic xenobiotics have been identified as one of the most important sources of pollution on the Mediterranean coast. These environmental pollutants constitute increasingly serious threats to the Mediterranean's marine ecosystems. The pollutants like heavy metals

introduced into the marine ecosystems are absorbed by aquatic organisms living in the environment. The hazard of these metals can be transferred to humans via the ingestion of these animals which have bioaccumulated pollutants to levels higher than the permissible contents established by the international organizations [5]. The bivalve molluscs have been successfully utilized as an indicator of environmental quality [6]. Programs such as the 'Mussel watch', which was first implemented in 1975 [7] illustrate the usefulness of this approach to monitor the status and the trends of chemical contamination of United States coastal waters. Recent monitoring of heavy metals based on mussels in Mediterranean coastal waters has also proven effective [8-10]. The marine mussels are known to concentrate these elements, providing an integrated indication at the time of environmental contamination [3]. The abiotic factors (e.g. salinity, pH, dissolved oxygen, temperature, and diet) and

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physiological state (e.g., reproductive cycle, age, and growth), are known to influence the metal bioaccumulation in the marine bivalves [11]. The objective of the present work was to investigate the trace element (Cd, Zn and Cr) compositions in mussels (*Mytilus galloprovincialis*) and seawater samples, in order to discuss the seawater parameter impacts on the contents of the metallic element in bivalves. These parameters were monitored monthly from the Al Hoceima coastal area, North of Morocco. The study may provide data on the use of Mediterranean mussels, as sentinel organisms in metal Biomonitoring studies in the Moroccan Mediterranean coastal area.

2 Material and methods

2.1 Study area and sampling methods

The seawater and *Mytilus galloprovincialis* were sampled monthly from January to December of 2018, from five sites (A, B, F, J and I) of the study area that are located in the Al Hoceima coasts

(Figure 1). Once sampled, the seawater and the bivalves (*M. galloprovincialis*) were transported to the laboratory and prepared for chemical analyses as described in a previous work by Azizi et al. [9].

2.2 Environmental parameters of seawater

To provide information about the water quality of Al Hoceima coasts, some abiotic factors (dissolved oxygen, salinity and temperature) were determined *in situ* at each site using a multiparameter instrument (HQd Series portable meters, HACH, Safety Mark). The chlorophyll *a* was analyzed according to the method established in our laboratory [9, 12].

2.3 Metal determinations

The metal concentration of Cadmium, Zinc and Chromium were analyzed in seawater and tissues of *M. galloprovincialis*, as well described in a previous study by Azizi et al. [9].

