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Environmental Contamination and Toxicology

Preliminary Study of the Clam *Ruditapes decussatus* Exposed *In Situ* to Metal Contamination and Originating from the Gulf of Gabès, Tunisia

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The gulf of Gabès is situated in the southeastern coast of Tunisia and is exposed to pollution due to anthropogenic and industrial activities. This represents a danger for the marine ecosystem and specially for many molluscs abundant in this area. Among these molluscs, there is *Ruditapes decussatus* which is a filter-feeding marine bivalve, widely present in the gulf of Gabès. This species represents a natural fishery resource which is mainly exported to Europe and also consumed in Tunisia (CRDA 2002). *Ruditapes decussatus* is sedentary and tolerate wide ranges of contaminant concentrations.

In the gulf of Gabès, only few studies have dealt with metal in bivalves (Hamza-Chaffai et al. 1999, 2000; Machreki-Adjimi 2002) and practically no studies have treated metals in the whole soft tissues of *Ruditapes decussatus* originating from different sites along the studied area. The objectives of this work are to determine metal concentrations in the whole body of *Ruditapes decussatus* collected in five sites and to examine the environmental impact of the extensive industries setting up in this region.

MATERIALS AND METHODS

The five studied sites (covering 150 kilometres) are presented in Fig. 1. The species richness, biodiversity and the nature of sediment sites are given according to Machreki-Adjimi (2002).

Sidi Mansour (SM) is situated at 12 kilometres north of the city of Sfax and near an active fishing port. The stock of clams collected from this site is very important and represents about 16.8% of the total clams collected from different sites in Tunisia (CRDA 2002). Few species are present at this site. The sediment is muddy and black, covered with a thin layer of sand. At this site, it was shown that the surficial sediment was contaminated with Pb and Cr (Illou 1999).

The site of El Hofra (EH) is situated to the south of the fishing port of Sfax, surrounded by the industrial effluents and the stock of phosphogypsum. Dead bivalves and shellfish were noticed at this site. The substrate is sandy and muddy.

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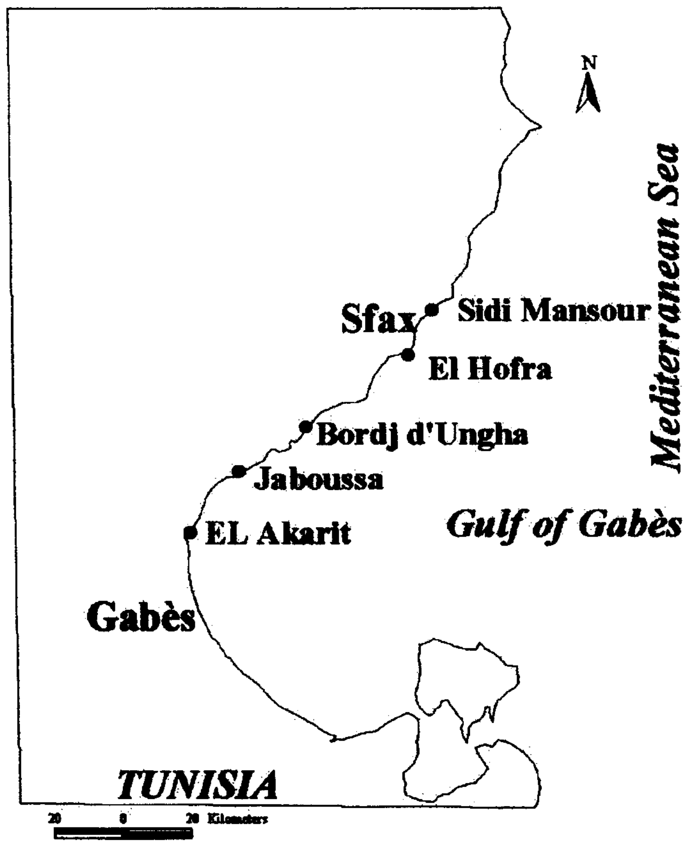


Figure 1. Location of the sampling stations in the gulf of Gabès

The site Bordj d'Ungha (BU) is situated 44 kilometres to the south of Sfax. It is rich of species (fish, bivalve molluscs and cephalopods). The substrate is muddy and black.

