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Assessment of the Trace Metal Levels in Two Species of Mussels from the Agadir Marine Bay, South of Morocco

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The southern Atlantic coasts of Morocco are known for their fisheries resources and touristic beaches. Although, part of this ecosystem received several types of pollutants which are discharged in the seawater without any treatment. In previous studies, in which acetylcholinesterase and glutathione S-transferase activities were used as biomarkers of pollution in Agadir Bay, we demonstrated that marine organisms (*Donax trunculus*, *Mytilus galloprovincialis*, *Perna perna*) living at the sites receiving domestic and industrial wastewater were affected (Najimi et al. 1997; Kaaya et al. 1999; Lagbouri, 1997). The study of these organism's biology showed many perturbations in the reproductive cycle and growth (Id-Halla et al. 1997; Lagbouri, 1997).

In order to determinate whether metallic pollution was responsible for these perturbations, three heavy metals (zinc as essential metal, cadmium and copper known to have toxic effects) were analyzed in mussels. In fact, heavy metals were waste products of many industrial processes and were important causes of environmental pollution (Viarengo, 1985; Viarengo, 1989). Data about heavy metals in marine organisms from Moroccan coasts were rare and limited to the north Atlantic coasts (El Hraiki et al. 1994, Cheggour et al. 1999). The purpose of the present study was to assess the trace-metal content (Cd, Cu and Zn) in two species of mussels, the Mediterranean mussel *M. galloprovincialis* and the Africans mussel *P. perna*, collected in the Agadir bay (South of Morocco). The accumulation and transformation capabilities of pollutants make these sentinel organisms suitable for marine pollution monitoring (Lower and Kendall, 1990). The choice of Cd, Cu and Zn in this study, resided in the fact that they were the most heavy metals present in the wastewater in the area studied (Bari, 1994).

Otherwise, this work constituted also a contribution in the assessment of the heavy metal level in the African North-West coasts which were characterized by an important upwelling. It completes the studies realized in the Mauritanian part of these coasts, by Roméo and Gnassia-Barelli (1988) and Sidoumou et al. (1992, 1999).

Considering that many factors could affect the trace metals bioaccumulation in mollusks, e.g. physiological state, size and season (Roméo and Gnassia-Barelli

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1988), our study investigated the metallic concentrations according to the season, animal size and distribution in organs. It was realized in two different sites of the Agadir bay.

MATERIALS AND METHODS

Samples of standardized shell size (40 to 60 mm) of *Mytilus galloprovincialis* and *Perna perna*, were collected monthly between January and December 1994. Two types of sites, representative of the Agadir bay, were considered : i) Cap Ghir, located at 50 km to the North of Agadir far from any source of pollution, is considered as reference site, and ii) Anza located at 10 Km to the North of Agadir and received domestic and industrial untreated waste waters. Animals collected were washed in fresh seawater, rapidly transported to laboratory and frozen at -30 °C until analysis. After thawing, soft tissues (whole animal or organs) were separated from shell, rinsed with a Tris-glycine buffer (1M glycine, 10 mM Tris-HCl, pH 7.4), and cut into small pieces. Samples were dried at 60°C to a constant weight and digested in nitric acid (65%) in teflon pressure vessels in a microwave oven. Trace-metal analyses (Cd, Cu and Zn) were carried out on the digestate solution using a flame atomic absorption spectrophotometer (GBC 904 AA) equipped with an air-acetylene flame (for Cu and Zn analysis) or with a graphite furnace (for Cd analysis). Deuterium background correction was used when necessary. The analytical procedure was checked using a standard reference material (TORT-2 lobster hepatopancreas) provided by the National Research Council of Canada. The results are shown in Table 1.

Table 1. Analysis of referential material TORT-2 (hepatopancreas of lobster from Canadian National Council of Research). The values indicate mean ± standard deviation (n=6).

Metal	Certified values (µg/g dry weight)	Our values (µg/g dry weight)
Cd	26.7± 0.6	25.9 ± 0.6
Cu	106 ± 10	100 ± 10
Zn	180 ± 6	177 ± 2

The differences between groups of means were tested with the analysis of variance (ANOVA) and then with the least significant difference. A regression analysis was developed to assess the influence of body size on metal concentrations.

RESULTS AND DISCUSSON

The results are presented below according to the season, animal size and organ distribution in the two sites.

