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Assessment of Metallothioneins in Tissues of the Clam *Megapitaria squalida* as Biomarkers for Environmental Cadmium Pollution From Areas Enriched in Phosphorite

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Abstract The aim of this study was to evaluate the use of metallothionein (MT) concentrations in tissues of the clam *Megapitaria squalida* as biomarkers of environmental cadmium (Cd) pollution from phosphorite enrichments in the marine environment, which resulted from mining activities in La Paz Bay, Baja California Sur, Mexico. Cd and MT were quantified in gills, digestive gland, and kidney of clams exposed to 0.2 or 0.5 mg Cd l^{-1} for 10, 20, or 30 days. In addition, clams from four strategically selected natural sites of La Paz Bay were collected for analysis. In tissues of bioassayed and untreated clams, the gradient of Cd concentrations was digestive gland >> gills > kidney, whereas that of MT was digestive gland > gills > kidney. Digestive gland of the clams exposed to 0.5 mg Cd l^{-1} for 30 days showed the highest concentrations of Cd (16.3 ± 3.9 μg Cd g^{-1}). The highest statistically significant MT concentrations were found in digestive gland at 10 days of exposure to Cd. In the untreated clams, one of the highest Cd concentrations, but not MT levels, was found in digestive glands of the organisms collected from the area close to phosphorite mining activities. For environmental monitoring, MT levels in digestive gland can be used as a first approximation of the presence of high levels of divalent metals in the environment. However, in this study, MT levels did not correlate with high Cd levels in clams that had been collected from areas associated with phosphorite enrichment.

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Metallothioneins (MTs) are low molecular–weight, metal-binding, cytosolic proteins (Mouneyrac et al. 1999) with a high number of sulfhydryl groups due to their cysteine content. MT are involved in several metabolic functions, including the homeostasis of essential metals (e.g., copper [Cu] and zinc [Zn]) and the detoxification of nonessential metals (e.g., cadmium [Cd] and mercury [Hg]) (Klaassen et al. 1999). These proteins have been associated with important cellular protective processes, such as inactivation of hydroxyl radicals and protection against immunotoxins, hematoxins, and nephrotoxins (Bordin 2000). Because of these characteristics, MT has been proposed for use as a biomarker for heavy-metal pollution (Dabrio et al. 2002).

For most studies of MT induction, bivalves have been used because these sedentary, filter-feeding organisms have subcellular systems that are involved in the accumulation of metals (Bebianno and Serafim 1998). Induction of MT can occur in several tissues, depending on the exposure period as well as the chemical presentation and concentration of the inducer. In the clam *Corbicula fluminea* from freshwater lakes (Aquitaine, France), gills are the main organ of MT synthesis (Baudrimont et al.1997). In *Ruditapes decussates*, the highest MT levels were found in digestive gland (Bebianno and Serafim 2003; Serafim and Bebianno 2007).

In bivalves, divalent metals, such as Cd and Zn, are accumulated in gills, digestive gland (Boutet et al. 2002; Tanguy et al. 2003; Serafim and Bebianno 2007), mantle (Serra et al. 1995; Kádár 2007), and kidney (In-Young et al. 2001; Blackmore and Wang 2004). Dabrio et al. (2002) demonstrated that Cd is the major inducer of MT in

mollusks. Cd constitutes a major environmental health problem because Cd increases oxidative stress, mainly causing renal injury, which leads to renal dysfunction (Morales-Martín et al. 2004).

One of the most important sources of Cd in aquatic ecosystems is that of mineral deposits, such as phosphorite banks, which contain heavy metals (such as Cd) as common impurities (Mann and Ritchie 1995). Therefore, close to areas containing the tailings from phosphorite mines, it is common to find increased levels of Cd in sediments, water, plants, and marine organisms (Gnandi et al. 2006). One of the largest natural phosphorite banks in the world is located in the peninsula of Baja California, Mexico (Riley 1989). In La Paz, at a site close to one of these phosphorite deposits, Méndez et al. (2006) found high Cd concentrations in the clam *Megapitaria squalida* (2.22 μg Cd g^{-1} wet weight [ww]). These levels are higher than the concentration (2 μg Cd g^{-1} ww) considered by international agencies with jurisdiction over seafood, such as the Australia New Zealand Food Authority (Abbott et al. 2003) and the Hong Kong Food and Environmental Hygiene Department (Copes et al. 2008), as being the maximum concentration permissible in clams for human consumption.

