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As, Cd, Cu, Mn, Pb, and Zn Contents in Sediments and Mollusks (*Hexaplex trunculus* and *Tapes decussatus*) from Coastal Zones of a Mediterranean Lagoon (Mar Menor, SE Spain) Affected by Mining Wastes

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Abstract Individuals of *Hexaplex trunculus*, *Tapes decussatus*, and associated sediments were collected from 16 coastal sampling plots of the Mar Menor lagoon (SE Spain), and the metal and As concentrations were determined. The sediments had maximum values (in milligrams per kilogram dry weight [d.w.]) of 7,132 for Zn; 6,975 for Pb; 5,039 for Mn; 501 for As; 74 for Cu; and 9.1 for Cd. Specimens of *H. trunculus* could be collected from all the sampling plots, and it was found that concentrations of Zn (between 883 and 3,130 mg kg^{-1} d.w.), Pb (between 0.09 and 222 mg kg^{-1} d.w.), Mn (between 7.6 and 17.7 mg kg^{-1} d.w.), As (between 144 and 418 mg kg^{-1} d.w.), and Cd (between undetectable and 8.4 mg kg^{-1} d.w.) in soft tissues significantly increased when concentrations in sediments increased. *H. trunculus* apparently regulated Cu assimilation (concentrations between 17.7 and 47.2 mg kg^{-1} d.w.) in its soft tissues. *T. decussatus* was very scarce or even absent from sites with higher metal and As contents in the sediments. Hence, *H. trunculus* could be used as a bioindicator of metals and As pollution, but not *T. decussatus*. Based on our results, a human health risk exists because the species analyzed are collected from the studied zone and so are consumed by the population.

Keywords Coastal lagoons · Polluted sediments · Mining wastes · Gastropods · Bivalves

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1 Introduction

Anthropogenic inputs are important sources of metals in marine ecosystems, particularly near the shoreline and in bodies of water with restricted circulation, such as coastal lagoons. The latter environments are characterized by being isolated from the open sea, which makes them highly vulnerable to impacts. Within determined thresholds, these ecosystems can show a certain buffer capacity to external stresses, but high levels over prolonged periods can lead to their irreversible deterioration (Viaroli et al. 2007). The existence of pollutants can affect sediments and organisms and be a risk to human health when uncontrolled activities occur in contaminated sites. From this point of view, studying the relationships between the concentrations of pollutants in the sediments and corresponding mollusks can be a valuable tool to assess the contamination levels and the risk to the population.

Marine organisms, especially mollusks, have the ability to accumulate metals and As from the environment in which they live (Roméo et al. 2005). Mollusks are considered reliable bioindicators for identifying biologically available metals (Szefer 1986), and their usefulness as sentinel organisms in metal biomonitoring studies is widely recognized (Cravo et al. 2004; Rainbow and Philips 1993). Bivalves are filter feeders and thus obtain elements not only from food and water but also from the ingestion of inorganic particulate materials (El-Sikaily et al. 2004). The metal concentrations can be magnified in the trophic chain if bivalves are consumed by predators such as some marine snails.

The aims of this study were: (1) to determine the levels of As and trace elements in shallow sediments of the shoreline of the Mar Menor lagoon and in mollusks living in these sediments; (2) to assess the risk of toxicity and trophic transfer due to the As and metal concentrations since the mentioned areas are used for recreational activities which include hand-fishing and hand-collecting of mollusks for personal consumption; and (3) to study if the species of mollusks analyzed could be acceptable biomonitors of metal pollution in the Mar Menor lagoon and other similar systems.

2 Materials and Methods

2.1 Study Zone

The Mar Menor, SE Spain with a superficial area of 135 km^2, is one of the biggest coastal lagoons in Europe and the Mediterranean Sea (Fig. 1). This hypersaline lagoon is relatively shallow with a mean depth of 3.5 m and a maximum depth of just over 6 m. It is isolated from the Mediterranean Sea by a 22-km long and 100- to 900-m wide sand bar (La Manga), which is intersected by five shallow channels. Hence, it can be classified as a choked lagoon with restricted circulation and long water residence times (Gilabert 2001). The lagoon receives runoff from various temporary watercourses, called "ramblas," mainly when the sporadic and torrential rainfall occurs. The territory has a typical semi-arid climate with annual means of 275 mm rainfall, 17°C temperature, and 856 mm evapotranspiration.

