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Biomonitoring heavy metals (Cu, Li and Mn) in the Marchica Lagoon of Morocco using *Mytilus galloprovincialis*

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Abstract. Concentration of Copper, Lithium and Manganese were determined in the whole soft tissues of *Mytilus galloprovincialis*, collected from the two sites (Bni Ansar and Kariat Arekmane) of the Marchica lagoon of Morocco. The mussels were sampled on December and July of 2019. The ability of mussels to accumulate metals was arranged in the following order: Li < Cu < Mn. The levels of heavy metals in *M. galloprovincialis* were higher ($P<0.05$) in December (7.38, 2.63 and 11.10 mg/kg d.w., for Cu, Li and Mn, respectively) than July (5.56, 1.85 and 7.24 mg/kg d.w., for Cu, Li and Mn, respectively) because of the environmental parameters of the seawater and the physiological status of the animal. The trends of accumulations of investigated metals in mussel were higher ($P < 0.05$) in samples from Bni Ansar than from Kariat Arekmane sites, because of the urban and industrial discharge that submitted the zone of lagoon near to the Bni Ansar city. The Mn concentration in the mussel exceeded the acceptable guidelines limits indicated by international organization, which suggests that consumption of bivalves represents a threat to human health. The studied mussel is suitable biomonitors to investigate heavy metals contamination in the coastal area of the Moroccan Mediterranean coasts.

1 Introduction

On a global scale, coastal lagoons occupy nearly 13% of the coastline and are present on all continents [1]. These lagoon ecosystems are subjected to strong pressures by anthropic activities.

The human activities around the lagoons produce organic and inorganic contaminants such as heavy metals that reach the marine ecosystems [2].

These metallic elements are known to be toxic and to cause serious human health problems when the levels of these metals exceed the accumulation thresholds in marine organisms as reported by various international organizations [3, 4].

In this sense, the accumulation of heavy metal in shellfish from the marine coasts must be monitored regularly to give information on the risk levels for consumer. Monitoring programs remain among the best tools to know and control metal pollutants in aquatic ecosystems [5-8]. The mussels, sessile species, have been widely investigated and used to monitor metal pollution in aquatic environments and to reveal the bioavailability of metals by marine biota [9, 10].

For this purpose, several biomonitoring investigations carried out in Moroccan coastal zones using *M. galloprovincialis* revealed that the main anthropogenic sources of metal contaminant result from domestic, agriculture, and industrial inputs [11-15].

In the present work, we studied the possibility of using *Mytilus galloprovincialis* as bioindicators of environmental pollution in the Marchica lagoon. The effect of spatio-temporal variations on heavy metal concentrations in the wild blue mussel, *M. galloprovincialis*, was determined.

The potential human risk associated with bivalve consumption was also evaluated.

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2 Materials and methods

2.1. Study Area and sampling strategy and analyses

The lagoon of Nador or the Marchica lagoon, also called Sebkha Bou Areg is the largest Mediterranean lagoon in Morocco, with an area of 115 km². It is located in the north-eastern Mediterranean of Morocco, between 'Cap des Trois Fourches' and 'Cap de l'eau'. Marchica lagoon is considered among the most productive areas of the Moroccan Mediterranean and has been identified as a 'Site of Biological and Ecological Interest' of great importance. This wetland has also been classified as a RAMSAR site since 2005 [16, 12]. A new channel of 300 meters in width and 6.5 meters in depth was opened to improve the water quality of the aquatic ecosystem.

The *Mytilus galloprovincialis* were collected from two locations (Bni Ansar and Kariat Arekmane) of the Marchica lagoon (Figure 1) in the wet (December) and summer (July) seasons of 2019.

