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## Assessment of heavy metal pollution in the gulf of Gabes (Tunisia) using four mollusc species

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### Abstract

Since the establishment of the 'Tunisian Chemical Group' in Gabes, Tunisia, no serious investigations have been carried out about heavy metal pollution in the Gulf of Gabes. In this study, the contents of four heavy metals were assessed in four mollusc species (two gastropods, *Gibbula ardens* and *Patella caerulea*, and two bivalves, *Pinctada radiata* and *Pinna nobilis*), collected from twelve coastal stations. In general, the results obtained show high concentrations of heavy metals in the central area of the Gulf of Gabes, near Gabes city in particular; in contrast, low concentrations were found at the edges of this gulf, which is probably due to the chemical pollution generated from the huge phosphoric acid industry in Gabes City. Comparing the results found with the four examined species, the lowest concentrations were noted with the two bivalve species *P. radiata* and *P. nobilis*. The highest heavy metal concentrations noted during this study are comparable to the findings of other authors in other areas, and they considerably exceed the standard levels. Remediation action is urgently required; the action could consist in either reducing the amount of heavy metals in the phosphogypsum discharged directly in the sea or discontinuing the operation of this harmful industry in order to protect marine life in the area.

**Keywords:** Heavy metals, pollution control, industrial waste, mollusc, Gulf of Gabes, Tunisia.

### Introduction

Due to increasing anthropogenic activities around the Mediterranean Sea, coastal environments are increasingly exposed to various contaminants, heavy metal pollution in particular, which is considered as a threat not only for marine biodiversity but also for humans through the consumption of heavy metal-polluted marine organisms. In fact, heavy metals can be found in urbanized and non-urbanized areas, as well in the water column and in sediments where they persist and can be diffused to marine species, and if they reach anomalous levels, they can constitute a potential environmental threat (Larsen, 1992; Readman *et al.*, 1993; Buchholtz Ten Brink *et al.*, 1996). Heavy metals can be adsorbed by inorganic and organic matter on which benthic species feed, and can then be incorporated in the food webs of marine ecosystems. Thus, heavy metals can be directly or indirectly accumulated in the tissues of marine species. The process of heavy metal bioaccumulation makes some marine species with specific ecological and biological characters useful as bioindicators of heavy metal pollution in marine habitats and can then be utilized to evaluate the "health status" of the

coastal environment (Gerhardt, 1999; Rainbow, 2006; Zhou *et al.*, 2008). In this context, molluscs are among the groups that have been used to assess the level of heavy metal pollution in aquatic environments and some species were reported to be good bioindicators (Boening, 1999; Bresler *et al.*, 2003a,b; Feldstein *et al.*, 2003).

The Tunisian coastline is about 1300 km long, and most urban concentrations are located on the coasts, thus leading to intense anthropogenic development, which certainly has negative effects on coastal areas. The south-eastern coasts of Tunisia, represented by the Gulf of Gabes, were reported to be the most exposed to pollution through the huge industrial activity based in this area, in particular in Gabes City. Large quantities of phosphogypsum (calcium sulphate) from the phosphoric acid and chemical product industry of Gabes are released into the Gulf of Gabes (Soussi *et al.*, 1995; Louati *et al.*, 2001; Zaghden *et al.*, 2005). In addition, other potential heavy metals transmitters, such as the salt, lead, ceramics, textile and building material factories, are found in other cities, Sfax in particular. This chemical pollution has had negative impacts on biodiversity and has triggered the disappearance, or at least the reduction, of plant

cover in the Gulf, *Posidonia oceanica* meadows in particular (Darmoul *et al.*, 1980; Darmoul, 1988; El Afli *et al.*, 2001). However, in spite of this situation, there is still a knowledge gap as regards heavy metal pollution in the Gulf of Gabes. In fact, the studies carried out about this topic are limited to one site in the Gulf of Gabes, and do not represent its entire area (Hamza-Chaffai *et al.*, 1995; Smaoui-Damek *et al.*, 2003; Banni *et al.*, 2005, 2007; Barhoumi *et al.*, 2009; Messaoudi *et al.*, 2009). In addition, none of these studies have examined the coastal area of Gabes City, which is the most exposed to various types of industrial pollution. Some of these works have reported that molluscs can be used as good indicators for monitoring heavy metal pollution in the Gulf of Gabes (Smaoui-Damek *et al.*, 2003; Banni *et al.*, 2005). In this context, this work was carried out in order to assess heavy metal pollution in the Gulf of Gabes, using four mollusc species: *Gibbula ardens*, *Patella caerulea*, *Pinctada radiata*, and *Pinna nobilis* originating from different sites of the Gulf of Gabes. Thus, the goals of this study are *i*) to analyze four heavy metal concentrations in the tissues of these species collected from different sites inside the Gulf of Gabes, and *ii*) to evaluate the pollution level of the coastal area of the Gulf of Gabes by comparing heavy metal concentrations between sampling sites and between the species considered.

## Material and Methods

### Study area and sampling

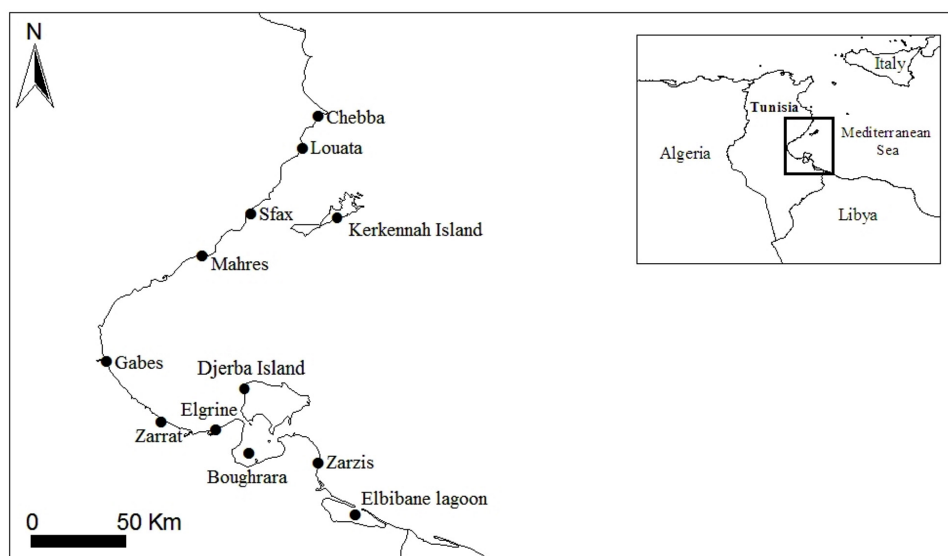
To achieve the goals of this study, four mollusc species were selected: two herbivorous gastropods *Gibbula ardens* (Linnaeus, 1758) and *Patella caerulea* (Linnaeus, 1758); and two filter-feeding bivalves, *Pinctada radiata* (Leach, 1814) and *Pinna nobilis* (Linnaeus, 1758). The two first molluscs are encountered in rocky substrata (or

in ports) along the beaches; however, the two latter species are typical of infralittoral areas. All these species live in the coastal area, which is supposed to be the most exposed to various pollution sources. Before proceeding with field sampling, twelve different sites were selected in the Gulf of Gabes, from its northern edge at Chebba to its southern edge at Elbibane lagoon, close to the Tunisian-Libyan borders. The sites, from north to south, are: *Chebba*, *Louata*, *Sfax*, *Kerkennah Island*, *Mahres*, *Gabes*, *Zarrat*, *Elgrine*, *Djerba Island*, *Boughrara*, *Zarzis* and *Elbibane lagoon* (Fig. 1). It is worth noting that due to the restricted geographic distribution of some species (*P. radiata* and *P. nobilis* in particular) in the Gulf of Gabes, it was not possible to collect all the considered species from all the sampling sites. Information about the GPS coordinates and species hosted at all the sampling sites are summarized in Table 1. *P. nobilis* and *P. radiata* were collected by free diving at a depth from 2 to 4 m; while *P. caerulea* and *G. ardens* were collected by hand (using a knife for *P. caerulea*) in rocky areas. The sampling survey was conducted from August to October 2011.