The quantification of MT has been used as a sensitive biomarker of anomalous levels of divalent metals, such as Cd. For the clam *Crassostrea gigas*, Boutet et al. (2002) recorded statistically significant differences between MT levels in organisms collected in Oléron compared with those collected from Pointe du Bendy (both sites in France): an average of 5.55 and 2.25 μg Cd g^{-1} dry weight (dw) (approximately 1.11 and 0.45 μg Cd g^{-1} wet weight [ww]), respectively.

There is little information related to MT in organisms from tropical or subtropical marine environments. Thus, in environmental monitoring along tropical coasts, the evaluation of MT levels in bivalves could be useful as a first approximation of the presence of toxic heavy metals, such as Cd.

The clam *M. squalida* is a sediment-burrowing, filter-feeding, bivalve mollusk (Schweers et al. 2006) that is abundant in La Paz Bay. This organism has a geographic distribution from Baja California, Mexico, to Mancora, Peru (Keen 1971). This species, which is economically important to the area of La Paz Bay, has been used as a bioindicator of Cd, nickel [Ni], iron [Fe], magnesium [Mn], lead [Pb], Cu, and Zn levels (Méndez et al. 2006; Cantú-Medellín et al. 2009). With the overall goal of evaluating the use of MTs as biomarkers of Cd contamination, we selected two concentrations of Cd, which we determined to be sublethal for adult *M. squalida*, to analyze the accumulation of this element in three different tissues with time. We compared the levels of MT found in these bioassays with the levels found in untreated organisms collected either close to or at a distance from areas with phosphorite mining activities.

Materials and Methods

Biologic Material

Adult *M. squalida* clams ($n = 400$) were collected in a natural area in the north of La Paz Bay. The harvesting site is located far (approximately 35 km) from the area of mining activities and undergoes no other anthropogenic activities. The clams were transported in seawater to the laboratory where they were placed in 40-l plastic tanks containing 2 μm filtered seawater (maintained at 21 ± 1°C and exchanged daily). During the course of 1 month, the clams were acclimated and their digestive systems depurated of sediment content. The clams were fed with the microalgae *Isochrysis galbana*.

In addition, sampling of adult *M. squalida* (15 clams/site) was performed at 4 sites along the shore of La Paz Bay: Animas, El Sausozo, El Quelele, and Balandra. Each site was visited once (Fig. 1). The organisms were transported to the laboratory on ice and frozen (−20°C) until they were assayed. The sampled sites were chosen for the following reasons: (1) In a previous study, the highest and

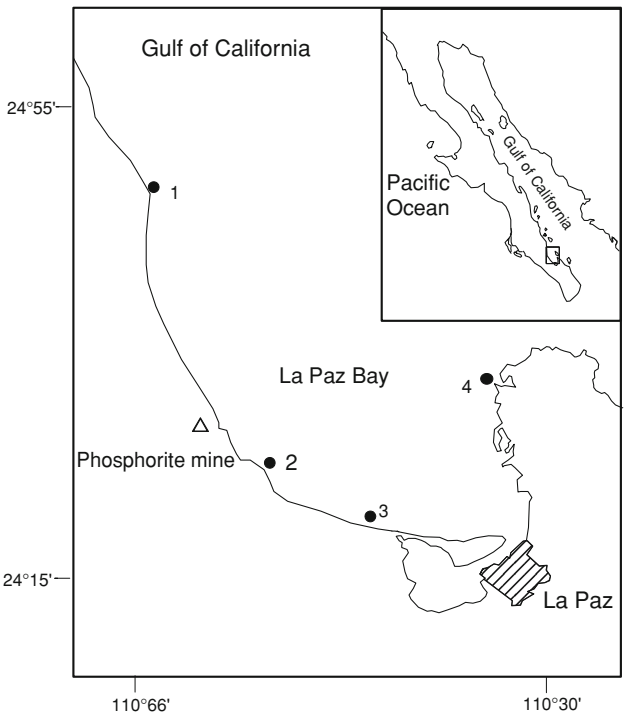

Fig. 1 Sampling sites along the coastline of La Paz Bay, Baja California Sur, Mexico. Clams (*M. squalida*) were harvested from four natural areas: Animas, El Sausozo, El Quelele, and Balandra

lowest levels of Cd in *M. squalida* were recorded at Animas and Balandra, respectively (Méndez et al. 2006); (2) El Sausozo is the site closest to the phosphorite mine (approximately 10 km); and (3) marine currents may transport marine sediments from El Sausozo to El Quelele (Monteforte and Cariño 1992), thereby increasing the levels of Cd in that area of La Paz Bay.

