

Biomonitoring study of trace metals (Al, As, Li) in mussels from Al Hoceima coastline of Moroccan Mediterranean Sea

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Abstract. *Mytilus galloprovincialis* are commonly used as heavy metal biomonitors across the world. In the present work, the contents of three elements (Al, As, Li) were analyzed in *Mytilus galloprovincialis* using Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES 720-ES). Samples of soft tissues are collected monthly in 2020, from three sites of the mussel farming facilities installed in the Al Hoceima Sea. The decreasing order of the mean element contents in mussel tissues was Al > As > Li. The lowest values of metallic elements were found in summer and the highest contents were indicated in winter. Positive correlations were indicated between these elements and chlorophyll *a* indicating the importance of diet for the bioaccumulation of metals in mussels. In addition, our results show that the temperature and the salinity increase with the decreasing of metals bioavailability suggesting the influence of chemical properties and physical kinetics changes in the solution. On the other hand, the strong correlations observed for metal contents in mussel tissues can be elucidated by their common sources. The results of this study may be useful in the use of *M. galloprovincialis*, in the environmental biomonitoring studies.

Keywords – biomonitoring, Al Hoceima coasts, *M. galloprovincialis*, abiotic parameters, monthly variations, heavy metals.

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1 Introduction

The cities of the marine coastal environment have often been identified as hot spots for various types of pollution [1]. Urbanization, agriculture and industrial developments are taking place at an accelerated rate in the coastal areas around the world [2]. These developments regenerate different sources of pollutants, which have been identified as one of the most important sources of contamination loading on the Mediterranean coast [3]. Heavy metals are a major anthropogenic contaminant in marine environment [4], because they cannot be degraded and persist in the environment; consequently, they accumulate in aquatic organisms [5]. Upon recognizing the danger of pollution from metallic elements and the negatives effects on the ocean's ecosystems, new ways to monitor and control this contamination have been found such as Mussel Watch programs used to assess contamination of the coastal environment since that they were proposed in the 1970s [6]. In fact, mussels are considered to be one of the best bioindicators [7, 8] due to their widespread use for biomonitoring for coastal marine pollution studies [9, 10]. Their specific life traits, such as a wide geographical distribution, a sessile, abundance, a relative resilience to contaminant and filter-feeding behaviour, display advantages for validating the use of mussels in metal monitoring studies [11]. Mussels such as *Mytilus* could accumulate substances in their tissues to levels significantly higher than the environment in which they live [12, 13]. Therefore they are the vectors of metals in the food chain trophic levels of the ocean's ecosystem [13-15], which explain the use of the *M. galloprovincialis* in the international Mussel Watch program for monitoring several pollution in the seas and oceans [16, 17].

The accumulation of trace elements in mussel tissues can be affected by many abiotic factors, such as, temperature, pH, salinity, dissolved oxygen and diet. The endogenous processes (e.g. growth, age and reproductive cycle) are also known to influence metal concentration and accumulation in these organisms [18]. Seasonal fluctuation have been linked to a large extent to seasonal variation in meat weight during the gonad tissue development [8, 19-21].

The objective of the present work is three-fold: (1) to analyze the trace element (Al, As and Li) compositions

of mussels (*Mytilus galloprovincialis*), (2) to discuss the impacts of seawater parameters (temperature, salinity and Chl *a*) on the trace metal concentrations in mussels, finally (3) to highlight the information of the use of *M. galloprovincialis* in biomonitoring studies of metals in the Mediterranean coasts of Morocco.

2 Material and methods

2.1 Sampling zone, sample collection and preparation

Soft tissues of the Mediterranean mussel, *M. galloprovincialis*, were obtained from three sampling sites (Fig. 1). Mussel and water samples were collected monthly during 2020. At the laboratory, 50 mussels (average value \pm standard error of the mean (SEM) of the whole weight, total width and length of samples was 30.2 ± 2.29 g and 40.1 ± 1.21 , 67.2 ± 2.02 mm) were dissected, and their soft tissues were removed from the shell and pooled into one sample for each sampling sites. The tissue samples were dried and homogenized with a mixer before chemical determinations as described in a previous study by Azizi et al. [8]. Samples of seawater were taken by hand into four bottles from each site. Mussel and water were transported at $+3$ °C in a cold box to the laboratory until analysis.

2.2 Abiotic factors of seawater

To provide information about the water quality of Al Hoceima coasts, some abiotic factors (salinity and temperature) were determined *in situ*, and other in the laboratory (chlorophyll *a*). All these analytical techniques have been well described in our previous works [8, 22].

2.3 Heavy metal analyzes

The metal (Aluminium, Arsenic, Lithium) concentrations were determined in soft tissues of *M. galloprovincialis*, as we well described in a previous study by Azizi et al. [8].

