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Accumulation of Cadmium and Lead in *Cerastoderma glaucum* Originating from the Gulf of Gabès, Tunisia

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Among the Tunisian littoral zone, the Gulf of Gabès area has received most attention especially with regard to aquatic resources, environmental conditions and risks. In fact, this coast contributes significantly to the national production of fish and other marine organisms. Nevertheless, important industrial and agricultural activity as well as demographic and urban pressure is exposing coastal waters and marine organisms to increasing contaminant (Hamza-Chaffai et al. 1996; Zairi and Rouis 1999; Illou 1999; Sarbaji 2000). Toxic metals such as cadmium and lead are present in the anthropogenic sources. When introduced in the marine environment, they are accumulated by organisms and could play an important disrupting role leading to ecological and toxicological consequences.
Marine bivalves such as mussels and oysters are widely used in international monitoring programs. In the case of Tunisia these bivalves are available only in a limited northern area. Alternatively, the cockle *Cerastoderma glaucum* is an interesting species. In fact, this filter feeding organism is available and widely distributed. Moreover, it possesses many attributes required of bioindicators of metal contamination (Szefer and Szefer 1985; Szefer and Wolowicz 1993; Arjonilla et al. 1994; Szefer et al. 1999).

The lack of information about the Gulf of Gabès pollution state as well as the potential of toxic metals for damaging the fragile ecological equilibrium of this ecosystem justifies the importance of our study.
In the present work, we validate *Cerastoderma glaucum* as a bioindicator organism reflecting the pollution state of the Tunisian coast and its possible usefulness in the national monitoring program. In seven sites along the Gulf of Gabès area, we have investigated the accumulation of cadmium (Cd) and lead (Pb) in the whole soft tissues and in the different organs. We also studied metal variation linked to size.

MATERIALS AND METHODS

Organisms were collected from seven sites in February 2001. Six sites covered 120 km along the Gulf of Gabès area. The seventh one (Kerkennah) was located at 20 kilometers from in the eastern part of Sfax (Fig. 1).

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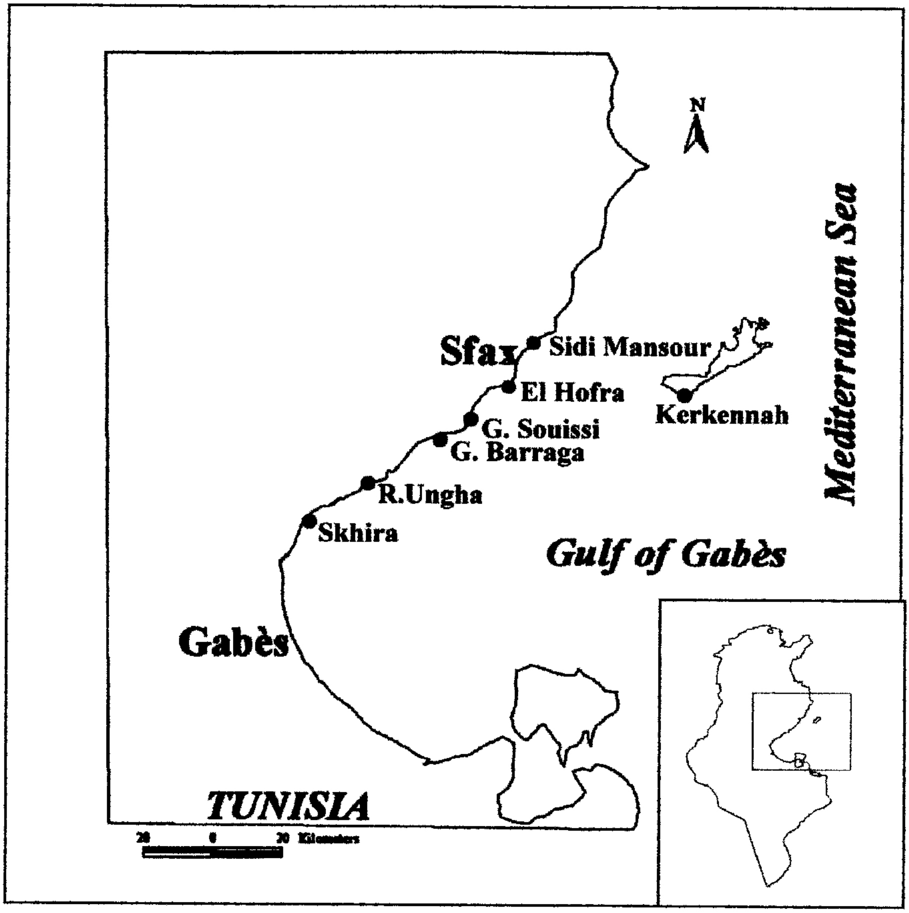