All clams used in this study ranged between 6 and 8 cm in length and were harvested, at a depth of 3–6 m, from sediment that consisted of sand containing <2% organic carbon (Rodríguez-Castañeda 2001). The areas of collection share the same arid climate. They generally do not receive fresh water, with the only exception being episodic discharges of ephemeral water streams ("arroyos") that are formed after rare but heavy rains (average rainfall 175 mm $year^{-1}$; average salinity 36 psu) (Gonzalez-Acosta et al. 2006).

Experimental Cd Exposure

Bioassays and Tissue Extracts

To obtain sublethal doses of Cd for adult *M. squalida*, a 30-day bioassay was performed. Of the clams that were viable after the acclimation period, 324 (all between 6 and 8 cm in length) were used for this purpose, with the remainder being discarded. Clams were exposed for 15 days to $CdCl_2$ dissolved in 2 μm filtered seawater at final concentrations of 0.1, 0.2, 0.5, 0.7, 1, 2, 3, or 6 mg Cd l^{-1} (0.89 to 53.38 μM Cd). Twelve clams were exposed to each concentration, with treatments run in triplicate. An untreated group (36 clams) was included as the control group. Water in each tank (20 ± 1°C and 38 psu) was changed twice a week, and Cd dosing was repeated at each change; clams were fed with *I. galbana*. Based on the results of the bioassay, two concentrations that had proven to be sublethal were selected, and treatment of the clams exposed to these concentrations was continued for ≤30 days.

Thus, the clams that had been treated with either 0.2 or 0.5 mg Cd l^{-1} (1.77 or 4.44 μM Cd, respectively), as well as those in the control group, were used for the MT and Cd studies. On completion of 10, 20, or 30 days of exposure, three clams from each group were taken to quantify MT induction and Cd concentrations. To this end, gills, digestive gland, and kidney were dissected from each clam, and each organ was processed individually. Each organ was weighed and homogenized in three volumes of Tris buffer (20 mM, pH 8.4) in an ice bath, and each resulting mixture was centrifuged (at 10,000 rpm; 4°C; 1 h). Each recovered supernatant was heated (80°C; 10 min) and centrifuged (10,000 rpm; 4°C; 1 h). Each resultant supernatant was recovered for analysis of Cd and MT levels (Bebianno and Serafim 1998; Wolf et al. 2000).

Natural Cd Exposure

Tissue Extracts

Of the 15 untreated clams per site, 9 were thawed, and each was individually treated, as previously described, to determine the levels of MT and Cd in gills, digestive gland, and kidney.

Whole-Clam Extracts

For the analysis of total Cd and MT, the whole tissue of each of the remaining untreated clams (six per site) was processed individually. After being thawed, each clam was weighed, dried at 70°C, digested in a microwave oven (CEM model Mars 5X; CEM, Matthews, NC), and extracted (Méndez et al. 2006).

MT Analysis

The MT concentration (μg g^{-1} ww) in each of the supernatants was estimated by reverse-phase high performance liquid chromatography (HPLC) on an HPLC chromatograph (Agilent, HP Agilent 1100, Waldbronn, Germany) using a Supelcosil LC-318 column; the detector diode array was configured at 230 nm. The eluants were 0.1% trifluoroacetic acid (TFA) in water at pH 3 (solvent A) and 0.1% TFA in acetonitrile (solvent B). Commercial rabbit liver MT (Sigma-Aldrich, St. Louis, MO) dissolved in Tris–HCl buffer (20 mM, pH 8.4) was used as calibration standard as described by Bordin et al. (1996). For each sample, supernatant (5 μl) was loaded, and the separation was performed at a flow rate of 1 ml min^{-1}. Fractions (1 ml) were collected for Cd quantification as described later in text. Results are expressed as mean ± SE.

Cd Quantification

To quantify the Cd levels (all data expressed as ww), each fraction collected from the high-pressure liquid chromatograph (see previous section) and analyzed directly by atomic absorption spectrophotometry (AAS) using an Avanta air-acetylene flame (GBC Scientific Equipment, Dandenog, Australia). The detection limit was 0.04 μg l^{-1}. Concentrations were measured with a relative precision of 2%. For tissue analysis, certified standard reference material TORT-2 (National Research Council of Canada, Ottawa) was used for accurate calibration (95% recovery).