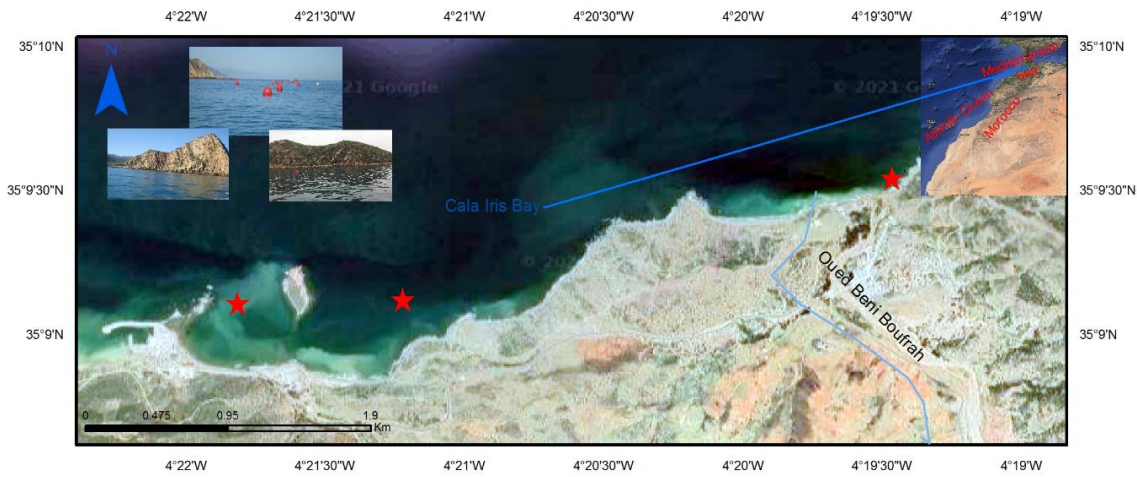


Fig. 1. Sampling map of mussels from the Al Hoceima coastline (Moroccan Mediterranean Sea).

3 Results and Discussion

3.1 Metal Al, As, Li contents in *Mytilus galloprovincialis* soft tissues

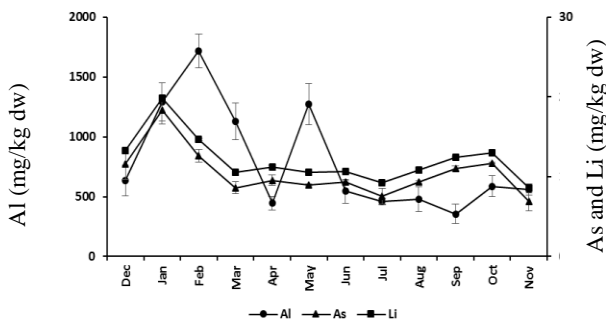


Fig. 2. The mean \pm SEM contents (mg/kg dry weight (DW)) of metals (Aluminium, Arsenic, Lithium) in soft tissues of *Mytilus galloprovincialis*.

The monthly metal (Al, As and Li) concentrations in *M. galloprovincialis* were indicated in figure 2. The metal contents showed a decreasing order: Al > As > Li. The maximum average concentrations for Al (1215.0 mg/kg), As (14.2 mg/kg) and Li (1.74 mg/kg) were observed in the winter season, while the minimum mean contents for Al (494.9 mg/kg), As (8.74 mg/kg), and Li (1.46 mg/kg) were indicated in the summer period. Similar results have been found by many researchers [23-29]. Bouthir et al. [25] indicated in the Moroccan Atlantic Ocean, higher contents of metallic elements in tissues of *M. galloprovincialis* during the wet period (winter), whereas lower contents were observed in the hot period (summer). The seasonal fluctuations in metal levels in Al Hoceima coast may be due to a combination of factors, directly related to weight (reproductive cycle, sexual maturity, abundance of food and environmental conditions) [30, 31], but also others, independent, such as the biogeochemical cycle change and the bioavailability of metals in the seawater [32]. Numerous investigations have indicated that physiological processes, such as the gametogenic cycle, affect the seasonal fluctuation in

heavy metal contents in the soft tissues of bivalves [24, 25]. However, another possible explanation for the pattern of metallic bioaccumulation in mussel tissue could be found under climatological conditions which characterize the Mediterranean basin, with hot and dry summers, and wet and rainy winters. Indeed, the increase in heavy metal contents in mussels during the wet period (winter) maybe affected mainly by human activities developed in the studied area, which is related to the soil drainage during the rainy period. Trace elements can reach runoff from nearby rivers and contaminate the seawater of Al Hoceima coasts. Once in the study area, they can be absorbed and accumulated in mussels. Similar results have been found by the authors (Kaimoussi et al. [24]; Fowler and Oregioni [33]). Previous work, Fowler and Oregioni [33] suggested that the maximum trace metal content in mussels from the northwestern Mediterranean Sea occurred at the end of winter when precipitation is very abundant. The same results were obtained by the authors (Szefer et al. [19]; Mubiana and Blust [20]; Giarratano et al. [34]), who attributed their observation to the effect of salinity. Food availability may also explain the bioaccumulation of metallic elements in mussels. In fact, the maximum content of metals in winter coincides with the maximum contents of phytoplankton, as it was described in our work (Fig. 3). Azizi et al. [35] found that metallic elements could be incorporated into molluscs through the phytoplankton. Several authors (Wang and Rainbow [36]; Azizi et al. [37]) observed that the quality and quantity of food could influenced the diet assimilation and the rate of metal ingestion by marine biota.