The site of Jaboussa (JA) is in the region of Skira, whose port is known for exporting oil and phosphate. Finally, El Akarit (EA) is an estuarian zone influenced by effluents carrying industrial pollutants which result from industrial activities situated in the north of the city of Gabès.

Clams (*Ruditapes decussatus*) were collected in April 2000 at the study sites in the gulf of Gabès (n=15 for each site). The shell length of the bivalves was measured. Samples were selected of similar size (37-40mm) in order to minimize the effect of body weight on metal concentrations. The soft tissues were separated from the shell, dried and weighed.

For metal analyses, the soft tissues from each individual were dried, then digested with suprapure nitric acid until the solution was clear. Metals were then analyzed by flame (Cu, Fe, Mn, Ni, Zn) and flameless (Ag, Cd, Pb) atomic absorption spectrophotometry with a graphite furnace equipped with Zeeman background correction. The spectrophotometers used were HITACHI Z6100 (Cu, Fe, Mn, Ni, Zn) and Z8200 (Ag, Cd, Pb). Analyses were achieved according to the method of standard additions in an iso-medium (Amiard et al. 1987). Standard reference material (fish homogenate MA-B-3/TM) was used to control the quality of our analysis. The analytical method has been validated by external intercalibration in sediment (Coquery and Horvat 1996) and in biological matrix (Campbell et al. 2000).

For statistical analyses, significant differences in metal concentrations between clams originating from the five sites were evaluated by an analysis of variance (one-way ANOVA) with significance level of 0.05. Then, a confidence interval (95% level) was used to compare the significance of differences in metal concentrations between clams from the site Bordj d'Ungha and those from the other sites.

RESULTS AND DISCUSSION

When we compared the total metal concentration in *Ruditapes decussatus* originating from the five studied sites (Table 1), we noticed significant differences between sites for all metals except for Zn.
According to Fig. 2, we noticed that specimens originating from El Hofra site presented high concentrations of Ag, Cd and Cu. Differences between El Hofra and the other sites were significant only for Cu.
However, the lowest and significant concentrations of Cd were obtained for clams collected from the site Bordj d'Ungha. At the same time, the concentrations of Ag and Cu were low for clams from the site (BU) but they were not always significant. Ni concentrations were high for the specimens collected from Bordj d'Ungha. In opposition, they were low and significant for clams from the site El Hofra. In clams originating from Sidi Mansour, Pb concentrations were high. They differ significantly from all the other sites except for El Akarit. Nevertheless, Fe and Mn were low in samples taken from Bordj d'Ungha. They were significant only for Mn.

From a human health point of view, at the El Hofra site, Cd concentrations (1.57 µg/g dry wt) were high but did not reach the norm value of 2 µg/g dry wt (Jorf 1995) (Fig. 2). Metal concentrations obtained in the other sites were all lower than the norm values (the norm values are 2 µg/g dry wt for Cd and Pb; 5µg/g dry wt for Cu and 100 µg/g dry wt for Zn). Therefore, Cd, Cu, Pb and Zn concentrations in clams do not for the moment represent a danger for the consumer.

Silver, a non-essential element, is naturally occurring as a trace element in all the compartment of the environment. But, it can also be toxic for organisms exposed to high concentrations. Silver has strong affinity with metallothionein, protein engendered in the detoxification of this metal. Indeed, experimental studies

(Berthet et al. 1992) revealed that the exposure of bivalves for Ag caused disturbance of the behavior in the mussels and the scallop and severe injury to the siphons of clams *Scrobicularia plana*. In our study a significant and highest concentration of Ag were observed in clams from the site El Hofra. This can reflect the metal level in the environment since important industrial activities (industry of phosphate; chemical and tannery industries) are developed in this area and can release metallic wastes.