Statistical Analyses

Statistical significance ($p < 0.05$) was assessed using Kruskal–Wallis nonparametric significance test. For all

statistical methods, STATISTICA (version 7; StatSoft, Tulsa, OK) was used. The level of significance (p) is indicated when statistical differences are recorded between the samples.

Results

MT Induction Assay

During the course of the bioassay, mean survival of the clams that had been exposed to 1 and 2 mg Cd l^{-1} was 50 and 0%, respectively. Thus, 0.2 and 0.5 mg Cd L^{-1} were selected for use in the MT induction assay. No mortality was observed during the course of the induction study.

For the 0.5 mg Cd L^{-1} treatment, the Cd concentration in gills showed significant differences between controls and the groups exposed for 20 ($p = 0.042$) or 30 days ($p = 0.011$), with the highest levels (2.8 ± 0.8 μg Cd g^{-1}) found in clams exposed for 30 days (Fig. 2a). This Cd concentration was significantly higher ($p < 0.05$) than the levels found in clams (0.8 ± 0.2, 1.5 ± 0.5, and 1.0 ± 0.7 μg Cd g^{-1}) exposed to 0.2 mg Cd L^{-1} for 10, 20, or 30 days, respectively (Fig. 2a). In the digestive gland of the clams exposed to 0.2 mg Cd l^{-1}, the Cd concentration at 20 days was significantly higher ($p = 0.045$) than that at 10 days (10.8 ± 2.8 μg Cd g^{-1} vs. 5.4 ± 1.7 μg Cd g^{-1}, respectively). However, at 30 days, the Cd concentration decreased but not significantly (Fig. 2b). Digestive gland of the clams exposed at 0.5 mg