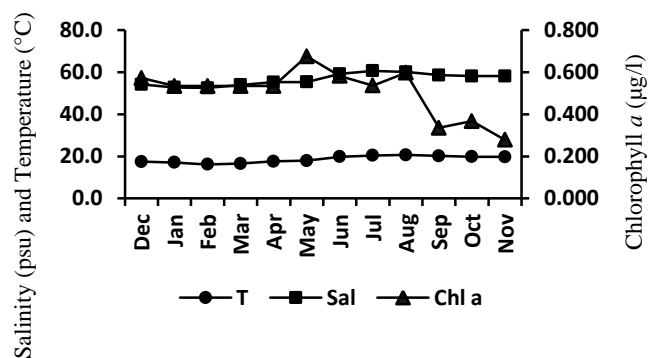


Fig. 3. Annual variations of salinity, temperature, and chlorophyll *a* in seawater from Al Hoceima coastline of 2020.

3.2 correlation coefficients between metal levels in *M. galloprovincialis* and abiotic parameters of Al Hoceima seawater

Table 1. Pearson correlation coefficients between content of the elements (Aluminium, Arsenic and Lithium) in *M. galloprovincialis* and abiotic parameters of Al Hoceima seawater

	Al	As	Li	Sal	T	Chl <i>a</i>
Al	1	0.318	0.698**	-0.525**	-0.488*	0.758**
As		1	-0.053	-0.556*	-0.174	0.798**
Li			1	-0.212	-0.261	0.467*
Sal				1	0.872**	-0.131
T					1	-0.354
Chl <i>a</i>						1

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Sal: salinity; T: temperature; Chl *a*: chlorophyll *a*.

Table 1 shows the correlation analyses between abiotic factors determined in seawater and investigated metal content in soft tissues of bivalves from the studied coasts. Li was significantly correlated with Chl *a* ($r = 0.467$, $P < 0.05$), As with Chl *a* ($r = 0.798$, $P < 0.01$) and Sal ($r = -0.556$, $P < 0.05$), and Al with Chl *a* ($r = 0.758$, $P < 0.01$), T ($r = -0.488$, $P < 0.05$), and Sal ($r = -0.525$, $P < 0.01$). The studied metals were strongly correlated with the chlorophyll *a* indicating the importance of phytoplankton in the incorporation and bioaccumulation of heavy metals in the flesh of mussels. Similar trends were observed by Bocchetti and Regoli [38] and Mubiana and Blust [20] studying the effect of temperature on metal assimilation by these marine organisms. The effects of temperature on absorption are largely due to changes in the physicochemical properties of water, which favours higher absorption at low temperatures, which are in agreement with the results mentioned in Table 1. The salinity gradient also affected the seasonal changes of metal incorporations by bivalves [39]. In fact, mussels absorb metals better at low salinity than at high content, which coincides with our results reported in Table 1 indicating that the mussels absorbed metallic elements at low salinities.

Pearson correlation coefficients for investigated metals show moderate correlations observed in Al *versus* Li ($r = 0.698$), at the significance level 0.01. Our results from Table 1 on the positive correlations between the metallic trace elements in the flesh of the mussel can be explained by a synergistic interaction between metals or by a common source of these pollutants [40]. In the present work, the common source of metallic elements, which are associated with effluents coming from domestic and industrial activities developed in the region, could explain the strong correlations observed for metal levels in bivalves [41, 42].

4 Conclusion

This work provides significant results and database for biomonitoring studies in coastal environments. The concentration of metals in mussels *M. galloprovincialis* show the decreasing order: Al > As > Li. The heavy metal contents showed significant variation between seasons. The highest values found in mussels during the winter period, and the lowest contents indicated in the summer period were due to the climatological conditions that characterize the Mediterranean basin and to the human activities developed in the Al Hoceima coasts. In fact, during the rainy period (winter), the runoff water drain the metallic elements coming from the anthropogenic activities and reach to the seawater of the studied region. These pollutants can be uptake and bioaccumulate in the bivalves. The diet availability can also affect the seasonal variations in heavy metal contents observed in *M. galloprovincialis*. The variation in body weight of *M. galloprovincialis*, which is related to the development of gonads, affects the seasonal variations of metals in mussels. The common origin of these metallic elements in tissues of mussels which is associated with the anthropogenic activities developed in the study area, is indicated by the correlations between them. Pearson correlations revealed that the heavy metal studied in tissues of molluscs were correlated to the environmental factors of seawater, especially for chlorophyll *a*, indicating the importance of food for the accumulation of the elements in the *M. galloprovincialis*. The results of the present investigation could serve as a reference for future assessments of possible chemical contaminations in the marine environment of Al Hoceima.

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