Table 1. Results of statistical analyses of variance (one-way ANOVA, 0.05). Critical value of F= 2.5

Parameters	df	Probability	F measured
Differences between sites	4		
Differences within sites	70		
Total variation	74		
Metals			
Ag		$1.12\ 10^{-05}$	8.55*
Cd		$9.75\ 10^{-8}$	12.79*
Cu		$9.48\ 10^{-06}$	8.68*
Fe		$3.16\ 10^{-09}$	15.7*
Mn		$1.77\ 10^{-04}$	6.4*
Ni		$2.55\ 10^{-08}$	13.9*
Pb		$4.22\ 10^{-08}$	13.27*
Zn		0.41	0.9

df: degree of freedom, (*) significant

Cadmium bioaccumulation by marine organisms has been the subject of considerable interest in recent years. In fact, high levels of Cd may have detrimental effects on the marine organisms and may create problems in relation to human consumption. Previous studies (Henry et al. 1984) revealed that Cd was toxic to the clam at the level of 7.03 ± 3mg/l (LD_{50} 96h 14°C). Besides, Cd bioaccumulation kinetics in clams indicates that *Ruditapes decussatus* may be classified as a high Cd affinity species.

When we compare the concentrations of Cd in *Ruditapes decussatus* originating from El Hofra site, we noticed that they were similar to those obtained in Portuguese clam (1.09) (Bebianno et al. 1993) and lower than those of other molluscs (Table 2). This site is influenced by industrial effluents and is contaminated by the stock of phosphogypsum, main source of cadmium and other trace elements such as lead, zinc, mercury, copper and molybdenum (Zairi and Rouis 1999). In addition to the high concentrations of cadmium observed in the surficial sediment, Illou (1999) showed that the concentrations of Cd were higher at a water depth of 900m from beach line, therefore the accumulation of Cd persists and could have disastrous consequences.

Table 2. Comparison of trace metal concentrations (µg/g dry wt) in some bivalves from estuaries in different locations of the world (Brown and Depledge 1998) with metal concentrations in *Ruditapes decussatus* originating from the gulf of Gabès (present study*).

Taxon	Location	Cd	Cu	Fe	Mn	Ni	Pb	Zn
Crassostrea virginica	California	12-40	273-1510	148-260	13-34	-	-	3570-19300
Mytilus galloprovincialis	NW Mediterranean sea	0.4-6	2.4-15.5	149-2220	3-70	1-14	2.7-117	97-644
Mytilus edulis	SW England	0.8-2.6	4-13.6	152-401	5.2-35.5	1-3.5	30-105	57-199
**Ruditapes decussatus* (gulf of Gabès)	-Bordj d'Ungha	0.5	0.5	64	0.8	1.9	0.014	13.7
	-El Hofra	1.57	1	65	1.1	0.7	0.01	14.5

Table 3. Metal concentrations of the sediment in the northern region of Sfax (Illou 1999) [1], in an unpolluted sediment (Mediterranean sea) (Augier et al. 1980) [2] and the threshold of contamination (Laurent et al. 1977) [3]