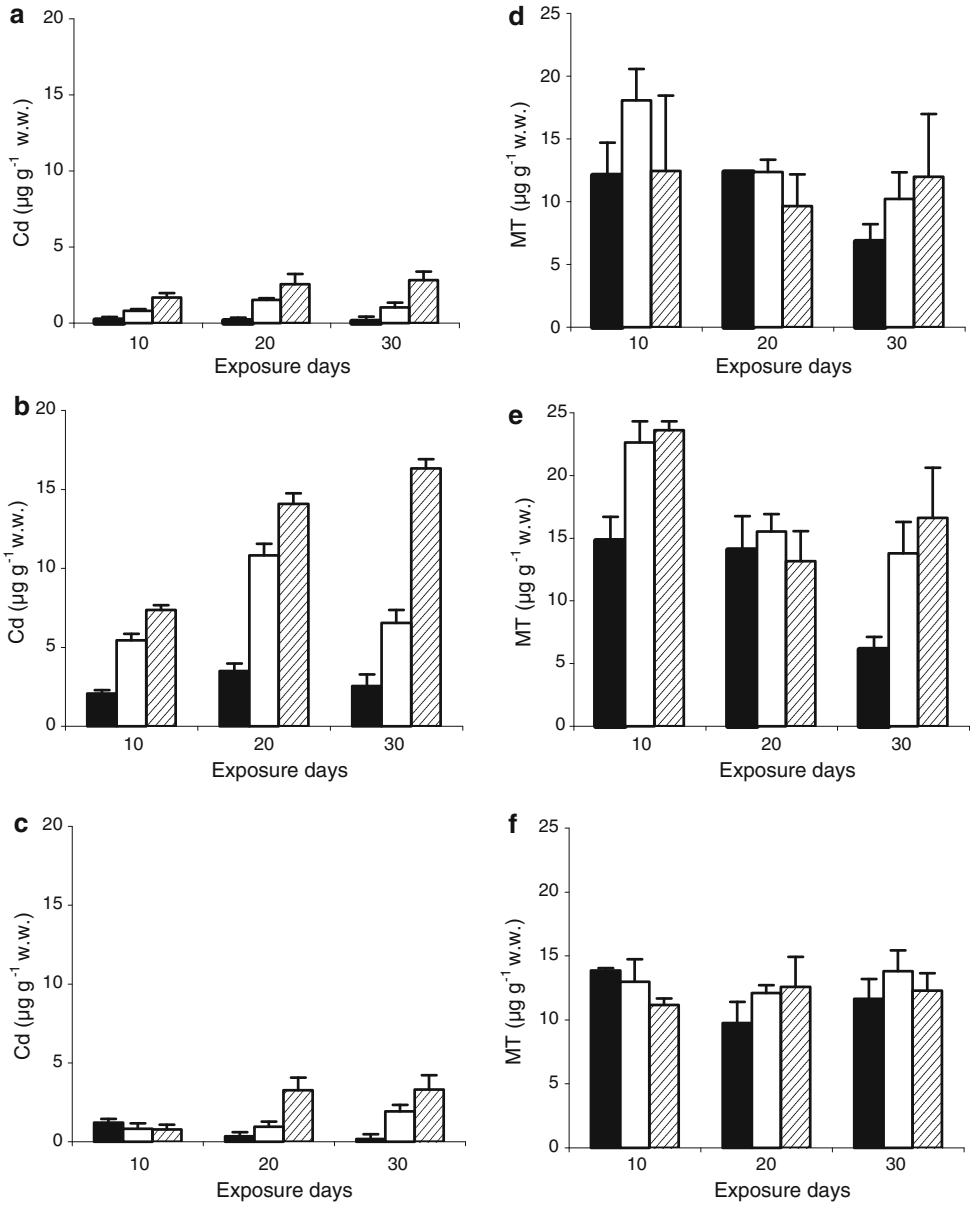

Fig. 2 Cd and MT concentrations in three organs of clams *M. squalida* from La Paz Bay, Baja California Sur. Control clams (*black bar*) or clams exposed for 10, 20, or 30 days to either 0.2 (*white bar*) or 0.5 (*hatched bar*) mg Cd l^{-1}. Shown are Cd concentrations in gills (**a**), digestive gland (**b**), and kidney (**c**), as well as MT concentrations in gills (**d**), digestive gland (**e**), and kidney (**f**)

Cd l^{-1} showed a significant increase over controls at 20 ($p = 0.045$) and 30 days ($p = 0.049$) of exposure (14.1 ± 0.5 and 16.3 ± 3.9 μg Cd g^{-1}, respectively). In kidney, significant differences ($p < 0.05$) in Cd levels were found between controls and clams exposed to 0.5 mg Cd l^{-1} for 20 or 30 days (3.3 ± 1.2 and 3.3 ± 0.9 μg Cd g^{-1}, respectively) (Fig. 2c).

In reverse-phase HPLC, MT was detected at minute 2.7. The sample fraction having the highest Cd concentration had the same retention time as did a peak for the MT standard, which is known to contain a Cd-binding protein. For the three tissues analyzed, all MT-related peaks were detected at the same retention time.

In general, higher variability and lower concentrations of MT were found in kidney (Fig. 2f) compared with the other two tissues. No significant differences in MT levels were observed in gills or kidney compared with controls (Figs. 2d and 3f). The highest MT levels (22.6 ± 1 and 23.6 ± 0.08 μg MT g^{-1}) were found in digestive gland of clams at 10 days of exposure to 0.2 or 0.5 mg Cd l^{-1}, respectively (Fig. 2e). In digestive gland, although Cd concentrations increased at 20 and 30 days of exposure, MT tended to show decreasing levels with time; however, the latter values were not statistically significant (Fig. 2).

Cd and MT Concentrations in Untreated Clams

In untreated clams, the pattern of Cd accumulation in the three organs studied was similar to that observed in clams in the MT induction assay, with digestive gland having the highest Cd concentration (Fig. 3). Of the four sites, the Cd levels in digestive gland in organisms from Quelele were significantly lower ($p < 0.05$) than those from the other sites. The lowest levels of Cd in kidney were found in organisms from Balandra (0.02 ± 0.05 μg Cd g^{-1}). When whole organisms were analyzed, higher Cd levels ($p < 0.05$) were found for clams from Animas (1.10 ± 0.16 μg Cd g^{-1}) and El Sausozo (1.12 ± 0.16 μg Cd g^{-1}) than for those recorded in Quelele (0.77 ± 0.11 μg Cd g^{-1}) and Balandra (0.69 ± 0.11 μg Cd g^{-1}).

No relation was found between the concentrations of MT and Cd in untreated clams. MT concentrations in gills from untreated clams (Fig. 4) were lower ($p < 0.05$) for those from El Sausozo (9.2 ± 1.2 μg MT g^{-1}) than for those from Balandra (14 ± 1.4 μg MT g^{-1}). MT levels in digestive gland of clams from Animas were higher ($p < 0.05$) (13.9 ± 1.2 μg MT g^{-1}) than those from El Sausozo (10.2 ± 1.3 μg MT g^{-1}). MT levels were higher ($p < 0.05$) in kidney of clams from Animas and Balandra (14.3 ± 2.3 and 12.9 ± 1.3 μg MT g^{-1}, respectively) compared with those from El Sausozo and Quelele (6.9 ± 1.3 and 5.1 ± 0.8 μg g^{-1}, respectively) (Fig. 4).