Fig 1. Location of the sampling stations in the Gulf of Gabès.

All samples were frozen until analysis. *In toto* analysis were performed on 15 specimens of standardized shell size (28±2 mm). When studying the accumulation of metals according to the tissues, 20 individuals were dissected; the gills, digestive glands and remainder (mantle, muscle and gonads) were isolated. To assess the trace metal content in the whole soft tissues as a function of size, analyses were carried out on 30 samples (shell size between 19 and 34mm).
For each individual, soft tissues were oven–dried (80°C) to constant weight. The samples were digested with concentrated nitric acid (2 ml of nitric acid per 0.1 g of dry tissue) until the solution was clear. Metals were then analyzed by flame (Pb) and flameless (Cd) atomic absorption spectrophotometry with graphite furnace equipped with Zeeman background correction. The standard addition method was used to correct the matrix effect. Concentrations of elements were expressed as µg/g on a dry weight basis. Standard reference material (fish homogenate MA-B-3/TM and mussel tissue SRM-2976 were used to control the quality of our analysis. Data obtained are shown in table 1.

Table 1. Comparison of metal concentration (µg/g dry weight) in standard reference material and our values.

	Cd	Pb
Fish homogenateMA-B-3/TM		
Our value	-	4.14 (0.90)
Certified values	-	4.62 (1.28)
Recovery %		89.61%
Mussel tissue, SRM-2976		
Our values	0.75(0.12)	1.00 (0.15)
Certified values	0.82 (0.16)	1.19 (0.18)
Recovery %	91.14%	84.03%

For statistical analyses, significant differences in metal concentrations between cockles originating from the seven sites were evaluated by an analysis of variance (one-way ANOVA) with significance level of 0.05. Multiple comparisons between localities were based on the Scheffé test. The SPSS software was used.

RESULTS AND DISCUSSION

In the whole soft tissues of *Cerastoderma glaucum* collected at the studied sites, metal concentrations varied between 0.08 and 2.5 µg/g dry weight for cadmium and from 0.13 to 1.07 µg/g dry weight for lead.

The analysis of variance revealed that the cadmium concentrations in *Cerastoderma glaucum* originating from the seven studied sites differ significantly (ANOVA $p < 0.05$) (Table 2).

According to Fig. 2, we noticed that cockles from EL Hofra (E.H) presented the highest cadmium concentrations (2.5 µg/g dw). Cadmium levels in this station were significantly different from those from the other sites (Scheffé test significant at $p<0.05$). Relatively high Cd concentrations were observed at Sidi Mansour (S.M) site. Intermediate levels were recorded at stations of G. Souissi (G.S), G. Barraga (G.B) and Skhira (SK). However the significant lower concentrations were observed at Ras Ungha (R.U) and Kerkennah (Kr) (Scheffé test significant at $p < 0.05$).

In the studied area, cadmium concentrations decreased significantly from the north (El Hofra site (EL.H), which is the most contaminated one), to the south (Ras Ungha (R.U), showing the lowest concentrations) (Sheffé test significant at $p < 0.05$). This could be explained by the influence of marine streams carrying pollution from the north to the south. However, relatively high cadmium concentrations observed in Skhira (SK) could be linked to the presence of an important plant, transforming crude phosphate and discharging cadmium in its effluent.