### Laboratory analyses

After being sampled, the individuals were immediately stored in plastic bags and transported separately to the laboratory, where the calcareous shells were removed, and the visceral masses of all specimens were rinsed with abundant distilled water to remove sediments or surface debris.

Because of the variability in the ability of mollusc tissues to accumulate pollutants (Bargagli *et al.*, 1986; Catsiki, 1986; Uthe & Chou, 1987), heavy metal contents were analyzed in different tissues of *P. nobilis* and *P. radiata*. Hence, the specimens of *P. nobilis* were separated into mantle, gills, muscle, and hepatopancreas; those of *P. radiata* were separated into muscle and hepatopancre-



**Fig. 1:** Sampling sites in the Gulf of Gabes.

**Table 1.** GPS Localization of sampling sites and the species found in each site.

Sampling sites	GPS Coordinates		Species found
<b>Chebba</b>	110947.82 E	351358.08 N	<i>Patella caerulea</i> + <i>Gibbula ardens</i> + <i>Pinna nobilis</i> + <i>Pinctada radiata</i>
<b>Louata</b>	110204.43 E	350233.21 N	<i>Patella caerulea</i> + <i>Gibbula ardens</i> + <i>Pinna nobilis</i> + <i>Pinctada radiata</i>
<b>Sfax</b>	674744.08 E	3860236.47 N	<i>Patella caerulea</i> + <i>Gibbula ardens</i> + <i>Pinctada radiata</i>
<b>Kerkennah Island</b>	111405.83 E	344211.99 N	<i>Pinna nobilis</i> + <i>Pinctada radiata</i>
<b>Mahress</b>	102632.18 E	342900.65 N	<i>Gibbula ardens</i> + <i>Pinctada radiata</i>
<b>Gabes</b>	100709.52 E	335341.28 N	<i>Patella caerulea</i> + <i>Pinctada radiata</i>
<b>Zarrat</b>	626253.05 E	3729871.79 N	<i>Patella caerulea</i> + <i>Gibbula ardens</i> + <i>Pinctada radiata</i>
<b>Elgrine</b>	645099.11 E	3723970.30 N	<i>Patella caerulea</i> + <i>Gibbula ardens</i>
<b>Djerba Island</b>	671709.91 E	3751359.66 N	<i>Patella caerulea</i> + <i>Gibbula ardens</i> + <i>Pinna nobilis</i> + <i>Pinctada radiata</i>
<b>Boughrara</b>	677870.13 E	3721216.69 N	<i>Patella caerulea</i> + <i>Gibbula ardens</i>
<b>Zarzis</b>	696228.23 E	3706137.95 N	<i>Patella caerulea</i> + <i>Gibbula ardens</i> + <i>Pinctada radiata</i>
<b>Elbibane lagoon</b>	710957.36 E	3686004.86.N	<i>Patella caerulea</i> + <i>Gibbula ardens</i> + <i>Pinna nobilis</i> + <i>Pinctada radiata</i>

as. In contrast, it was not possible to follow the same procedure for the two gastropod species (*P. caerulea* and *G. ardens*) because their visceral masses are too small to allow heavy metal concentration measurements in different parts of their bodies. For this reason, all the soft parts of these gastropods were considered while analyzing heavy metal content. In addition, for *P. nobilis*, two specimens were used for the preparation of one pooled sample; for *P. radiata*, 4 individuals correspond to one sample; while for *P. caerulea* and *G. ardens*, each pooled sample was prepared using 8 individuals. Three samples were prepared for each species, at each sampling site.

The samples obtained were thereafter placed, separately, in a muffle furnace at 550°C for 24 hours. A total of 105 ash samples were obtained (30 for *G. ardens*, 30 for *P. caerulea*, 30 for *P. radiata* and 15 for *P. nobilis*). The metal content of these samples was determined using Atomic absorption flame emission spectroscopy (AAS). The targeted heavy metals are mercury, lead, cadmium, and chromium. These heavy metals were chosen as they are considered to be the most harmful and because they are found in phosphogypsum discharged in the sea by the phosphoric acid industry in Gabes. Prior to analysis, fifty milligrams of Fe<sup>3+</sup>-doped TiO<sub>2</sub> samples were transferred to Teflon flasks and then completely dissolved in H<sub>2</sub>SO<sub>4</sub>-HNO<sub>3</sub> solution (30/70% in volume). After dissolution, the mixture was diluted with 100 mL of deionized water and analyzed by AAS.

### Statistical analyses

The data collected were expressed in µg g<sup>-1</sup>, and the comparison between sites and species (and also the different body parts of each species) was conducted using one-way ANOVA, and Post-Hoc comparison using Tukey's test, after testing the homogeneity and normality of variables with the use of the Shapiro-Wilk test. Besides, principal component analysis (PCA) and hierarchic classifica-

tion were also conducted using the data obtained with all species in order to represent the sampling sites of the Gulf of Gabes in space. Statistical analyses were performed using SYSTAT software 11.0 (SPSS, Richmond, CA, USA).

### Results

Average concentrations of the four analyzed heavy metals in the four mollusc species are summarized in Figure 2. The distribution of these heavy metals in the Gulf of Gabes appears to follow an almost similar pattern for all four mollusc species. The highest concentrations were found in the area between the Gabes and Zarzis sites. For *G. ardens*, the lowest average concentration of mercury, lead, cadmium, and chromium were respectively 0.042±0.018 µg<sup>-1</sup> g (in Elbibane lagoon), 0.633±0.107 µg<sup>-1</sup> g (in Chebba), 0.244±0.067 µg<sup>-1</sup> g (in Chebba), and 0.041±0.091 µg<sup>-1</sup> g (in Chebba); while the highest were found to be 0.181±0.020 µg<sup>-1</sup> g (in Zarzis), 2.543±0.527 µg<sup>-1</sup> g (in Djerba Island), 3.005±0.117 µg<sup>-1</sup> g (in Djerba Island), and 1.664±0.246 µg<sup>-1</sup> g (in Djerba Island) respectively (Fig. 2A). As for the heavy metal contents assessed in *P. caerulea*, they were found to vary between 0.031±0.001 µg<sup>-1</sup> g (in Elbibane lagoon) and 0.679±0.0846 µg<sup>-1</sup> g (in Louata) for mercury, between 0.823±0.0575 µg<sup>-1</sup> g (in Elbibane lagoon) and 2.123±0.7075 µg<sup>-1</sup> g (in Djerba Island) for lead, between 0.800±0.0288 µg<sup>-1</sup> g (in Elbibane lagoon) and 4.300±0.3398 µg<sup>-1</sup> g (in Gabes) for cadmium, and between 0.321±0.0106 µg<sup>-1</sup> g (in Chebba) and 2.171±0.1394 µg<sup>-1</sup> g (in Gabes) for chromium (Fig. 2B).

Regarding the bivalve *P. radiata*, although the distribution pattern of the analyzed heavy metals appeared similar in both animal tissues (i.e. muscle and hepatopancreas), some slight variations were found. The lowest average concentrations of mercury, lead, cadmium, and chromium in *P. radiata* muscle were estimated to be 0.040±0.0095 µg<sup>-1</sup> g (in Chebba), 0.722±0.0757 µg<sup>-1</sup> g