Discussion

The Cd concentrations used in this study are similar to those employed in comparable studies (Bebianno et al. 1993; Boutet et al. 2002). The differences between the present work and other MT studies were not only in the organisms employed but also in the temperatures of the seawater from which the organisms were collected as well as that in which the clams were maintained and tested. Temperature increases the filtration rate of the clams, thus increasing the Cd levels that can be accumulated in their tissues (Bebianno and Serafim 2003; Croteau et al. 2005). Annual recorded temperatures for La Paz Bay range from 20 to 31°C (Gonzalez-Acosta et al. 2006), whereas for most other MT studies the stated temperatures were

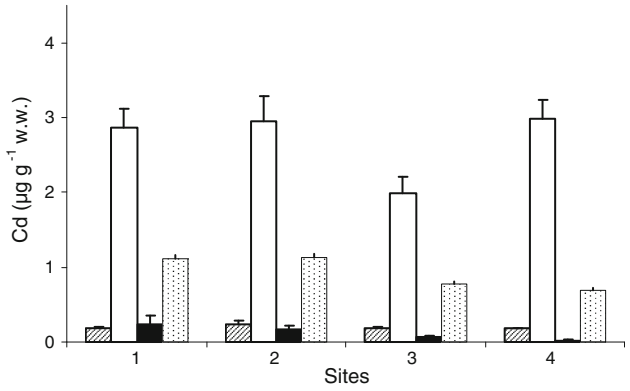

Fig. 3 Cd concentrations in whole tissue and three organs of untreated clams *M. squalida* collected from four sites of La Paz Bay. Shown are gills (*hatched bar*), digestive gland (*white bar*), kidney (*black bar*), and whole tissue (*point bar*) of clams from four natural areas: Animas, El Sausozo, El Quelele, and Balandra

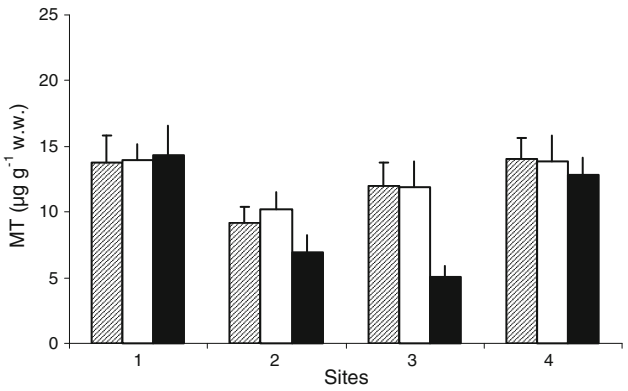

Fig. 4 MT concentrations in three organs of untreated clams *M. squalida* collected from four sites of La Paz Bay. Shown are gills (*hatched bar*), digestive gland (*white bar*), and kidney (*black bar*) of clams from four natural areas: Animas, El Sausozo, El Quelele, and Balandra

between 10 and 15°C (Bebianno et al. 1993; Boutet et al. 2002; Podgurskaya and Kavun2005).

The highest concentrations of Cd and MT in digestive gland are probably due to dietary exposure, a major route by which metals enter marine organisms (Rainbow 2002; Wang 2002). In this route of exposure, metals arrive first to the digestive gland, activating mechanisms (such as MT synthesis) involved in cellular regulation and detoxification of essential (such as Zn) and nonessential (such as Cd) metals (Rainbow 2002). Cd is mainly bioaccumulated by aquatic invertebrates bound to MT in the cytosol of the organ predominantly used for accumulated Cd (Bebianno et al. 1993), which in this study was found to be the digestive gland, which is in agreement with results reported previously by several other investigators (Amiard et al. 1989; Boutet et al. 2002; Bebianno and Serafim 2003; Machreki-Ajmi et al. 2008). In the present study, digestive gland showed the highest MT concentration in clams exposed for 10 days to both experimental concentrations; however, the levels decreased at 20 days. A similar response was reported in *C. gigas* (Boutet et al. 2002) and *M. edulis* (Bebianno and Langston 1991). The latter study suggested an overall decrease in digestive-gland metabolic rate due to the cellular toxicity of the heavy metal (Geret et al. 2002). This decrease in MT levels could be explained by hormesis, which is characterized by low-dose stimulation/high-dose inhibition of the detoxification mechanism. However, it is also important to consider that the half-life of MT is estimated to be between 4 and 20 days, depending on the time and concentration at which the organisms are exposed to Cd, and then the degraded MT is rebound to newly synthesized MT (Roesijadi and Robinson 1994) or detoxified in phosphate granules (Rainbow 2002).