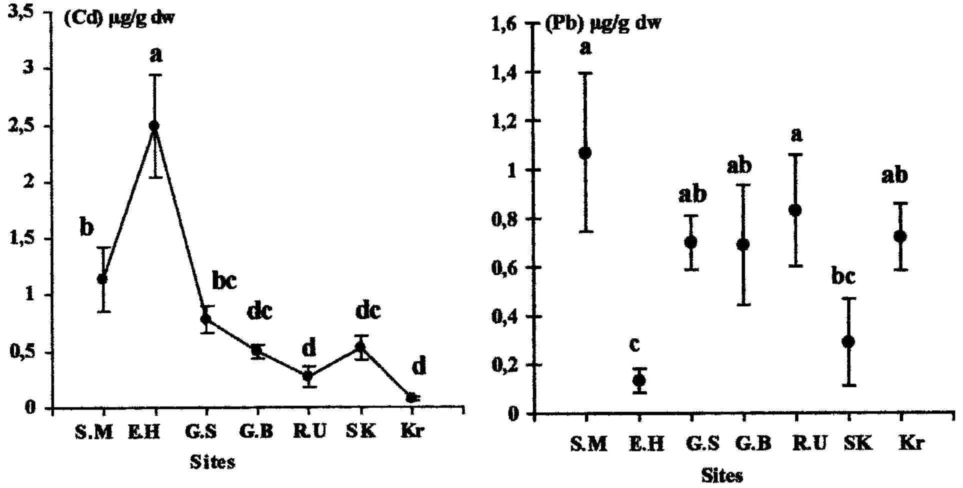

Figure 2. Comparison of Cd and Pb concentrations in the whole soft tissues of the cockle *Cerastoderma glaucum* collected from different areas at standardized size. Mean values and confidence intervals (95 % level) are shown. **S.M** = Sidi Mansour; **E.H** = EL Hofra; **G.S** = Gargour Souissi; **G.B** = Gargour Barragas; **R.U** = Ras Ungha; **SK** = Skhira; **Kr** = Kerkennah.
Sites with the same superscript indicate that they did not differ significantly at 95% level (Sheffé test).

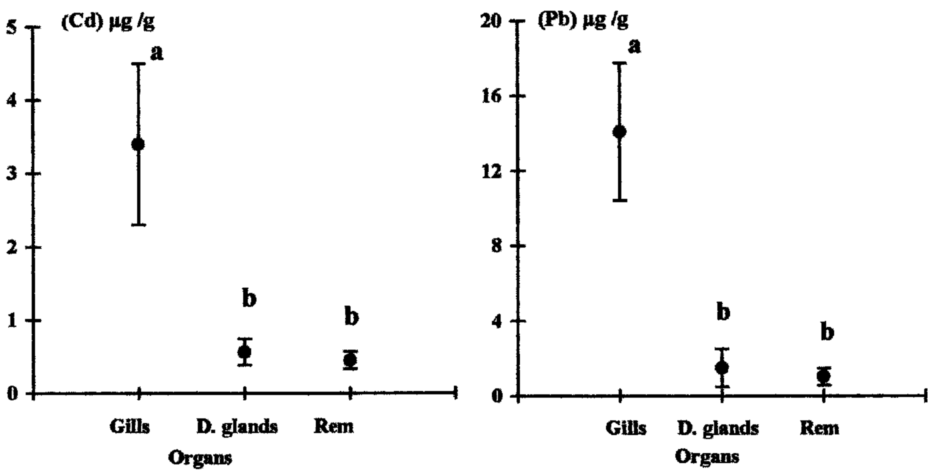

Figure 3. Concentrations (µg/g) of Cd and Pb in the different organs of *Cerastoderma glaucum*. **D. glands**: Digestive glands; **Rem**: Remainder
Organs with the same superscript indicate that they did not differ significantly at 95% level (Sheffé test).