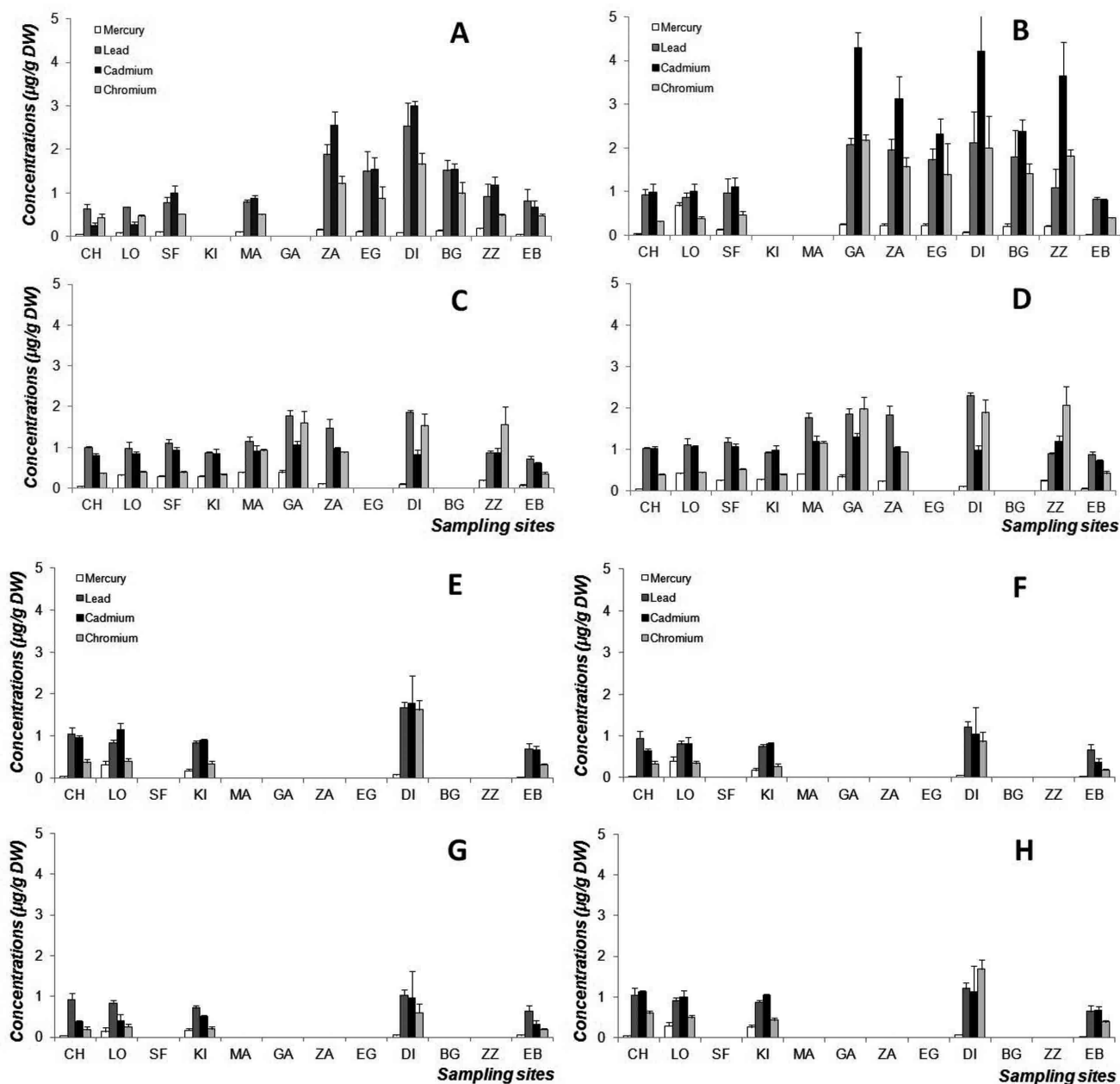
(in Elbibane lagoon),  $0.605 \pm 0.0252 \mu\text{g}^{-1} \text{g}$  (in Elbibane lagoon), and  $0.335 \pm 0.008 \mu\text{g}^{-1} \text{g}$  (in Kerkennah Island) respectively. However, the highest levels of the same heavy metals in pearl oyster muscle were found to be  $0.387 \pm 0.0127 \mu\text{g}^{-1} \text{g}$  (in Mahres),  $1.848 \pm 0.0711 \mu\text{g}^{-1} \text{g}$  (in Djerba Island),  $1.065 \pm 0.0858 \mu\text{g}^{-1} \text{g}$  (in Gabes), and  $1.605 \pm 0.2859 \mu\text{g}^{-1} \text{g}$  (in Gabes) respectively (Fig. 2C). With *P. radiata* hepatopancreas, the results of the heavy metals analysis have shown that the lowest contents of the four heavy metals were found as follows:  $0.044 \pm 0.0070 \mu\text{g}^{-1} \text{g}$  for mercury (in Elbibane lagoon),  $0.860 \pm 0.0607 \mu\text{g}^{-1} \text{g}$  for lead (in Elbibane lagoon),  $0.732 \pm 0.1877 \mu\text{g}^{-1} \text{g}$  for cadmium (in Elbibane lagoon), and  $0.390 \pm 0.0214 \mu\text{g}^{-1} \text{g}$  for chromium (in Chebba). In contrast, the highest metal concentrations were recorded in Louata for mercury ( $0.420 \pm 0.0990 \mu\text{g}^{-1} \text{g}$ ), in Djerba Island for lead ( $2.288 \pm 0.2791 \mu\text{g}^{-1} \text{g}$ ), in Gabes for cadmium ( $1.295 \pm 0.1729 \mu\text{g}^{-1} \text{g}$ ), and in Zarzis for chromium ( $2.066 \pm 0.2222 \mu\text{g}^{-1} \text{g}$ ) (Fig. 2D).

As for *P. nobilis* samples, different patterns of heavy metal distribution were obtained with the four analyzed tissues of this animal. Considering the mantle, the average concentrations of mercury were found to vary between a minimum of  $0.027 \pm 0.0025 \mu\text{g}^{-1} \text{g}$  (in Elbibane lagoon) and a maximum of  $0.312 \pm 0.0974 \mu\text{g}^{-1} \text{g}$  (in Louata); those of lead were comprised between  $0.691 \pm 0.1435 \mu\text{g}^{-1} \text{g}$  (in Elbibane lagoon) and  $1.682 \pm 0.1348 \mu\text{g}^{-1} \text{g}$  (in Djerba Island). Regarding cadmium, average concentrations fluctuated between  $0.666 \pm 0.0960 \mu\text{g}^{-1} \text{g}$  (in Elbibane lagoon) and  $1.790 \pm 0.6480 \mu\text{g}^{-1} \text{g}$  (in Djerba Island). For chromium, the lowest average content was recorded in Elbibane lagoon ( $0.321 \pm 0.0297 \mu\text{g}^{-1} \text{g}$ ), while the highest was found in Djerba Island ( $1.627 \pm 0.2248 \mu\text{g}^{-1} \text{g}$ ) (Fig. 2E). With *P. nobilis* gill samples, the lowest average concentrations of mercury, lead, cadmium, and chromium, were  $0.026 \pm 0.0050 \mu\text{g}^{-1} \text{g}$  (in Chebba),  $0.654 \pm 0.0764 \mu\text{g}^{-1} \text{g}$  (in Elbibane lagoon),  $0.374 \pm 0.0681 \mu\text{g}^{-1} \text{g}$  (in Elbibane lagoon), and  $0.183 \pm 0.0638 \mu\text{g}^{-1} \text{g}$  (in Elbibane lagoon) respectively. Moreover, the highest heavy metal contents recorded were as follows:  $0.399 \pm 0.0385 \mu\text{g}^{-1} \text{g}$  for mercury (in Louata),  $1.221 \pm 0.2257 \mu\text{g}^{-1} \text{g}$  for lead (in Djerba Island),  $1.039 \pm 0.1460 \mu\text{g}^{-1} \text{g}$  for cadmium (in Djerba Island), and  $0.870 \pm 0.1008 \mu\text{g}^{-1} \text{g}$  for chromium (in Djerba Island) (Fig. 2F). Considering *P. nobilis* muscle, the average concentrations of mercury were found to vary between  $0.041 \pm 0.0083 \mu\text{g}^{-1} \text{g}$  (in Chebba) and  $0.169 \pm 0.048 \mu\text{g}^{-1} \text{g}$  (in Kerkennah Island), those of lead between  $0.643 \pm 0.0814 \mu\text{g}^{-1} \text{g}$  (in Elbibane lagoon) and  $1.032 \pm 0.1659 \mu\text{g}^{-1} \text{g}$  (in Djerba Island); cadmium average concentrations varied from  $0.312 \pm 0.0960 \mu\text{g}^{-1} \text{g}$  (in Elbibane lagoon) to  $0.966 \pm 0.1524 \mu\text{g}^{-1} \text{g}$  (in Djerba Island), and those of chromium from  $0.192 \pm 0.0096 \mu\text{g}^{-1} \text{g}$  (in Elbibane lagoon) to  $0.604 \pm 0.0777 \mu\text{g}^{-1} \text{g}$  (in Djerba Island) (Fig. 2G). Finally for *P. nobilis* hepatopancreas, the lowest concentrations of the four analyzed heavy metals were recorded in Elbibane lagoon ( $0.033 \pm 0.0100 \mu\text{g}^{-1} \text{g}$  for mer-