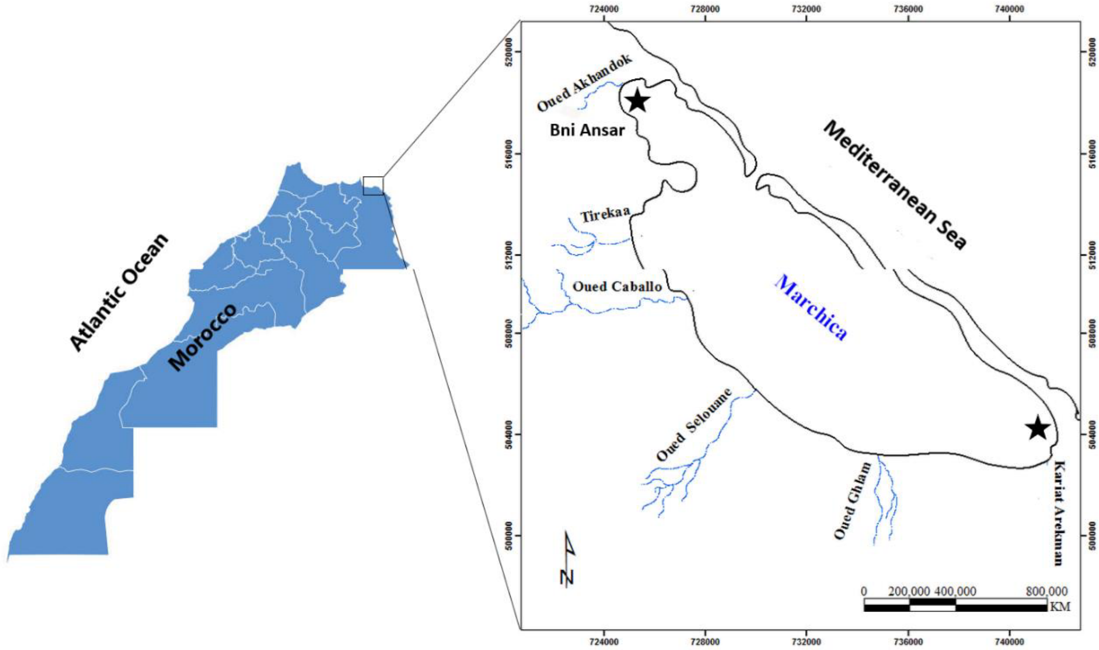

Fig. 1. Mussel sampling sites at the Marchica lagoon.

About 60 Mediterranean mussel, 7-8 cm in length and 28- 30 g in weight were taken from each sampling sites, to ensure homogeneous distribution of samples. 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. The whole soft tissues of mussels were separated from the shells to avoid contamination, and were dried in an oven for 48 hours at a temperature of 72 °C [17, 18].

For each site and season, the tissues of approximately 10 mussels were combined then ground in a glass blender which was equipped with a stainless steel cutter for chemical analyses as described in previous works [17, 10].

The concentration of Cu, Li and Mn in the 0.5 g of ground and homogeneous soft tissues of mussel samples was determined by inductively coupled plasma – optical emission spectrometry (ICP-OES 720-ES) method, (Agilent Technologies, USA) as described in previous work by authors [17, 19]. To study the effect of time and space on the heavy metal concentrations, we used one-way analysis of variance (ANOVA) followed by Tukey's test. ANOVA data with a $P < 0.05$ was statistically significant.

3 Results and Discussion

3.1. Temporal variation of Cu, Li and Mn in whole tissues of mussels

Figure 2 shows the seasonal variations of Cu, Li and Mn concentrations in mussels (*M. galloprovincialis*) from the Marchica lagoon coasts. Heavy metal indicate a clear temporal fluctuation ($P < 0.05$). Highest Cu, Li and Mn values were observed in December (7.38, 2.63 and 11.10 mg/kg d.w., for Cu, Li and Mn, respectively), and lowest contents were observed in July (5.56, 1.85 and 7.24 mg/kg d.w., for Cu, Li and Mn, respectively).

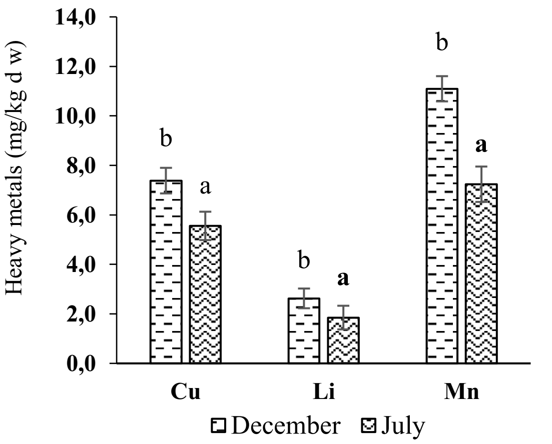

Fig. 2. Temproral variations of heavy metal (Cu, Li and Mn) concentrations (mg/kg d.w.) in *M. galloprovincialis* from the Marchica lagoon. Results expressed on mean values ± standard deviation, values marked with different letters (a and b) indicate significant differences (P < 0.05) between seasons.

Similar results were obtained by Azizi *et al*. [13] in the Cala Iris sea of Al Hoceima (Morocco), in which the metallic elements values were higher in winter and lower in summer.