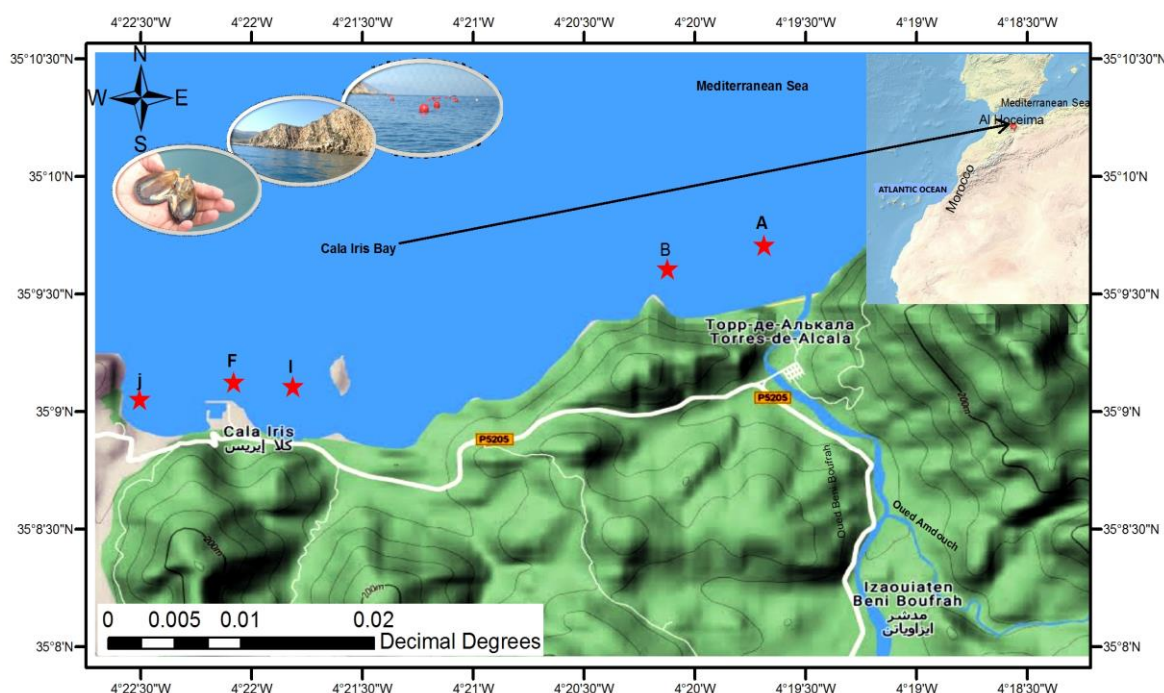


Fig. 1. Geographical location of the study sites in the cultured mussels from Al Hoceima coasts.

3 Results and Discussion.

3.1 Abiotic parameters

The mean monthly salinity, temperature, dissolved oxygen and Chlorophyll *a* concentration (\pm SD) from January to December 2018 are shown in Figure 2. The lowest salinity was observed in January (35.62 ± 0.226 practical salinity unit (psu)),

the salinity began to rise in June, and the peak salinity of (40.17 ± 0.569 psu) was recorded in July. Our finding coincided with the values obtained by Kayhan et al. [13] in the seawater of Tuzla Shipyard area of Turkey. The seawater salinity was influenced by the freshwater influx coming from the neighboring rivers during the rainy periods [14, 15].

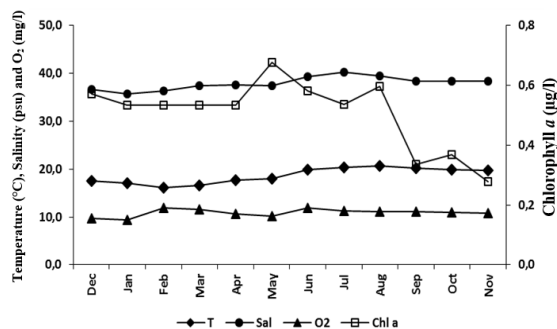


Fig. 2. Monthly fluctuations of physico-chemical parameters in seawater from Al Hoceima coastline of 2018.

Water temperature began to rise in February, and the peak temperature of 20.67 ± 0.153 °C was recorded in August. Our results coincided with the values obtained by Kayhan et al. [13], in the seawater from Tuzla Shipyard area of Turkey, where the maximum levels of temperature were observed in summer and the minimum in winter. The lowest dissolved oxygen was obtained in winter season with a peak in January (9.45 ± 0.338 mg/l), and the highest content was recorded in June (11.93 ± 0.217 mg/l). Similar results have been observed by various authors [14, 16] on different coasts of the Mediterranean countries. Chlorophyll *a* content fluctuated between 0.135 µg/l in June, and 1.58 µg/l in September. Our values for chlorophyll *a* were higher than results obtained by Giarratano et al. [17] from the coastal waters of the Beagle Channel of Argentina, and lower than values obtained by Lacroix et al. [18] from the Bay of Brest of France. Previous investigators [16] have shown that physicochemical parameters, in the coastal marine environment, are relevant for the influence of the degree of toxicity and the uptake of metallic elements by marine invertebrates.