cury,  $0.645 \pm 0.1336 \mu\text{g}^{-1} \text{g}$  for lead,  $0.674 \pm 0.1575 \mu\text{g}^{-1} \text{g}$  for cadmium,  $0.386 \pm 0.0565 \mu\text{g}^{-1} \text{g}$  for chromium), while the highest were found in Louata for mercury ( $0.285 \pm 0.0517 \mu\text{g}^{-1} \text{g}$ ), in Djerba Island for lead ( $1.209 \pm 0.2000 \mu\text{g}^{-1} \text{g}$ ) and chromium ( $1.687 \pm 0.3027 \mu\text{g}^{-1} \text{g}$ ), and in Chebba for cadmium ( $1.121 \pm 0.0905 \mu\text{g}^{-1} \text{g}$ ) (Fig. 2H).

The analysis of variance, applied in order to check the differences between the sampling sites, for each mollusc species (and tissues in cases of *P. radiata* and *P. nobilis*) and each heavy metal analyzed, showed highly significant differences between study localities (Table 2). As for the results of the combined post-hoc comparison, based on the Duncan test, they allowed classification of the sampling sites, for each species (and tissue) and each heavy metal. Since the four mollusc species were not sampled at all twelve sampling sites, the classification of these latter differed among species and analyzed metals. For mercury, the post-hoc comparison showed that the less affected sites were Elbibane lagoon, Chebba and Djerba Island with almost all the species and tissues examined. However, the most affected sites varied for *G. ardens*, *P. caerulea*, and *P. radiata* (for both muscle and hepatopancreas), whereas the results obtained with *P. nobilis* tissues have shown that the most affected localities were Louata and Kerkennah Island (Table 2). As for the results obtained with lead considering *G. ardens*, *P. caerulea* and *P. radiata* samples, they show that the less polluted localities were Chebba, Louata, Sfax, Mahres, Kerkennah Island, Zarzis and Elbibane lagoon; while the most-polluted sites were found to be Gabes, Zarrat and Djerba Island. With respect to *P. nobilis* samples, post-hoc comparison allowed to define Kerkennah Island and Elbibane lagoon (and in some cases Louata) as the sites with the lowest lead concentrations and Chebba and Djerba Island as those with the highest contents (Table 2). As for cadmium, the results obtained showed that Chebba, Louata, Sfax, and Elbibane lagoon were mainly among the less polluted sites, for most of the four examined mollusc species. However, the localities with the highest cadmium contents were found to be Gabes, Zarrat, Elgrine, Zarzis, and Djerba Island in particular (Table 2). Finally, considering chromium analyses, the results obtained showed that Chebba, Louata, Kerkennah Island, Sfax, Mahres, Zarzis, and Elbibane lagoon were the localities with the lowest heavy metal content, with *G. ardens*, *P. caerulea*, and *P. radiata* samples; while the most polluted sites were mainly, Gabes, Djerba Island, and Zarzis. With *P. nobilis* samples, the sites with the highest chromium concentrations were mainly Chebba, Louata, Kerkennah Island, and Elbibane lagoon; in contrast, Djerba Island was defined as the locality with the highest cadmium content (Table 2).

Otherwise, the analysis of variance was also applied in order to test for differences between the examined tissues of the two bivalve species *P. radiata* and *P. nobilis*, and the results are summarized in Table 3. For *P. radiata*,



**Fig. 2:** Variations of average concentrations of the four heavy metals analyzed in the four mollusc species and their tissues: **A** *Gibbula ardens*, **B** *Patella caerulea*, **C** *Pinctada radiata* muscle, **D** *Pinctada radiata* hepatopancreas, **E** *Pinna nobilis* mantle, **F** *Pinna nobilis* gills, **G** *Pinna nobilis* muscle, **H** *Pinna nobilis* hepatopancreas.

most of the analyses performed, taking into account the different tissues examined and sampling sites, were not significantly different. For mercury, only one significant difference was highlighted between the heavy metal concentrations recorded in the muscle and hepatopancreas of the species, in Zarrat ( $F = 11.504$ ;  $p = 0.027$ ). For lead, only one significant difference was obtained between the two different tissues analyzed in Mahres ( $F = 9.689$ ;  $p = 0.036$ ). As for cadmium, ANOVA results have shown a significant difference in Louata ( $F = 21.749$ ;  $p = 0.010$ ). Besides, regarding chromium, the statistical analysis highlighted that the contents of the heavy metal in the two different tissues of the animal were significantly different in Sfax only ( $F = 29.388$ ;  $p = 0.006$ ). In addition,

most of the cases analyzed showed no significant differences (Table 3). As for *P. nobilis*, the results of ANOVA, performed with mercury data, showed significant differences in two sampling localities only, Louata ( $F = 7.832$ ;  $p = 0.009$ ) and Elbibane lagoon ( $F = 5.921$ ;  $p = 0.020$ ). At both sites, post-hoc comparison has allowed to classify the examined fan shell tissues in two subsets, separating the “muscle” from the three other tissues i.e. “mantle”, “gills”, and “hepatopancreas” (Table 3). As for lead, significant differences were found in both Kerkenah ( $F = 4.742$ ;  $p = 0.035$ ) and Djerba Islands ( $F = 6.763$ ;  $p = 0.014$ ), and post-hoc comparison resulted globally in separating between “muscle-gills” and “mantle-hepatopancreas” (Table 3). Concerning cadmium, the statistical

**Table 2.** Analysis of variance of the data obtained among the sampling sites. The homogenous subsets were determined by post-hoc comparison using Duncan's test, and for  $\alpha = 0.05$ . CH, Chebba; LO, Louata; SF, Sfax; KI, Kerkennah Island; MA, Mahres; GA, Gabes; ZA, Zarrat; DI, Djerba Island; BG, Boughrara; ZZ, Zarzis; EB, Elbibane.