The induction mechanisms by which the metal will be bioaccumulated could differ among organisms (Rainbow 2002). Therefore, two species that live in a same place can differ in the types and concentrations of metals they accumulate (Rainbow 2002; Wang 2002). However, in the present study, *M. squalida* showed a pattern of bioaccumulation similar to that of other bivalves, such as the clam *R. decussatus* (Bebianno and Serafim 2003), the cockle *Cerastoderma glaucum* (Machreki-Ajmi et al. 2008), and the oyster *Ostrea edulis* (Tanguy et al. 2003). In all of these studies, higher levels of Cd and MT were recorded for digestive gland than for gills. The investigators attributed their results to the fact that although gills and digestive gland act as the main reservoirs of metals, as described by Boutet et al. (2002) and Tanguy et al. (2003), these organs have different physiologic roles: The gills are in direct contact with the surrounding environment and reflect short-term metal exposure, and the digestive glandwhere several number of metalloenzymes bind, metabolize, and accumulate excess metal concentrationsacts as a storage organ, thus reflecting long-term metal exposure (Amiard et al. 1989; Bebianno et al. 1993). These results seem to indicate that the physiologic function of each tissue is independent (Bebianno and Serafim 2003).

In the present study, during the bioassays, kidney showed the lowest degree of MT induction. This is in agreement with Rainbow (2002), who established that not all organisms exhibit the same pattern of metal bioaccumulation and detoxification. For example, after 4 weeks of exposure to 0.5 μg Cd ml^{-1} seawater, the clam *Scapharca inaequivalvis* accumulated more Cd (with concentrations ≤725.70 μg Cd g^{-1} dw) bound to a low molecular–weight (10,000 Da) protein in kidney compared with the levels in digestive gland or gills (Serra et al. 1995).

In the current study, the lack of induction of MT in kidney and in gills could be attributed to the low Cd accumulation in these tissues. It has been suggested that the MT induction depends on the concentration of divalent metals in the tissue. However, although kidney, as the primary organ of metal excretion, is most exposed to the effect of accumulated toxins, MT expression was in general lower in this tissue than in digestive gland (Podgurskaya and Kavun 2005).

MT and Cd Levels in Untreated Clams

Experimental conditions significantly differ from natural conditions. The concentration of metals in water usually exceeds the content in nature, metal input from food is usually not taken into account, *etc*. (Croteau et al. 2005; Podgurskaya and Kavun2005). Our results showed a specific response in the three tissues analyzed in this study. Higher MT concentrations were found in digestive gland than in gills or kidney of the untreated clams as well as those included in the bioassays. This coincides with the results reported by Boutet (2002). MT levels recorded in untreated clams were similar to MT levels obtained at 20 and 30 days of the bioassay at both Cd concentrations tested (0.2 and 0.5 μg Cd ml^{-1}). However, the highest MT concentrations in digestive gland were obtained at day 10, regardless of the Cd concentration used in the bioassay. Under these conditions, MT concentrations were almost two times higher than those recorded in untreated clams. This was probably caused by induction mechanisms being drastically different in clams moving from an environment with concentrations <0.006 μg Cd ml^{-1} (natural conditions used during the acclimation) to one with concentrations ≤0.5 μg Cd ml^{-1} (concentrations used during the bioassays).

In gills of untreated clams collected from the four study sites, the content of Cd was low and not significantly different among samples. Similar results were found in gills of *C. gigas* collected from Gironde Estuary (France). High

levels of Cd were found both in oysters and sediment, supporting the idea that gills are not a storage site for this metal (Boisson et al. 2003). Figure 3 shows the difference found in the present study between gills, a short-term storage organ, and digestive gland, a tissue that accumulates and stores toxic metals (Amiard et al. 1989). The high Cd levels in digestive gland of clams from Animas and El Sausozo may be explained by their proximity to the phosphorite banks and, especially, to the mining activities at the second site. Both Animas and Balandra are influenced by currents that transport Cd (among other elements) as a result of upwellings (Monteforte and Cariño 1992) that supplies not only nutrients but also heavy metals to the surface waters (Kavun 2008). The highest and lowest Cd levels ($\leq$10.05 and 1.74 g g^{-1} dw) in total tissue were found in El Sausozo and in Balandra, respectively, which is in agreement with the data obtained by Méndez et al. (2006) for *M. squalida*.