The environmental parameters of waters comply with a fundamental role in the bioaccumulation of metallic elements by marine biota [19].

3.2 Heavy metals in seawater

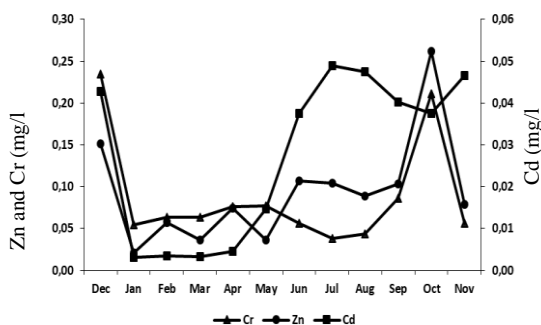


Fig. 3. The average variations in metals Zn, Cr and Cd in seawater from the Al Hoceima coasts of 2018.

The mean variations in metallic elements in seawater from the studied region are shown in Figure 3. The heavy metal contents in seawater decreased in the order of $Cr > Zn > Cd$. Chromium (Cr) in seawater presents a peak in December (0.2344 ± 0.026 mg/l). The highest Zn concentration was recorded in autumn with a peak in October (0.262 ± 0.032 mg/l), and the minimum value was observed in January (0.0209 ± 0.004 mg/l). The lowest value of Cd content was also observed in January (0.0030 ± 0.0003 mg/l) and the maximum values during June-December. The values of heavy metals found in seawater of the study zone were similar to those obtained in seawater from Northwest Spain by Pérez-López et al. [20]. The local human activities that regenerated the urban, industrial and agricultural effluents influenced the metal pollutants in the region. According to the World Health Organization (WHO), concerning water quality standard to metal pollutant levels, the heavy metal contents observed in the present work were lower than the maximum permissible limits recommended by WHO [21, 22]. The metallic pollutants observed in the tissues of the marine invertebrates, as indicated in Figure 4, are much higher than those observed in the environment. Similar results have been observed by various authors [19, 20].

3.3 Metal concentration of Zn, Cd and Cr in *Mytilus galloprovincialis* soft tissues

Figure 4 shows the monthly heavy metal contents in *Mytilus galloprovincialis* samples collected from the mussel farming located in the coasts of Al Hoceima. The average values of the heavy metal contents in the marine invertebrates decreased in the order of Zn (159.9 ± 5.906 mg/kg) > Cr (3.121 ± 0.192 mg/kg) > Cd (0.812 ± 0.022 mg/kg).

The Cd concentrations in the bivalve soft tissues present higher and similar contents during the wet period (winter-spring), and the minimum values were obtained in summer (July) (0.610 ± 0.016 mg/kg). The mean values of Cr were higher in winter with a peak in February (4.898 ± 0.080 mg/kg) and the lowest value was observed in summer and early autumn. The minimum values of Zn were observed in June (142.13 ± 0.83 mg/kg). In our study, the seasonal variations in the metallic pollutants observed in the mussels, with higher levels obtained in the winter season, and lower concentrations indicated in summer period have long been considered [9-11]. In the Al Hoceima coasts, the bioaccumulation of metallic pollutants in marine biota have been influenced by the abiotic variables and the biological states [9]. In relation to the environmental parameters, the effect of seasonal variations in trace metal accumulations in bivalves could be explained by fluvial inputs. Authors

(Rzyski et al. [23]; Azizi et al. [9]) found that the variations in metals in molluscs were highly correlated with the flow of streams. In winter, the high-flow river promotes an increase in metallic elements in the coastline regions. In the Al Hoceima coasts, the anthropogenic activities developed in the region regenerated effluents and by-products that reach seawater by the neighboring rivers (Amadouch and Beni Boufrah) during the rainy months of winter. Another explanation can be attributed to the salinity gradient. Author Johansson [24], indicated that the bioaccumulation of metals in mussels decreases with increasing salinity levels in marine waters, depending on the contents of free element ion species encountered in the water.