SPECIES (TISSUE)	HEAVY METAL	F	P	HOMOGENOUS SUBSETS
<i>G. ardens</i> (total)	Mercury	18.815	< 0.001	(EB, CH) - (LO, DI, SF, MA, EG) - (EG-BG) - (BG-ZA) - (ZZ)
	Lead	15.401	< 0.001	(CH, LO, SF, MA, EB, ZZ) - (EG, BG, ZA) - (DI)
	Cadmium	85.217	< 0.001	(CH, LO) - (EB, MA) - (MA, SF, ZZ) - (EG, BG) - (ZA) - (DI)
	Chromium	20.101	< 0.001	(CH, LO, EB, ZZ, MA, SF) - (EG, BG) - (BG, ZA), (DI)
<i>P. caerulea</i> (total)	Mercury	57.402	< 0.001	(EB, CH, DI) - (DI, SF) - (ZZ, BG, EG, ZA, GA) - (LO)
	Lead	6.610	< 0.001	(EB, LO, CH, SF, ZZ) - (EG, BG, ZA, GA, DI)
	Cadmium	22.616	< 0.001	(EB, CH, LO, SF) - (EG, ZZ, ZA) - (ZA, ZZ) - (ZZ, DI, GA)
	Chromium	13.034	< 0.001	(CH, LO, EB, SF) - (EG, BG, ZA, ZZ, DI) - (ZA, ZZ, DI, GA)
<i>P. radiata</i> (muscle)	Mercury	83.786	< 0.001	(CH, EB) - (EB, DI) - (DI, ZA) - (ZZ) - (SF, KI, LO) - (GA, ZA)
	Lead	34.692	< 0.001	(EB, ZZ, KI) - (ZZ, KI, LO, CH) - (LO, CH, SF, MA) - (ZA) - (GA, DI)
	Cadmium	5.056	0.001	(EB) - (CH, DI, KI, LO, ZZ, MA, SF, ZA) - (MA, SF, ZA, GA)
	Chromium	23.751	< 0.001	(KI, EB, CH, SF, LO) - (ZA, MA) - (DI, ZZ, GA)
<i>P. radiata</i> (hepatopancreas)	Mercury	21.754	< 0.001	(EB, CH, DI) - (ZA, ZZ, SF, KI) - (ZZ, SF, KI, GA) - (GA, MA, LO)
	Lead	12.547	< 0.001	(EB, ZZ, KI, CH, LO, SF) - (MA, ZA, GA) - (DI)
	Cadmium	1.964	0.100	(EB, DI, KI, CH, ZA, LO, SF) - (DI, KI, CH, ZA, LO, SF, MA, ZZ, GA)
	Chromium	29.647	< 0.001	(CH, KI, EB, LO, SF) - (ZA, MA) - (DI, GA, ZZ)
<i>P. nobilis</i> (mantle)	Mercury	17.688	< 0.001	(EB, CH, DI) - (KI) - (LO)
	Lead	30.412	< 0.001	(EB, KI, LO) - (KI, LO, CH) - (DI)
	Cadmium	5.911	0.010	(EB, KI, CH, LO) - (DI)
	Chromium	75.665	< 0.001	(EB, KI, CH, LO) - (DI)
<i>P. nobilis</i> (gills)	Mercury	79.116	< 0.001	(CH, EB, DI) - (KI) - (LO)
	Lead	9.734	0.002	(EB, KI, LO) - (KI, LO, CH) - (DI)
	Cadmium	8.267	0.003	(EB, CH) - (CH, LO, KI) - (LO, KI, DI)
	Chromium	44.783	< 0.001	(EB, KI) - (KI, CH, LO) - (DI)
<i>P. nobilis</i> (muscle)	Mercury	7.055	0.006	(CH, DI, EB) - (LO, KI)
	Lead	7.915	0.004	(EB, KI) - (KI, LO) - (LO, CH) - (CH, DI)
	Cadmium	29.333	< 0.001	(EB, CH, LO) - (CH, LO, KI) - (DI)
	Chromium	54.362	< 0.001	(EB, CH, KI, LO) - (DI)
<i>P. nobilis</i> (hepatopancreas)	Mercury	38.402	< 0.001	(EB, CH, DI) - (KI, LO)
	Lead	7.843	0.004	(EB, KI) - (KI, LO, CH) - (CH, DI)
	Cadmium	10.669	0.001	(EB) - (LO, KI, DI, CH)
	Chromium	44.199	< 0.001	(EB, KI, LO, CH) - (DI)

analyses showed significant differences between the four analyzed tissues, for most of the sampling localities (For Chebba:  $F = 50.653$ ;  $p < 0.001$  – For Louata:  $F = 24.057$ ;  $p < 0.001$  – For Kerkennah Island:  $F = 11.503$ ;  $p = 0.003$  – For Elbibane lagoon:  $F = 9.065$ ;  $p = 0.006$ ). Different post-hoc classifications were obtained, with respect to the sampling localities; but in all cases, “muscle” tissue was separated from the rest of analyzed tissues. In addition, ANOVA applied with chromium data showed significant differences for all the sampling sites considered (For Chebba:  $F = 20.998$ ;  $p < 0.001$  – For Louata:  $F = 14.436$ ;  $p = 0.001$  – For Kerkennah Island:  $F = 15.451$ ;  $p$

$= 0.001$  – For Djerba Island:  $F = 22.310$ ;  $p < 0.001$  – For Elbibane lagoon:  $F = 14.395$ ;  $p = 0.001$ ). As for the cadmium analyses, the results of post-hoc comparison also differed among sites; however, they generally separated “muscle” or “muscle-gills” from the rest of the *P. nobilis* tissues tested (Table 3).

Principal component analysis coupled with cluster analysis, applied taking into consideration all data obtained at all the sampling sites, has allowed grouping into 6 clusters at a distance of 2.6: cluster 1 consisted of Chebba, Louata, Kerkennah Island, and Elbibane lagoon; cluster 2 was formed by Djerba Island; Cluster 3 was rep-

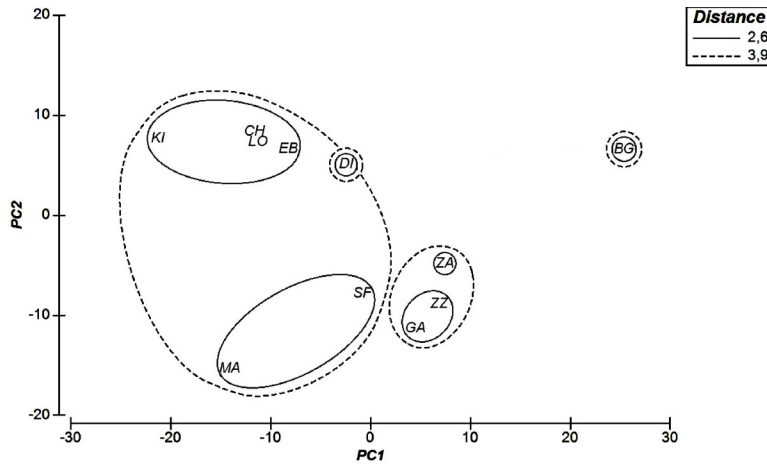
**Table 3.** Analysis of variance of the data obtained among the tissues of *P. radiata* and *P. nobilis*. The homogenous subsets were obtained, only for *P. nobilis* samples, by post-hoc comparison using Duncan's test, and for alpha = 0.05. Post-hoc comparison was not performed (*np*) for *P. radiata* samples because the analyzed tissues (groups) were fewer than three. CH, Chebba; LO, Louata; SF, Sfax; KI, Kerkenah Island; MA, Mahres; GA, Gabes; ZA, Zarrat; EG, Elgrine; DI, Djerba Island; BG, Boughrara; ZZ, Zarzis; EB, Elbibane.