For lead analysis, the highest concentrations were observed in Sidi Mansour and Ras Ungha, followed by G. Souissi, G. Barraga, and Kerkennah. The lowest level was recorded at El Hofra (Sheffé test significant at $p < 0.05$). The observed high levels could be linked to the navigation activity and the presence of petroleum plants especially in Kerkennah Islands. In the other sites, the diversity of the industrial activities mainly painting, plastic plant, PCV as well as the agricultural ones could be a major source of lead. Even if these activities are mainly concentrated in the northern sites (S.M), the southern ones are influenced indirectly by marine streams.

Table 2. Results of statistical analyses of variance.

Parameters	df	Probability	F Measured
Differences between sites	6		
Differences within sites	98		
Total variation	104		
Metals			
Cd		$< 10^{-29}$	57.55*
Pb		$< 10^{-7}$	10.21*

df: degree of freedom, (*) significant, (one-way ANOVA, 0.05) Critical value of F= 2.19

The high obtained cadmium and lead levels in *Cerastoderma glaucum* is in agreement with previous *in situ* studies about the contamination of the superficial sediment with Cd, Pb and other toxic metals (Illou 1999).

The average of metal concentrations obtained in *Cerastoderma glaucum* from the Gulf of Gabès is, in general, higher than those found for *Ruditapes decussatus* from the same area (El Menif-Trigui 1995; Hamza-Chaffai et al. 2000; Smaoui-Damak et al. 2003) and in the northern part of Tunisia (Chouba et al. 2001). Cd concentrations at El Hofra (2.5 µg/g dry wt) are similar to those obtained in Gulf of Lion (France) (Szefer et al. 1999) but much higher than those observed in the south of Spain (1.5 µg/g) (Arjonilla et al. 1994).

From a human health point of view, except for El Hofra, total metal concentrations obtained in the whole soft tissues of *Cerastoderma glaucum* from different stations remain below the acceptable levels (2 µg/g dry wt for Cd and Pb) (JORF 1995) and thus do not present any risk to human health.

Concerning the metal variation according to organs, our results showed that Cd and Pb were mainly accumulated by the gills and follow the following order: gills >> digestive gland ≈ remainder (Fig. 3). When comparing cadmium and lead accumulation according to the organs (Table 3), significant differences were observed between gills and other organs (digestive gland and remainder) (Sheffé test significant at $p < 0.05$).
The gills, which constitute only 1.5 % of total tissue dry weight, accumulated 77%

Table 3. Results of statistical analyses of variance.

Parameters	df	Probability	F measured
Differences between tissues	2		
Differences within tissues	42		
Total variation	44		
Metals			
Cd		$< 10^{-7}$	29.22*
Pb		$< 10^{-13}$	71.52*

df: degree of freedom, (*) significant, (one-way ANOVA, 0.05) Critical value of F= 3.21

Table 4. Metallic accumulation models in the different organs.

Organisms	Cadmium	lead	Reference
Ruditapes decussatues	G = DG >R	-	Roméo and Gnassia-Barelli (1995)
Ruditapes decussatus	G > DG >R	-	Bebianno et al. (1994)
Donax rygosus	G > DG > R	-	Sidoumou et al. (1994)
Cerastoderma glaucum	-	G > other organs	Szefer et al. (1999)
Cerastoderma glaucum	G > DG ≈ R	Br > DG ≈ R	Present Work

G: Gills; **DG**: Digestive Gland; **R**: Remainder. >: Superior, ≈ : No significant difference

of total cadmium and 85 % of total lead. They are- therefore the principal tissue absorbing these two metals, and the first organ directly in contact with the external medium. The abundance of toxic metal in the gills was mentioned in many bivalves (Amiard et al. 1986; Bebianno et al. 1994; Sidoumou et al. 1994; Roméo and Gnassia - Barelli 1995; Szefer et al. 1999) (Table 4)

In order to study the metallic variation according to size, Cd and Pb were determined in the soft part of individuals over a wide size range (19-34). Results are represented in Figure 4. Concentration of cadmium increases significantly with increasing size of *Cerastoderma glaucum* (n=30; r=0.48). This positive correlation indicates that the metal accumulation rate is faster than the excretion rate of the organism. However, lead variation shows a curvilinear relationship.
Lead concentration increases in the small and middle–sized cockles and decreases in the large-sized individuals. Maximum concentration is registered in middle-size cockles (25–28 m).