Seasonal variations of Cd, Cu and Zn concentrations, in *Mytilus galloprovincialis* and *Perna perna*, were presented in Figure 1. Generally, in the reference and polluted sites, similar seasonal profile was showed for the three metals : the highest levels were observed in winter and spring and the lowest in summer and autumn. For example, in *M. galloprovincialis* from the reference site, the mean values (n=6) were 4.6 ± 1.5, 5.5 ± 1.5 and 189 ± 43 µg/g dw respectively

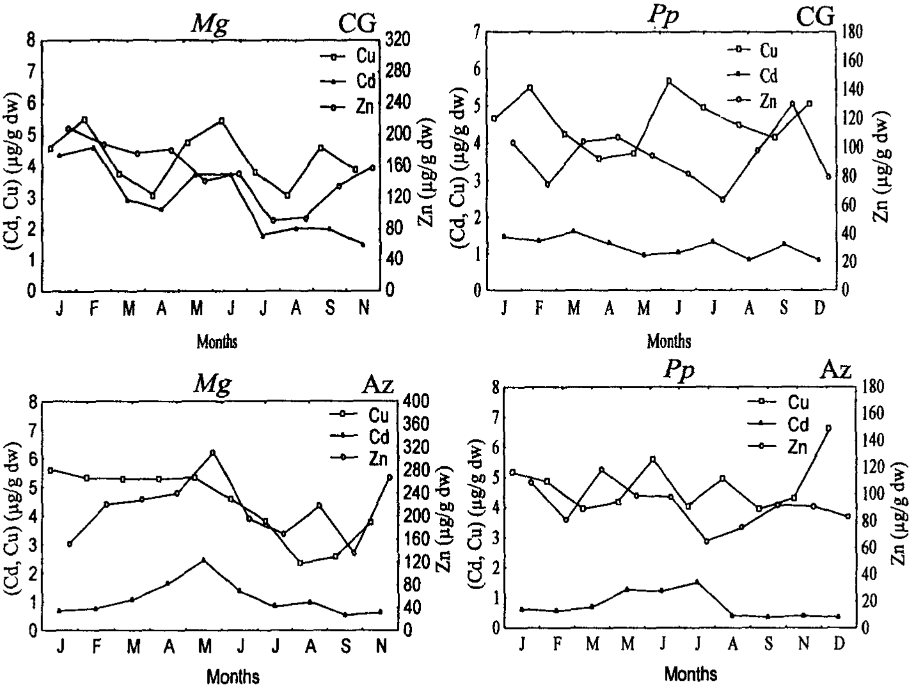

Figure 1. Seasonal variations of Cd, Cu and Zn concentration (µg/g dry weight) of *Mytilus galloprovincialis* (*Mg*) and *Perna perna* (*Pp*) sampled in the reference site Cap Ghir (CG) and in the polluted site Anza (Az).

for Cd, Cu and Zn in the winter (February) against 2.03 ± 0.9, 3.08 ± 0.8 and 95 ± 25 µg/g dw in the summer (August). Similar seasonal variations of heavy metals concentrations were also reported in *M. galloprovincialis* from the Northern Moroccan coasts (El Hraiki et al. 1994) and French coasts (Majori et al., 1978; Amiard et al 1986). Recently, Cheggour et al. (1999) noted the same variations in *Crassostrea gigas* from Oualidia lagoon (Moroccan Atlantic coasts). These variations could be linked to the mussels reproductive cycle already described by Id Halla et al. (1997) in Anza and Cap Ghir. The low concentrations in summer could be explained by the metal dilution in the organism which caused by weight increase resulted to the gonad development. In contrast, the release of genital products caused a weight decrease and a metal reconcentration in mussels.

According Roméo and Gnassia-Barelli (1988), many factors, such as body size, could affect the concentration of trace-metals in mollusks. Boyden (1974) reported that the total trace metal content per individual (Y in µg/g) could be related to body weight (W) as the power function $Y = aWb$, which after logarithmic transformation yields a linear regression of the form: $\log Y = \log a + b \log W$ (with W = dry weight in g, b = regression coefficient and a = Y intercept). In order to establish this relation for *M. galloprovincialis* and *P. perna* from the Agadir bay, we have considered all samples collected during the annual cycle. Equation parameters for these two species were reported in Table 2. These results showed that, except the case of the Cu in *P. perna* ($b \approx 0$), for the three metals,

Table 2. Parameters of equation of Boyden, relating Cd, Cu and Zn concentrations (µg/g) to dry weight of whole soft parts of *M. galloprovincialis* (A) and *P. perna* (B) sampled in the reference site, Cap Ghir (CG) and polluted site, Anza (AZ).