Species	Sampling site	Heavy metal	F	P	Homogenous subsets	
<i>P. radiata</i>	CH	Mercury	1.383	0.305	<i>np</i>	
		Lead	0.089	0.780	<i>np</i>	
		Cadmium	2.400	0.196	<i>np</i>	
		Chromium	3.047	0.156	<i>np</i>	
	LO	Mercury	3.203	0.148	<i>np</i>	
		Lead	1.536	0.283	<i>np</i>	
		Cadmium	<b>21.749</b>	<b>0.010</b>	<i>np</i>	
		Chromium	1.794	0.251	<i>np</i>	
	SF	Mercury	0.811	0.419	<i>np</i>	
		Lead	0.598	0.483	<i>np</i>	
		Cadmium	6.795	0.060	<i>np</i>	
	KI	Chromium	<b>29.388</b>	<b>0.006</b>	<i>np</i>	
		Mercury	0.466	0.532	<i>np</i>	
		Lead	1.031	0.367	<i>np</i>	
		Cadmium	2.849	0.167	<i>np</i>	
	MA	Chromium	6.538	0.063	<i>np</i>	
		Mercury	0.397	0.563	<i>np</i>	
		Lead	<b>9.689</b>	<b>0.036</b>	<i>np</i>	
		Cadmium	1.656	0.268	<i>np</i>	
	GA	Chromium	1.599	0.275	<i>np</i>	
		Mercury	1.403	0.302	<i>np</i>	
		Lead	0.045	0.842	<i>np</i>	
		Cadmium	4.260	0.108	<i>np</i>	
	ZA	Chromium	1.468	0.292	<i>np</i>	
		Mercury	<b>11.504</b>	<b>0.027</b>	<i>np</i>	
		Lead	3.475	0.136	<i>np</i>	
		Cadmium	2.163	0.215	<i>np</i>	
	DI	Chromium	1.025	0.369	<i>np</i>	
		Mercury	0.030	0.870	<i>np</i>	
		Lead	7.000	0.057	<i>np</i>	
		Cadmium	2.040	0.226	<i>np</i>	
	ZZ	Chromium	1.597	0.275	<i>np</i>	
		Mercury	2.873	0.165	<i>np</i>	
		Lead	0.140	0.728	<i>np</i>	
		Cadmium	3.416	0.138	<i>np</i>	
	EB	Chromium	3.094	0.153	<i>np</i>	
		Mercury	0.408	0.558	<i>np</i>	
		Lead	<b>6.064</b>	<b>0.070</b>	<i>np</i>	
	<i>P. nobilis</i>	CH	Cadmium	1.342	0.311	<i>np</i>
			Mercury	2.670	0.119	-
			Lead	0.895	0.485	-
			Chromium	<b>50.653</b>	<b>&lt; 0.001</b>	(muscle) - (gills) - (mantle) - (hepatopancreas)
LO		Chromium	<b>20.998</b>	<b>&lt; 0.001</b>	(muscle) - (gills, mantle) - (hepatopancreas)	
		Mercury	<b>7.832</b>	<b>0.009</b>	(muscle) - (hepatopancreas, mantle, gills)	
		Lead	0.894	0.485	-	
		Cadmium	<b>24.057</b>	<b>&lt; 0.001</b>	(muscle) - (gills, hepatopancreas) - (hepatopancreas, mantle)	
KI		Chromium	<b>14.436</b>	<b>0.001</b>	(muscle) - (gills, mantle) - (hepatopancreas)	
		Mercury	2.255	0.159	-	
		Lead	<b>4.742</b>	<b>0.035</b>	(muscle, gills, mantle) - (mantle, hepatopancreas)	
		Cadmium	<b>11.503</b>	<b>0.003</b>	(muscle) - (gills, mantle, hepatopancreas)	
DI		Chromium	<b>15.451</b>	<b>0.001</b>	(muscle, gills) - (gills, mantle) - (hepatopancreas)	
		Mercury	2.158	0.171	-	
		Lead	<b>6.763</b>	<b>0.014</b>	(muscle, hepatopancreas, gills) - (mantle)	
		Cadmium	<b>3.658</b>	<b>0.063</b>	(muscle, gills, hepatopancreas) - (mantle)	
EB		Chromium	<b>22.310</b>	<b>&lt; 0.001</b>	(muscle, gills) - (mantle, hepatopancreas)	
		Mercury	<b>5.921</b>	<b>0.020</b>	(mantle, gills, hepatopancreas) - (muscle)	
		Lead	0.119	0.947	-	
		Cadmium	<b>9.065</b>	<b>0.006</b>	(muscle, gills) - (mantle, hepatopancreas)	
			Chromium	<b>14.395</b>	<b>0.001</b>	(gills, muscle) - (mantle, hepatopancreas)

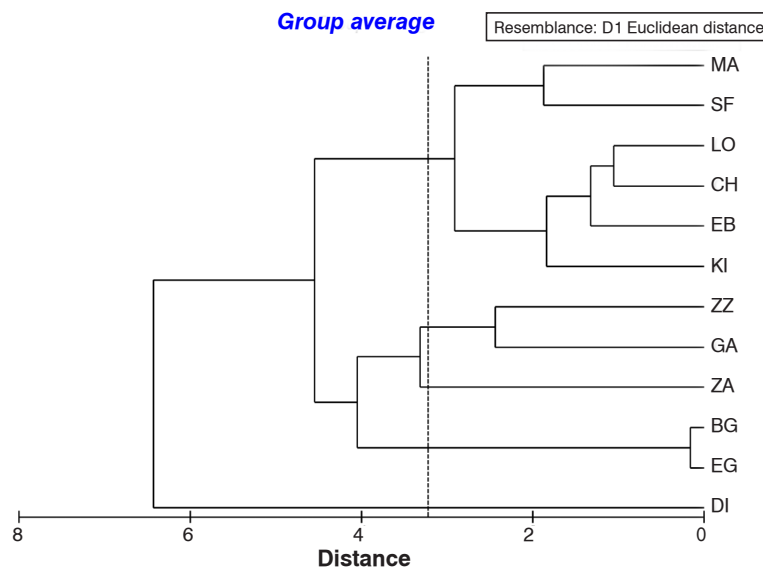


represented by the sites of Sfax and Mahres; cluster 4 was formed by Gabes and Zarzis; cluster 5 by Zarrat; while cluster 6 was represented by Elgrine and Boughrara sites. At a distance of 3.9, the PCA and clustering dendrogram allowed to classify the sites into 4 different groups: A, B, C and D. Group A was composed by clusters 1 and 3; group B consisted of cluster 2; group C was represented by clusters 4 and 5; and finally group D was represented by cluster 6 only (Figs. 3, 4).

ingestion of contaminants adsorbed by phytoplankton, detritus, and sediment particles. Because of their benthic and/or sessile way of life, they can be used as good models that better reflect local contaminant concentrations, compared to other taxa such as crustaceans and free-swimming finfish. Marine gastropods and bivalves, oysters and mussels in particular, have been extensively used as model organisms in environmental studies (Wang *et al.*, 1996; Griscom *et al.*, 2000).



**Fig. 3:** Scatterplot of the sampling sites in the axis1/axis2 plan of the Principal Component Analysis applied using the data obtained with all species and the four heavy metals analyzed.



**Fig. 4:** Hierarchic classification of the sampling sites, based on Euclidean distance. The clustering was obtained considering all data obtained with the four heavy metals and the four mollusc species.

### Discussion

Marine molluscs, gastropods and bivalves, take up and accumulate pollutants, including heavy metals, either directly from the water column or indirectly via

This work is the first study dealing with the distribution of heavy metals in the Gulf of Gabes using different mollusc species. Since no similar studies have been carried out before in the Gulf of Gabes, it is not possible to make a comparative analysis of heavy metal distribution. The only published papers concern a single species (mol-

lusc or fish) and only one heavy metal, cadmium in particular, which is considered as one of the most toxic heavy metals (Burger, 2008; Barhoumi *et al.*, 2009). Cadmium contents recorded herein are the highest compared to the results of previous studies (Banni *et al.*, 2005; Smaoui-Damak *et al.*, 2006; Barhoumi *et al.*, 2009). In the same context, the findings of this work are also higher than those recorded in other parts of the Tunisian coastline (Banni *et al.*, 2007) and are comparable, for some heavy metals, with the results of the study carried out with *P. caerulea* by Belkhodja *et al.* (2012) in northern Tunisia.

With *P. nobilis*, the highest heavy metal contents were recorded in the hepatopancreas. A similar result has been reported by Catsiki *et al.* (1994) who explained that the hepatopancreas is the principal accumulation organ and can thus represent the highest concentrations of pollutants (Bryan, 1976; Catsiki *et al.*, 1994). Unfortunately, no other studies, dealing with the bioaccumulation of other heavy metals in *P. nobilis* have been carried out and, therefore, comparison with the results obtained herein is not possible. Otherwise, it is worth noting that the limited presence and distribution of the fan mussel can be related to the sensibility of this bivalve to marine pollution. In fact, a previous study on the distribution of this mollusc along the eastern and south-eastern (Gulf of Gabes) Tunisian coastline has revealed a relationship between the presence and density of *P. nobilis* and the distance from Gabes City as a source of pollution; the species was not found in the central area of the Gulf of Gabes and its density increased with distance from the City of Gabes, both southwards and northwards (Rabaoui *et al.*, 2010). This species was reported to be very sensitive to marine pollution (Vicente, 1990; Vicente & Moreteau, 1991; Richardson *et al.*, 2004). In this context, a recent study carried out by Cabanellas-Reboredo *et al.* (2010) revealed that the fan mussel may not discriminate between food sources in suspension, and that a large percentage of the organic matter consumed originates from resuspended sediments. Hence, resuspension of accumulated pollutants may intensify the uptake of toxic material by fan mussels, probably leading to the disappearance of this mollusc from polluted areas. Otherwise, a comparison of heavy metal concentrations found in Tunisian *P. nobilis* revealed that the cadmium, chromium and lead contents are higher than those obtained with other Pinnidae species i.e. *P. bicolor*, *P. deltodes*, *P. aturpurae* and *Atrina vexillum* (Baharuddin Pallan, 2010). However, the concentrations of mercury recorded during this study more or less differed from those recorded by the same author, with the latter Pinnidae species.