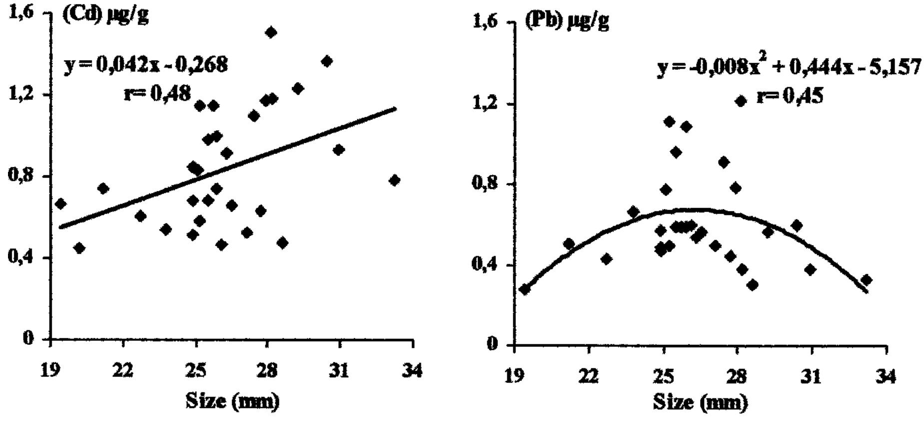

Figure 4. Relationship between metal concentrations in tissues (Cd, Pb) and body size of the cockle *Cerastoderma glaucum*

The reduction of metal concentration in the large sized individuals has often been observed in other bivalves. But there are no satisfactory explanations about the cause of this reduction. According to Bordin et al. (1992), inverse correlations can occur if metal uptake by small individuals is faster than uptake by large individuals. The reduction of Pb concentration in the large individuals may also be explained by tissue dilution effect due to increasing body weight (Rainbow 1996).

The first part of our study was based on analysis of cadmium and lead in the whole body of *Cerastoderma glaucum* collected from seven studied stations. It showed that the northern part of the Gulf was the most contaminated region. Indeed, this area is influenced by industrial and urban effluents generating metal pollution. This region is also contaminated by the stock of phosphogypsum, which is the main source of cadmium, lead and other toxic metals (Zairi and Rouis 1999).

In the second part, we demonstrated that the highest amount of cadmium and lead is accumulated by the gills. This may reflect the high levels of soluble metals in sea water. In fact, while gills are capable of more rapid integration and characterization of short-term pollution, the digestive gland is important for metal metabolism and it is considered as a long-term storage tissue, reflecting persisting contamination (Duquesne and all. 1995).

The size affects significantly metal accumulation and should therefore be taken into account. Throughout the third part of this work, we can see that *Cerastoderma glaucum* with middle size (25-28 mm) seems to be suitable for reflecting cadmium and lead pollution and for monitoring heavy metals in the Gulf of Gabès.

The Gulf of Gabès is not only the richest area in the Tunisian coast but also the most exposed to anthropogenic inputs and thus to the risk of alteration of marine resources. For this reason developing the national monitoring program is a matter of great interest.

This work fills the gap of information about the pollution state of this ecosystem by the mean of cadmium and lead analyses in whole soft tissues. Moreover, metal distribution in organs provides a more precise response about accumulation. As the size factor affects metal concentration it should be seriously taken into consideration in monitoring.

Cerastoderma glaucum offers an interesting biomonitor reflecting the available fraction of pollutants but more information is required about the physico-chemical forms of metals as well as some biochemical responses and their molecular regulation.

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