A

	Mytilus galloprvincialis									
	Loga		*b*		*R*		*n*		*P*	
	CG	AZ	CG	AZ	CG	AZ	CG	AZ	CG	AZ
Cd	0.192	- 0.860	- 0.44	- 0.47	- 0.505	- 0.344	60	57	<0.001	<0.01
Cu	0.949	0.644	- 0.25	- 0.43	- 0.421	- 0.457	60	57	<0.001	<0.001
Zn	3.817	4.741	- 0.58	- 0.33	- 0.472	- 0.310	60	57	<0.001	<0.05

B

	Perna perna									
	Loga		*b*		*R*		*n*		*P*	
	CG	AZ	CG	AZ	CG	AZ	CG	AZ	CG	AZ
Cd	- 0.405	- 1.11	- 0.44	- 0.74	- 0.487	- 0.506	60	57	<0.001	<0.001
Cu	1.535	1.507	- 0.03	- 0.04	0.044	- 0.088	60	57	NS	NS
Zn	3.932	4.299	- 0.49	- 0.22	- 0.605	- 0.359	60	57	<0.001	<0.01

(a = Y intercept, b = regression coefficient, r = correlation coefficient, n = number of samples and p = Student's test for b).

concentration and body weight were significantly correlated. The negative values of *b* indicated that the higher metal concentrations were in small individuals (Table 2). These negative correlation, more commonly observed than other correlations in mollusks, may be partially attributed to greater metabolic rate per biomass of smaller individuals or by a slight adsorption of metal by larger individuals (Blueweiss et al. 1978). Similar results has been reported in *M. edulis* (Boalch et al. 1981) and *Donax rugosus* (Sidoumou et al. 1992).

Table 3 presents Cd, Cu and Zn concentrations in *M. galloprovincialis* and *P. perna* collected in Cap Ghir and Anza. In order to minimize the influence of the body weight of animals on metal concentrations, comparison was made between the two sites with animals where no significant differences in weight were registered. *M. galloprovincialis* (n = 48) obtained from the polluted site contained significantly ($p<0.05$) more Cu and Zn than mussels obtained from the reference site (4.8 ± 1.3 and 212 ± 73 µg/g dw respectively for Cu and Zn in Anza but 4.2 ± 1.3 and 157 ± 61 µg/g dw for the two metals in Cap Ghir). On the contrary, higher Cd concentrations were noted in mussels collected in the reference site ($p<0.001$) in spite of its locality far from any human activity (3.2 ± 0.2 µg/g dw in Cap Ghir against 1.2 ± 0.7 µg/g dw in Anza). The high concentrations of Cu and Zn in mussels from the polluted site could be linked to the state of pollution in Anza. In fact, this site receives directly the discharges of an industrial (with oil refinery, cement factory, etc.) and domestic untreated wastewater. Data on pollutants nature in this site are still rare ; However, some preliminary results indicated important levels of heavy metals in sediment of this area (Id Halla 1997) and in wastewater released in the same site (Bari, 1994). For *P. perna* (n = 24), the Cu and Zn concentrations did not present a significant difference between polluted and unpolluted sites. However, as in *M. galloprovincialis*, Cd was higher in the

Table 3. Concentrations of Cd, Cu and Zn (µg/g dry weight) in whole soft parts of *Mytilus galloprovincialis* and *Perna perna* sampled at the reference site, Cap Ghir (CG) and polluted site, Anza (AZ). The values indicate mean ±SD.

	Mytilus galloprovincialis		*Perna perna*	
	CG	AZ	CG	AZ
Cd	4 ± 1.5	0.8 ± 0.3	1.5 ± 0.3	0.6 ± 0.2
Cu	4.6 ± 1.2	5.4 ± 1.4	4.8 ± 0.9	4.8 ± 0.8
Zn	195 ± 59	198 ± 55	110 ± 26	103 ± 29