As for *P. caerulea*, the only published study was carried out in northern Tunisia by Belkhodja *et al.* (2012). This study revealed high concentrations of cadmium, chromium and lead in soft tissue of the gastropod in Bizerta lagoon channel, compared to Sidi Rais and La Goulette (Gulf of Tunis). Based on the study of Belkhodja

*et al.* (2012), the highest average concentrations of cadmium was estimated to be  $2.81 \mu\text{g g}^{-1}$  in Bizerta lagoon channel, which is lower than the highest average cadmium concentration recorded with *P. caerulea* in Gabes (i.e.  $4.300 \pm 0.3398 \mu\text{g g}^{-1}$ ). Similarly, the highest average contents of chromium and lead recorded in this work (chromium:  $2.171 \pm 0.1394 \mu\text{g g}^{-1}$  in Gabes; lead:  $2.123 \pm 0.07075 \mu\text{g g}^{-1}$  in Djerba Island) are lower than those recorded in Bizerta lagoon channel ( $3.46 \mu\text{g g}^{-1}$  for chromium and  $4.78 \mu\text{g g}^{-1}$  for lead) by Belkhodja *et al.* (2012) (Table 4). A comparison of our results with the findings of other authors who have worked with *P. caerulea* showed that the highest concentrations of cadmium recorded herein are lower than those recorded in Ustica and Linosa Islands in Italy by Conti *et al.* (2007, 2010) and also than those recorded in Favignana Island in Sicily, Italy (Campanella *et al.*, 2001; Cubadda *et al.*, 2001) (Table 4); nevertheless, they were comparable to the values mentioned by Schiber & Shatila (1978) in Lebanon and by Conti & Cecchetti (2003) in the Tyrrhenian Sea. The highest cadmium concentrations reported in this study were, however, lower than those noted in the Gulf of Suez (Red Sea, Egypt) by Hamed (1996) and Hamed & Emar (2006) (Table 4).

With *G. ardens* samples, the concentrations of cadmium, chromium and lead recorded herein were much lower than those noted in the coast of Al-Hanyaa, Libya (Ramadan & Ahmed, 2010) (Table 4). The latter authors assessed the contents of the above-mentioned heavy metals in both a polluted and unpolluted area and the concentrations found were lower than those recorded herein.

Concerning the results obtained with *P. radiata* samples, no studies about heavy metal pollution using this bivalve have been carried out before in Tunisia. A comparison of the results obtained herein with the findings of other authors revealed some differences with respect to the study areas. Average cadmium contents recorded in the Gulf of Gabes (between  $0.605 \pm 0.0252$  and  $1.065 \pm 0.0858 \mu\text{g g}^{-1}$ ) were found to be higher than those recorded in Qatari waters, Arabian gulf (between  $0.030 \pm 0.14$  and  $0.85 \pm 0.18 \mu\text{g g}^{-1}$ ) by Al-Madfa *et al.* (1998), those noted in Saudi Arabia ( $0.66 \mu\text{g g}^{-1}$ ) by Fowler *et al.* (1993), those mentioned in Australia by Peerzada & Dickinson (1989) i.e. between 0.25 and  $0.78 \mu\text{g g}^{-1}$ , and those recorded in Akkuya Bay, İçel, Turkey ( $0.0058 \pm 0.00034 \mu\text{g g}^{-1}$ ) by Göksu *et al.* (2005) (Table 4). The latter authors have also recorded lower average cadmium content in *Brachidontes pharaonis* in the same study area (Göksu *et al.*, 2005). Previous studies carried out on *P. radiata* showed higher (extremely higher in some cases) cadmium concentrations, in Bahrain (Fowler *et al.*, 1993; Al-Sayed *et al.*, 1994), the United Arab Emirates (Fowler *et al.*, 1993), Oman (Fowler *et al.*, 1993), and Kuwait (Bou-Olyan *et al.*, 1995) in particular. In contrast, the average lead concentrations found herein with *P. radiata* samples (between  $0.722 \pm 0.0757$  and  $1.848 \pm 0.0711 \mu\text{g g}^{-1}$ ) were found to be lower than those

**Table 4.** Comparison of average heavy metals concentrations recorded during the present study with those noted in other areas with the same species and tissues and with the maximum limits fixed by the World Health Organization (WHO) and Food and Agriculture Organization (FAO). EB: Elbibane lagoon, DI: Djerba Island, CH: Chebba, GA: Gabes, LO: Louata, ZZ: Zarzis, MA: Mahres, KI: Kerkennah Island, GT: Gulf of Tunis, BL: Bizerta lagoon.

SPECIES	COUNTRY (SITE)	TISSUE	MINIMAL AND MAXIMAL AVERAGE HEAVY METALS CONCENTRATIONS				REFERENCES
			Mercury ( $\mu\text{g g}^{-1}$ )	Lead ( $\mu\text{g g}^{-1}$ )	Cadmium ( $\mu\text{g g}^{-1}$ )	Chromium ( $\mu\text{g g}^{-1}$ )	
<i>Gibbula ardens</i>	Tunisia (Gulf of Gabes)	soft body	0.042±0.018 (EB) - 0.181±0.020 (ZZ)	0.633±0.107 (CH) - 2.543±0.527 (DI)	0.244±0.067 (CH) - 3.005±0.117 (DI)	0.041±0.091 (CH) - 1.664±0.246 (DI)	Present study
	Lybia (Al-Hamyaa coast)	soft body	-	0.54±0.17	0.32±0.01	0.49±0.15	Ramadan & Ahmed (2010)
	Tunisia (Gulf of Gabes)	soft body	0.031±0.001 (EB) - 0.679±0.0846 (LO)	0.823±0.0575 (EB) - 2.123±0.07075 (DI)	0.800±0.0288 (EB) - 4.300±0.3398 (GA)	0.321±0.0106 (CH) - 2.171±0.1394 (GA)	Present study
	Northern Tunisia (Gulf of Tunis and Bizerta lagoon)	soft body	-	2.14 (GT) - 4.78 (BL)	0.65 (GT) - 2.81 (BL)	0.37 (GT) - 3.48 (BL)	Belkhdja <i>et al.</i> (2012)
	Italy (Ustica Island)	soft body	-	0.85 - 1.30	3.27 - 6.87	0.28 - 0.95	Conti <i>et al.</i> (2007)
	Italy (Linosa Island)	soft body	-	0.40 - 2.41	2.69 - 4.94	0.13 - 0.75	Conti <i>et al.</i> (2010)
	Italy (Sicily, Favignana Island)	soft body	-	0.14 - 1.52	3.3 - 6.3	0.19 - 0.46	Campanella <i>et al.</i> , 2001
	Italy (Sicily, Favignana Island)	soft body	-	0.06 - 2.18	1.7 - 11.8	0.1 - 1.01	Cubadda <i>et al.</i> , 2001
	Lebanon	soft body	-	6.8 - 95.6	0.4 - 4.7	-	Schiber & Shatila (1978)
	Tyrrhenian Sea	soft body	-	0.51 - 1.50	2.89 - 4.06	0.72 - 0.96	Conti & Cecchetti (2003)
<i>Pinctada radiata</i>	Egypt (Gulf of Suez, Red Sea)	soft body	-	1.12 - 1.15	0.06 - 0.17	0.36 - 0.51	Hamed (1996)
	Egypt (Gulf of Suez, Red Sea)	soft body	-	6.23 - 70.91	0.63 - 2.13	2.34 - 7.99	Hamed & Emara (2006)
	Tunisia (Gulf of Gabes)	muscle	0.040±0.0095 (CH) - 0.387±0.0127 (MA)	0.722±0.0757 (EB) - 1.848±0.0711 (DI)	0.605±0.0252 (EB) - 1.065±0.0858 (GA)	0.335±0.008 (KI) - 1.605±0.2859 (GA)	Present study
	Tunisia (Gulf of Gabes)	hepatopancreas	0.044±0.0070 (EB) - 0.420±0.0990 (LO)	0.860±0.0607 (EB) - 2.288±0.2791 (DI)	0.732±0.1877 (EB) - 1.295±0.1729 (GA)	0.390±0.0214 (CH) - 2.066±0.2222 (ZZ)	Present study
	Qatar (Arabian Gulf)	soft body	-	1.25±1.01 - 5.59±0.93	0.030±0.14 - 0.85±0.18	-	Al-Madfa <i>et al.</i> (1998)
	Turkey (Akkaya Bay, İcel)	soft body	-	-	0.0058 ± 0.00034	-	Göksu <i>et al.</i> (2005)
	Saudi Arabia	soft body	-	0.43	0.66	-	Flowler <i>et al.</i> (1993)
	Australia	soft body	-	nd - 1.9	0.25 - 0.78	-	Peerzada & Dickinson (1989)
							(continued)

(continued) Table 4.