Our results corroborate these observations, as indicated in Figure 2. In terms of biological factors, we suggest that the variations in heavy metal accumulation in bivalves from the Al Hoceima coasts were due to the changes in marine invertebrate physiology than changes in metals found in their marine environment, as indicated by Mubiana et al. [25]. The physiological states of animals such as the gametogenic cycle and growth were strongly associated with seasonal variations [9], and any changes in these biological factors can affect the metal contents in mediterranean mussels (*M. galloprovincialis*) from Al Hoceima seawater [18, 6]. The observed seasonal pattern of metal bioaccumulation in mussels of Al Hoceima coasts was similar to that presented in other studies (Gorbi et al. [26]; Piscopo [11]) with maximum concentrations during the wet period and minimum during the warm season. Authors indicated that high metal concentrations found in the cold period coincided with low body weight [9]. They indicated that the increased contents of microalgae in the wet seasons coincided with high metallic element contents in the tissue of mussels. Microalgae cells, may act as carriers of trace metals and carbon organic to the bivalves [27], due to their ability to absorb dissolved trace elements from marine waters [28]. Lee et al. [29] indicated that the phytoplankton can improve the trace elements' bioavailability for marine invertebrates. In relation to the reproductive cycle, Azizi et al. [10] observed that the bioaccumulation of pollutants in *Mytilus galloprovincialis* was higher in winter, authors indicated that the fluctuation in heavy metal contents in bivalves depended on the variation in the animals' weight during the period of reproduction.

In a previous work, Richir and Gobert [30] observed that the contribution of the gonadal tissues to the total body weight of bivalves was more pronounced in winter and early spring. Azizi et al. [9] found that during the release of gametes, up to 40% of the animal's weight can be lost, which

indicates the importance of the gonadal cycle in the physiological states of the animal. Dokmeci [31] suggested that in order to determine the existence of heavy metals in marine biota it must take into account different stages of sexual reproduction in animals.

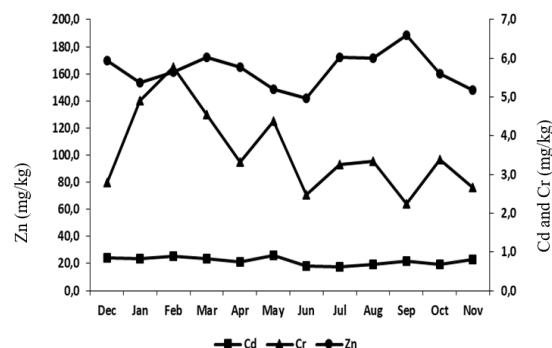


Fig. 4. Monthly variations (mg/kg dry weight) in metals Zinc, Cadmium and Chromium, in dried tissues of *Mytilus galloprovincialis* from Al Hoceima coastline. The levels of trace metals Cd, Zn and Cr of *Mytilus galloprovincialis*, obtained in our study are compared with the data from the literature (Table 1).

Table 1. Levels of metallic elements Zinc, Cadmium and Chromium in mussels obtained in several monitoring investigations and the guideline (values in mg/kg dry weight (d.w))

Location	Cd	Zn	Cr	References
Al Hoceima coastline	0.812	159.9	3.121	Present work
Mediterranean Sea, Italy	0.05-0.06	0.55-42.80	0.14-0.65	[32]
Algerian west coast	2.74-6.56	205.4-575.9		[8]
Southeastern Black Sea	0.16-0.84	8.59-43.39	0.09-0.28	[33]
Giresun, Turkey		69.06	0.56	[34]
Western Scheldt estuary	27	643	5.1	[25]
ElJadida, Morocco	25.3	612.3	20.6	[35]
Galicia, Cantabria Spain	4.54	470		[36]
Andalucia coast, Spain	1.25	424		[37]
Galicia, Gulf of Biscay, Spain	4.54	423		[38]
Croatian Coasts, Adriatic Sea	0.99	158.00	1.72	[39]
Montenegro Coasts, Adriatic Sea		119.60	3.04	[40]
Baleari Islands, Spain	2.83	234.16	0.53	[41]
Marmara Sea, Turkey	0.75	88.98	2.46	[31]
Guideline	1.4	200-500		[42]
Guideline	2.0	1000	1	[43]
Guideline	4		12	[44]