reference site (1.3 ± 0.4 against 1.01 ± 0.5 in Anza). The absence of difference for Cu and Zn in two sites could be explained by a specific bioaccumulation process in *P. perna*. The abnormal high accumulation of Cd in the two mussels species from reference site (Cap ghir), could be explained by high levels of this metal in the seawater mass of currents upwelling which took place in these site coats. As described in the California coats by Martin et al. (1976), these marine currents could enrich the surface seawater by the Cd from the profound water mass. The same trend with Cd was reported by Romeo and Gnassia-Barelli (1988) in the Mauritanian coasts where are located similar upwelling currents.
Results about organ distribution of metals in *M. galloprovincialis* and *P. perna*, sampled in the two studied sites, were presented in Figure 2. Excepted, the Zn more accumulated in muscle of *P. perna* from polluted site, the gills and digestive gland seem concentrate metals more than other organs. Most organotropic studies on mollusks have been focused on accumulation experiments. They indicated that, generally, tissues where absorption takes place (like gills), contained more metals than other tissues (Bebianno et al. 1993). In field experiments, a fairly uniform distribution of Cd, Cu and Zn among the different organs of *M. edulis* was described by Amiard et al. (1986). However, Sidoumou et al. (1999) reported recently a preferential accumulation of Cd, Cu and Zn in the gills and digestive gland of *Crassostrea gigas* from the Mauritanian coasts. The important accumulation of metals in gills and digestive gland could be related to the function of these organs. The gills are in contact with the external medium and considered responsible of the metal transfer to organism. The digestive gland plays also an important role in heavy metal metabolism and contribute to their detoxification (Viarengo 1989). This could be explained the high metal concentrations in these organs. Concerning the important concentration of Zn observed in the muscle of *P. perna* from polluted site, in the absence of detailed studies, this fact could be attributable to many factors, including the physicochemical comportment of the metal in this site and the physiological processes involved in its accumulation in this mollusk.

Our study constitutes a contribution in the trace metal level assessment in coasts of Morocco. It completes the metallic pollution studies realized in the African North-Atlantic coasts by Roméo and Gnassia-Barelli (1988) and Sidoumou et al. (1992, 1999) (in the Mauritanian part of these coasts) and by El Hraiki et al.

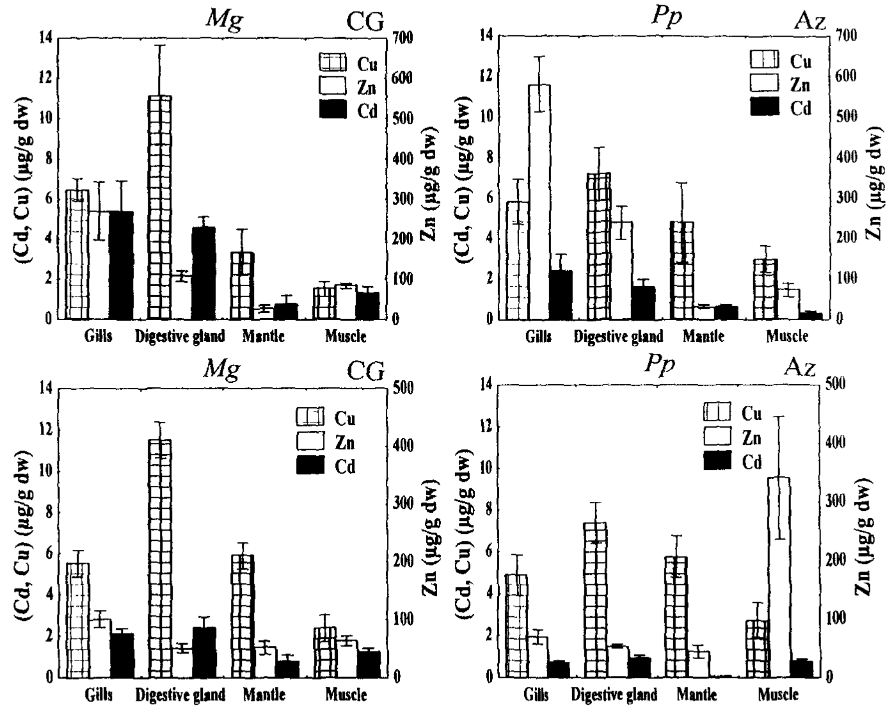

Figure. 2. Cd, Cu and Zn concentrations (µg/g dry weight) in organs. gills, digestive gland, mantle and muscle of *Mytilus galloprovincialis* (*Mg*) and *Perna perna* (*Pp*) sampled at the reference site, Cap Ghir (CG) and polluted site, Anza (Az). The values indicate mean ±SD, (n=6).

(1994) and Cheggour et al. (1999) (in the North of Moroccan part). Otherwise, the comparative study in the two mussels species showed that, like Mediterranean mussel *M. galloprovincialis* which was used as a sentinel organism in many European biomonitoring programs, African mussel *P. perna* which is widespread in the African coasts, could be used as a good sentinel species of the trace metal level assessment in African marine ecosystems. However, knowledge of the biology of this species and its trace-metals accumulation strategy must be more studied in order to make easy the interpretation of different results.

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