SPECIES	COUNTRY (SITE)	TISSUE	MINIMAL AND MAXIMAL AVERAGE HEAVY METALS CONCENTRATIONS				REFERENCES
			Mercury ( $\mu\text{g g}^{-1}$ )	Lead ( $\mu\text{g g}^{-1}$ )	Cadmium ( $\mu\text{g g}^{-1}$ )	Chromium ( $\mu\text{g g}^{-1}$ )	
	Bahrain	soft body	-	1.25 – 14.0	0.25 – 3.8	-	Al-Sayed <i>et al.</i> , 1994
	Bahrain	soft body	-	0.32 – 3.9	3.9 – 9.9	-	Flowler <i>et al.</i> , 1993
	UAE	soft body	-	0.14	11.1	-	Flowler <i>et al.</i> , 1993
	Oman	soft body	-	0.08 – 0.37	4.9 – 15.3	-	Flowler <i>et al.</i> , 1993
	Kuwait	soft body	-	10.68 – 18.66	1.85 – 3.16	-	Bou-Olyan <i>et al.</i> , 1995
	Tunisia (Gulf of Gabes)	mantle	0.027±0.0025 (EB) - 0.312±0.0974 (LO)	0.691±0.1435 (EB) - 1.682±0.1348 (DI)	0.666±0.0960 (EB) - 1.790±0.6480 (DI)	0.321±0.0297 (EB) - 1.627±0.2248 (DI)	Present study
	Greece (Gulf of Geras, Lesbos Island)	mantle	-	-	-	3.20±1.80 - 237.68±209.91	Catsiki <i>et al.</i> (1994)
	Tunisia (Gulf of Gabes)	gills	0.026±0.0050 (CH) - 0.399±0.0385 (LO)	0.654±0.0764 (EB) - 1.221±0.2257 (DI)	0.374±0.0681 (EB) - 1.039±0.1460 (DI)	0.183±0.0638 (EB) - 0.870±0.1008 (DI)	Present study
	Greece (Gulf of Geras, Lesbos Island)	gills	-	-	-	1.49±0.82 - 262.00±221.02	Catsiki <i>et al.</i> (1994)
<b><i>Pinna nobilis</i></b>	Tunisia (Gulf of Gabes)	muscle	0.041±0.0083 (CH) - 0.169±0.048 (KI)	0.643±0.0814 (EB) - 1.032±0.1659 (DI)	0.312±0.0960 (EB) - 0.966±0.1524 (DI)	0.192±0.0096 (EB) - 0.604±0.0777 (DI)	Present study
	Greece (Gulf of Geras, Lesbos Island)	muscle	-	-	-	0.89±0.68 $\mu\text{g g}^{-1}$ - 1.97±2.85 $\mu\text{g g}^{-1}$	Catsiki <i>et al.</i> (1994)
	Tunisia (Gulf of Gabes)	hepatopancreas	0.033±0.0100 (EB) - 0.285±0.0517 (LO)	0.645±0.1336 (EB) - 1.209±0.2000 (DI)	0.674±0.1575 (EB) - 1.121±0.0905 (CH)	0.386±0.0565 (EB) - 1.687±0.3027 (DI)	Present study
	Greece (Gulf of Geras, Lesbos Island)	hepatopancreas	-	-	-	3.53±1.75 - 153.10±166.16	Catsiki <i>et al.</i> (1994)
	-	-	Maximum limits for metals in mollusks, fixed by WHO and FAO				-
	-	-	0.5 (WHO); 0.5 (FAO)	2.0 (WHO); 0.5 - 6.0 (FAO)	1.0 (WHO); 0.05 - 5.5 (FAO)	50 (WHO); 1.0 (FAO)	WHO (1989); FAO (1992)

mentioned for the Qatari coasts (between  $1.25 \pm 1.01$  and  $5.59 \pm 0.93 \mu\text{g g}^{-1}$ ) by Al-Madfa *et al.* (1998) (Table 4).

Taking into account the impact of pollution, the comparison of the sampling sites in the Gulf of Gabes reveals an increasing pollution level, from the city of Gabes to the northern or southern sites. However, some southern sites, Djerba Island and Zarzis in particular, were found to be as polluted as the site of Gabes, i.e. the source of pollution. Such a result can be explained either by the special hydrodynamic features of the Gulf of Gabes, which are responsible for transporting the pollutants to these locations, or by the domestic waste from Zarzis and the cities on Djerba Island, Houmet Essouk in particular, from where the Djerba Island samples were taken. In fact, this city is among the well-developed cities (apart from Midoun and Gallala cities) on Djerba Island and is well-frequented by tourists; all domestic waste originating from urban areas, including tourism resorts, are thrown directly into the sea. Domestic pollution was reported to be a source of heavy metal pollution (Wei & Yang, 2010). Besides, although the Zarrat and Elgrine sites are closer to Gabes City, they were found to be less polluted than Djerba Island and Zarzis, and hence they were not clustered with Gabes City (Fig. 4). This could be due to the factors explained above, in particular hydrodynamics. Moreover, the Elgrine and Zarrat localities represent small urban areas with no considerable domestic pollution and no industrial activities. Otherwise, high levels of mercury were noted in Louata (northern Gabes City). The latter locality is far from Gabes City and hence the high contents of mercury recorded at this station do not seem to be due to the phosphoric acid industry in Gabes. Louata is however close to Sfax, which is characterized by an important coastal industrial zone. Alkali and metal processing, incineration of coal, and medical and other waste could be possible sources of mercury in this area.

This study has revealed the importance of using marine molluscs as bioindicators to assess heavy metal pollution in the Gulf of Gabes. *P. Pinna nobilis* and *P. radiata* were found to accumulate the targeted metals to a lesser extent than the two other gastropod species (*G. ardens* and *P. cearulea*). This may be due to the fact that these species are less important accumulators of heavy metals or because their trophic guild is lower (filter feeders) than the two gastropod species (algae and detritus feeders). Based on the results obtained herein, heavy metal pollution is certain in the Gulf of Gabes and it appears that pollution has reached critical levels since the highest pollutant concentrations exceeded the standards fixed by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) (Table 4). Based on a previous study on the impact of phosphogypsum on the environment in Sfax, it has been shown that phosphogypsum contains high levels of heavy metals that have negative effects on the environment and the marine ecosystem in particular, which is the final destination of phospho-

gypsum waste (Zairi & Rouis, 1999). Considering the present study, the phosphoric acid industry of Sfax does not seem to affect the marine environment in the localities close to this city (i.e. northern Gabes). This is because phosphogypsum waste from this industry is not discharged at sea, but deposited on the coast, near the company. Besides, the phosphoric acid industry of Sfax is considerably smaller than that of Gabes and, a few years ago, started reducing its activity with the objective to cease operating in the future. Although the ranking of study localities appears to be in general agreement with the hypothesis of this study, some localities appeared to be in discordance with the general pattern found. This is the case of Djerba Island, Zarzis, Elgrine, and Zarrat. This may be due to the hydrodynamics of the area, for some sites, or to other pollution sources, for other localities. These factors should be considered in other studies dealing with the same subject. Reaching this critical level of pollution in the Gulf of Gabes could be explained by the fact that the last political regime used to forbid studies on the impact of pollution on the ecosystem of the study area. Now, after a change in the political regime of Tunisia, the influence of pollution on the ecosystem of the Gulf of Gabes gulf should be seriously investigated, at various levels, by the scientific community. Besides, a political strategy should be designed aiming at solving, in the short and/or long term, the pollution problems in the Gulf of Gabes and restocking marine resources.

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