Authors [11, 12, 14, 15] attributed these changes to the physiological status of the animal and the environnmental parameters of the seawater. In this sense, Azizi *et al.* [19] reported that during the summer period, the bivalves increase their body weight, therefore, the concentration of heavy metals will be diluted in flesh of the mussels. Considering the environmental parameters, the bioaccumulation of metals was higher in winter, probably due to the slower growth at cooler temperature [13, 20, 21].

Mubiana and Blust [22] showed that low temperature favours higher uptake of metals by marine biota, which is our case, where the mussel absorbs more heavy metals in December.

3.2. Spatial variations of Cu, Li and Mn in whole tissues of mussels

Copper, Lithium and Manganese concentrations measured in mussels' soft tissues from Bni Ansar site (6.84, 2.56 and 9.65 mg/kg, for Cu, Li and Mn, respectively) were higher (P < 0.05) than those observed in *M. galloprovincialis* from Kariat Arekmane site (6.10, 1.92 and 8.69 mg/kg, for Cu, Li and Mn, respectively) (Figure 3). Ours results were consistent with that observed in bivalve tissues along the Moroccan Mediterranean Sea [19, 13]. This agrees well with recent studies that show that bivalves accumulate more heavy metals in polluted than in unpolluted areas [23, 15].

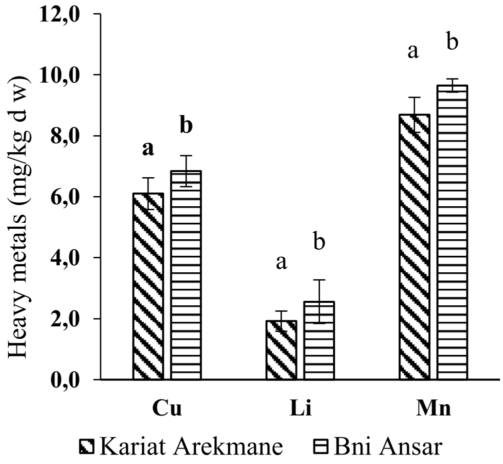

Fig. 3. Spatial variations of Cu, Li and Mn concentrations (mg/kg dry weight) in soft tissues of *M. galloprovincialis* from the studied areas. Results expressed as mean values ± standard deviation, values marked with different letters (a and b) indicate significant differences (P<0.05) between sites.

The Bni Ansar site in the Marchica lagoon undergoes considerable anthropogenic aggression by harbor activities and industrial and urban wastewater. The domestic discharges are discharges from the towns of Nador and Bni Ansar without prior treatment and from the recreational ports installed on the borders of Bni Ansar and Nador cities, which contribute to the deterioration of the quality of the marine environment of this area of the Marchica lagoon.

The rivers supplying the Nador lagoon are mainly the Oued Caballo crossing the Zegangan and Nador towns, and the Oued Selouane crossing the towns of Arouit and Selouane. The two Oueds also cross the various agricultural lands, thus transporting the chemical substances of various nature used in agriculture during the rainy seasons. However, in the Kariat Arkmane site, no eutrophication phenomenon was observed.

Table 1. Maximum limits for heavy metal levels in bivalves (mg/kg d.w) indicated by various international organizations.

International guidelines	Cu	Li	Mn
Present study	6.470	2.238	9.167
[4]	50-150		
[3]	70		
[24]	20		
[25]			5.4

The metal (Cu, Li and Mn) concentrations in the *M. galloprovincialis* and the criteria for these metals in international safety guidelines are summarized in Table 1. Among the evaluated metals, the Mn concentration in the bivalve (9.17 mg/kg, approximately 2 times which specified in the criteria) exceeded the safe and permissible limit of the international guideline (5.4 mg/kg, [25]). The Mn has been reported to cause disturbances in circulatory system, promote tumour development and hypotension [26]. For Mn concentration, the mussel was considered unsafe for human consumption. The concentration of Cu (6.47 mg/kg) in *M. galloprovincialis* from the studied area was below the acceptable standards set by international organizations (50-150 mg/kg, [4]); 70 mg/kg, [3]) and 20 mg/kg, [24]). For Li concentration, no value of international guidelines was found.

4 Conclusion

Based on the heavy metal (Cu, Li and Mn) levels found in *M. galloprovincialis* between different study areas from the Marchica lagoon, we concluded that mussel could be used to identify new emerging pollutants in coastal environments and to follow their loading variations in space and time. Concerning the heavy metals studied in the tissue of mussels, we observed that the levels of Mn exceeded the permissible limits. In order to reduce the human health risk related to the metal intoxication due to the consumption of bivalves, environmental laws and policies relating to the discharge of effluents into the marine ecosystem should be established and strictly enforced in all the Moroccan coasts.

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