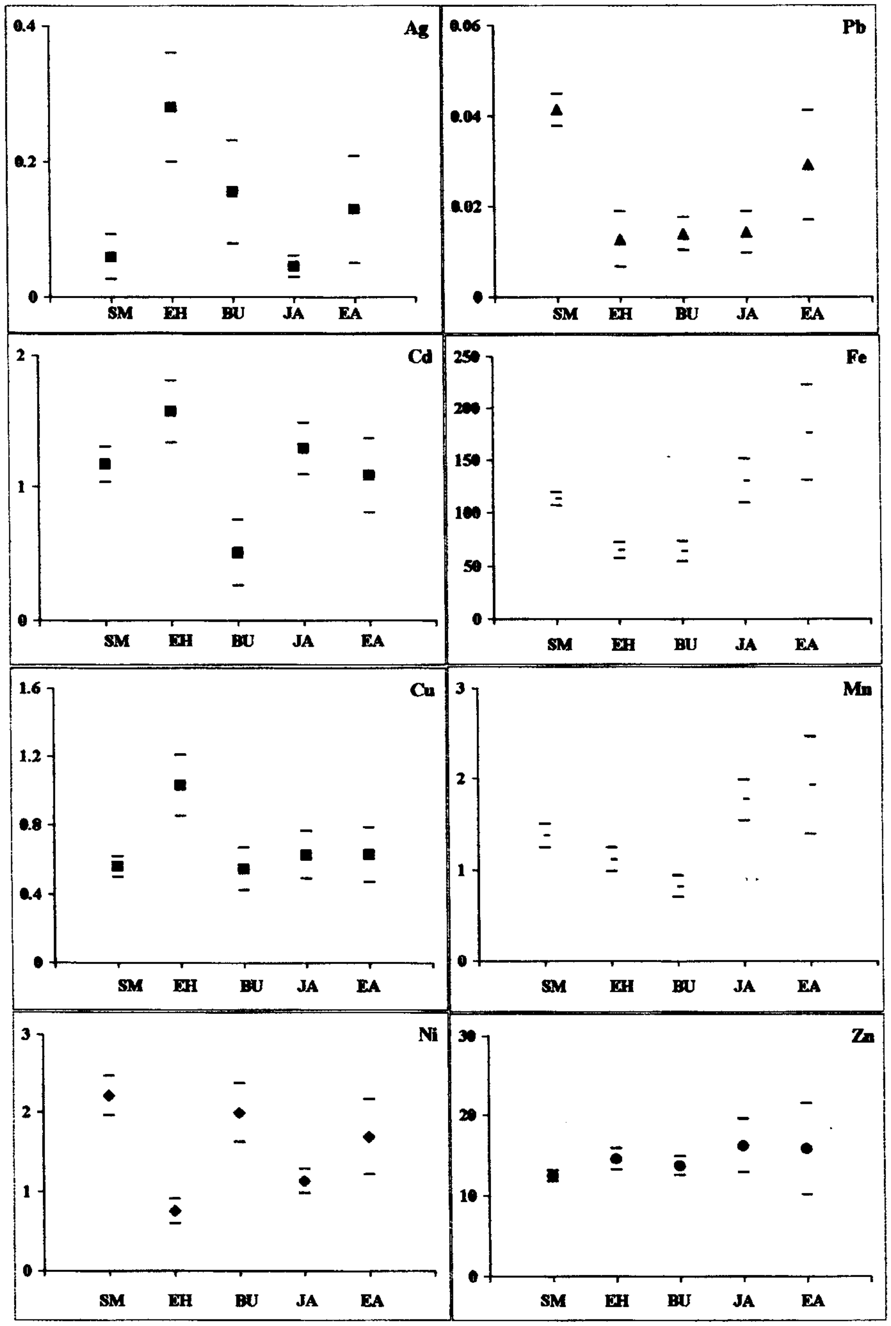
	Concentration of metals (µg/g dry wt) in sediment						
	Cd	Cu	Fe	Mn	Ni	Pb	Zn
[1]Sidi Mansour	7	170	18	160	10	113	280
[1]El Hofra	*70-230	250	26	201	24	70	*200-700
[2]Unpolluted sediment	0.9	6.9	-	-	9.2	21.5	41.2
[3]Threshold of contamination	3	30	-	-	50	60	60

*Minimum and maximum values

In Table 3, we notice that the distribution of trace metals in the sediment studied by Illou (1999) showed a high concentration of Cd in the northern region of Sfax (Sidi Mansour and El Hofra). Moreover, the concentrations of these metals exceeded the threshold values (Laurent et al. 1977). Therefore, the bioavailability of Cd in the environment could explain the increase of Cd uptake by clams in the site El Hofra.