The Cd, Zn and Cr are essential trace elements for human body. Food is the main source of human intake of these metals. However, in excess quantities, essential elements can also be poisonous and cause serious threats to human health [45]. Zn can cause gastrointestinal effects, induce Copper deficiency, and kidney and stomach damage [46]. Cd can be absorbed and bioaccumulated in the human body and pose a risk to human health by inducing reproductive deficiencies, kidney dysfunction and skeletal damage [47, 48]. For Cr, the major toxic and carcinogenic effects are those occurring in the skin, liver, kidneys and blood-forming organs [49]. The following is the comparison of our results with the maximum acceptable values set for Cd (1.4 mg/kg d.w - [42]; 2.0 mg/kg d.w - [43]; 4.0 mg/kg d.w- [44]), Zn (200-500 mg/kg d.w - [42]; 1000 mg/kg d.w - [43]), and Cr (1.0 mg/kg d.w - [43]; 12.0 mg/kg d.w - [44]). All the average contents of the determined metallic elements are lower than European Communities (EC) and United States (US) regulations and FAO limits for fishery products with the exception of Cr for the Food and Agriculture Organization [43]. Indeed, the concentrations of Cd found in our work were lower than those reported for Algerian west coast [8] and Galician and Cantabria coasts of Spain [36], but were higher than those reported for northwestern Mediterranean Sea of Italy [32]. On the other hand, our values for Cd were in agreement with ones reported for Marmara coast of Turkey [31] and southeastern Black Sea coast [33]. The levels of zinc (Zn) were lower than those indicated for Baleari Islands of Western Mediterranean of Spain [41], and El Jadida, Moroccan coast [35], but higher than those reported for Giresun coasts of the Black Sea of Turkey [33] and Montenegro Coasts of Adriatic Sea [40]. On the other hand, results for Zn concentrations were similar to those found by Kljaković-Gaspić et al. [39] from the Croatian Coasts of Adriatic Sea. Chromium (Cr) levels of the present study were greater than those studies reported by Gedik [33] from Southeastern Black Sea coast from Turkey. Our Cr findings were lower than those reported for El Jadida, Moroccan coast [35]. Chromium levels of the present study agreed well with the findings presented by Joksimovic et al. [40] for Montenegro Coasts of Adriatic Sea.

4 Conclusion

The present study reveals interesting results concerning bioaccumulation of Zn, Cr and Cd in *M. galloprovincialis* collected from the coasts of Al Hoceima during the period of 2018. In general, the content of metals in mussel tissues were decreased by season as Zn > Cr > Cd. The metallic element contents in tissues of bivalves displayed significant variation with season, being lowest in summer and

highest in winter. The highest values of metal bioaccumulations observed in the wet period were attributed to the local anthropogenic (industrial, agricultural and urban) activities which affect the abiotic factors of Al Hoceima seawater and also to the physiological states of mussels. *Mytilus galloprovincialis* from the studied region is safe concerning the contaminants investigated because metal concentrations remain within the permissible range established by various international organizations, with the exception of Cr for the Food and Agriculture Organization [43]. Nevertheless, the high value observed, for Cr, confirms that in seawater regions with mussel farming activity, contamination by heavy metals must not be underestimated, thus trace metal contents should be subjected to periodic biomonitoring for human and environmental health. Our work supports the importance of *M. galloprovincialis* as bioindicators for measuring the pollution levels of aquatic environment of the Moroccan Mediterranean coasts.

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