In the present study, we found that *M. squalida* clams from the four sites had Cd concentrations in whole tissue that was approximately 50% that in digestive gland. A similar relation was previously reported by Baudrimont et al. (1997), who found that Cd concentration in the whole organism of the freshwater clam *C. fluminea* was 40% less than those in organs or tissues that are known storage sites for metals. Although Cd levels in digestive gland and kidney were higher in clams from El Sausozo and Animas than those from Balandra, the clams from El Sausozo had the lowest statistically significant concentration of MT. This unexpected finding may be the result of a metabolic alteration, as was suggested in a study of *C. gigas* (Boutet et al. 2002). Boutet et al. (2002) found abnormally low MT levels in digestive gland of *C. gigas* collected in Royan, France, and concluded that the relation between MT induction and metal bioaccumulation is not linear, suggesting the existence of other mechanisms for metal sequestration. Roesijadi and Robinson (1994) demonstrated that in environments with high levels of metals (especially Cd), organisms have the capacity for partitioning the accumulated metals in noncytosolic compartments, such as phosphate granules. Nott and Nicolaidou (1990) showed that the high metal concentration in digestive gland is caused by the presence of $Mg_3(PO_4)$ in the granules, which serves as a ligand for binding metal ions.

In a previous study of *M. squalida* in La Paz Bay, significantly higher activity of glutathione S-transferase (GST) was found in organisms from Animas and Balandra than in those from El Sausozo (Cantú-Medellín 2006). GST, as MT, is associated with detoxification processes because of its involvement in xenobiotic metabolism, elimination of waste products, and regulation of hemolymph electrolyte composition (Gamble et al. 1995). Cantú-Medellín et al. (2009) found the activities of GST to be lower in clams from El Sausozo, although the levels of Cd in digestive gland and total content in clams were found to be as high as those from Animas, which is also in agreement with the results obtained in the present study. This suggests that mollusks from Animas and Balandra may be better adapted to an increased metal concentration in the environment than are the organisms from El Sausozo. The organisms at the latter site may be influenced by other chemical components present in the environment, thus decreasing their efficiency in maintaining certain processes, such as the homeostasis of elements and mechanisms of detoxification. For example, for the Chlorophyceae algae *Enteromorpha intestinalis*, Rodríguez-Castañeda et al. (2006) reported the highest levels of nine heavy metals, other than Cd, in those algae from an area closer to El Sausozo compared with levels in algae from other areas of La Paz Bay. As a probable cause of such levels of heavy metals in the studied algae, Rodríguez-Castañeda et al. (2006) suggested not only that the weathering of the natural rocks (mainly sedimentary and volcanic rocks) causes runoff of minerals into drainage basins but also that the nearby phosphorite mining operations influence the composition of the seawater and marine sediment.

In the present study, although Cd levels were low in the organisms from Quelele, MT concentrations in gills and digestive gland were high. In a previous monitoring study, levels of Ni, Cd, Mn, Zn, Cu, and Fe in clams from Quelele were not high (Méndez et al. 2006). However, in the digestive gland of *M. squalida* also collected in El Quelele, Cantú-Medellín et al. (2009) found a positive correlation between GST activity and superoxide dismutase (SOD) and Fe levels ($r^2 = 0.89$ and $r^2 = 0.97$, respectively; $p < 0.05$). MTs are considered an indicator of oxidative stress (Geret et al. 2002), as are GST and SOD. Therefore, when taken together, these results are indicative of free-radical production and antioxidant enzyme activities, perhaps caused by the presence of an organic compound (such as hydrocarbons not yet analyzed). Such a compound can enhance the liberation of Fe from ferritin (Winterbourn et al. 1991), thus causing free-radical production and the subsequent induction of MT.

The results of this study in the clam *M. squalida* showed that of the three organs analyzed (gills, digestive gland, and kidney), digestive gland was the most informative regarding both Cd accumulation and MT synthesis. Continuous monitoring of this tissue in the clam *M. squalida* could be used to provide information on Cd pollution. However, no specific relation between Cd content and MT induction was found for clams collected in an area influenced by phosphorite tailings. Therefore, these results indicate that MT levels cannot be used as unique and specific biomarkers of contamination by Cd or other elements associated with

phosphorite mine tailings in marine environments. More studies in relation to MT induction, such as simultaneously measuring other detoxification mechanisms of divalent metals, e.g., phosphate granules, must be carried out.

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