Zinc and copper are essential metals but can also be toxic depending on their concentrations. The abundance of Cu and Zn in *Ruditapes decussatus* is a result of the biological role of the two essential metals in homeostasis. The comparison between copper and zinc concentration in *Ruditapes decussatus* with other molluscs (Table 2) showed that our results were not in the same order and were much lower. This could be explained by the differences linked to the site, the feeding, the ecology of these bivalve molluscs and the kinetics of accumulation of a metal in each species.

Concerning Cu, clams originating from the site El Hofra showed high concentrations. This could be the result of the contamination of the environment by toxic metals. Indeed, studies of the sediment (Serbaji 2000) collected near the stock of phosphogypsum showed a pollution by trace metals and high level of contamination by the organic substances in this area. Moreover, from the site El Hofra when we compared the Cu concentration in the sediment (Illou 1999) and the Cu concentration observed in *Ruditapes decussatus* of the present study (Table 3), we noticed also the same distribution in both the sediment and *Ruditapes decussatus*. The same observations have been shown by Hummel et al. (1997) in the baltic clams *Macoma balthica* compared with *Ruditapes decussatus*. *Ruditapes decussatus* was considered a better indicator of copper pollution than the mussel or cockle.



SM: Sidi Mansour; **EH**: El Hofra; **BU**: Bordj d'Ungha; **JA**: Jaboussa; **EA**: El Akarit

Figure 2. Means of metals (µg/g dry wt) affected with confidence intervals (95% level) in *Ruditapes decussatus* collected from the five studied sites in the gulf of Gabès

Although nickel has many uses in industries (especially stainless steel products), it can cause environmental problems and is known to be carcinogenic. Clams originating from Sidi Mansour, presented high concentrations of Ni. In contrast, concentrations of Ni were low in clams from El Hofra. In the site El Hofra there are some industries which use Ni, but clams collected from these sites seem to be not affected. This is probably due to the fact that Ni is not retained in the sediment, it is put in solution or integrated by species (Illou 1999).

Lead as a pollutant, has a particular importance because it is toxic to almost all organisms. The highest concentration of lead was obtained in clams from Sidi Mansour site (Fig. 2). The presence of Pb is probably linked to the paint flaking from boats or the use of sewage sludge. According to Luoma and Bryan (1982), lead can be directly accumulated from sea water and sediment especially in clams (Castro et al. 1996).

Iron and manganese occur naturally in sea water and are essential for marine organisms. In *Ruditapes decussatus* concentration of Fe and Mn are lower than those observed in other molluscs (Table 2). This could result from the physiological differences of these species since Fe is used in oxygen transport and Mn plays an important role as cofactor in enzymatic processes (George 1990).

Sediment analysis are interesting because they could reflect a long-term contamination. However metal in sediment can be in an unavailable form for bivalves. That is why the marine bivalves such as *Ruditapes decussatus* constitute a better model reflecting the pollution state of one site and therefore could be used in the national monitoring program.

This study was based on analysis of metals in the whole body of *Ruditapes decussatus* collected from five sites in the gulf of Gabès differing by pollution levels. It showed that the site El Hofra was the most contaminated one but with a tolerable concentration of Cd, Cu, Pb, and Zn for human health according to the norm values. This study also showed that Bordj d'Ungha was the less contaminated site. Hence, we chose Bordj d'Ungha as a reference site comparatively to the site El Hofra which presented a high concentration of metals.

This work could contribute to fill the gap of information about metal distribution in the gulf of Gabès area. It was based on *Ruditapes decussatus* which is a good bioindicator of metal contamination. Therefore, research is still required to understand on the one hand if this species has an ability to regulate these essential metal concentration and, on the other hand, the processes of accumulation and decontamination of metals.

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