The Mar Menor lagoon and associated salt marshes are included in the Ramsar Convention of Wetlands. Also, it is a Special Protected Area of Mediterranean

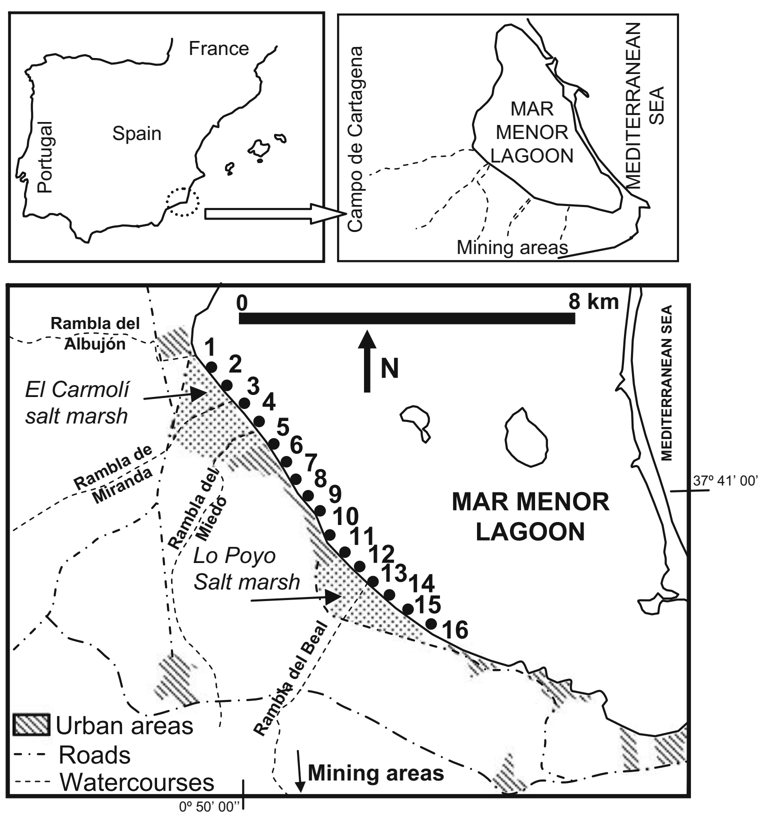

Fig. 1 Location map of the Mar Menor lagoon in SE Spain and the sampling plots (*black dots* and *numbers*) along the shoreline

Interest (SPAMI), a Special Protected Area (SPA) under the EU Wild Birds Directive, and a Site of Community Importance (SCI) to be integrated in the Nature 2000 Network (EU Habitats Directive). However, the ecology equilibrium of the lagoon ecosystem is threatened by massive urban growth on the shores, intensive agricultural activity, and the residues from former mining activities in the nearby Cartagena and La Unión area (Conesa and Jiménez-Cárceles 2007). The latter mine zone has been exploited for Ag, Pb, Zn, Cu, and Fe since the Phoenician and Carthaginian times (Oén et al. 1975; Robles-Arenas et al. 2006), but it was during the nineteenth and twentieth centuries when the most intensive extractions occurred. The negative effects of this activity were already noted during the nineteenth century due to the mining wastes which were dumped into the local streams draining the mine zone into the Mediterranean Sea and the Mar Menor lagoon. Extractions ceased in 1991, but up until today, the tailings remain in the mountains and continue to be eroded.

The three main watercourses carrying out mine wastes are the Rambla del Miedo, Rambla del Beal, and Rambla de Ponce (Fig. 1), which cause the sediments in the south area of the Mar Menor to present high concentrations of trace elements (Álvarez-Rogel et al. 2004; Jiménez-Cárceles et al. 2006; Rodríguez-Puente et al. 2001; Sanchiz et al. 2000). Concentrations of the main trace elements in the tailings of the mining area reach levels of 8,990 mg kg^{-1} (total Pb); 15,000 mg kg^{-1} (total Zn); and 560 mg kg^{-1} (total Cu) (Conesa 2005; Conesa et al. 2006). Robles-Arenas et al. (2006) indicated concentrations of soluble metals in the runoff from the mining zone of 77.2±40.42 mg L^{-1} Zn; 1.3±1.18 mg L^{-1} Cd; and 0.54±0.33 mg L^{-1} Pb. Marín-Guirao (2007) confirmed that, during strong rainfalls, the runoff in the Rambla del Beal is acidic and it contains high concentrations of dissolved (26.6 mg L^{-1} Zn, 0.89 mg L^{-1} Pb, 0.30 mg L^{-1} Cu, and 0.15 mg L^{-1} Cd) and particulate (31.65 mg kg^{-1} Zn, 141.1 mg kg^{-1} Pb, 1.64 mg kg^{-1} Cu, and 0.35 mg L^{-1} Cd) metals flowing from the acid mine drainage of the wastes deposited to the tailings.

2.2 Mollusks Sampling and Analysis

In February 2005, 16 coastal sampling plots of about 100–150 m^2 were located every 500 m between the mouths of the Rambla del Albujón and the Rambla de Ponce (Fig. 1). In each plot, three subsamples of ≈10 specimens of a marine snail, *Hexaplex trunculus* Linnaeus, 1758 (Mollusca, Gastropoda), and a bivalve, *Tapes decussatus* Linnaeus, 1758 (Mollusca, Bivalvia), were collected by hand from the seabed at a depth ≈50 cm (the depth at which people normally collect them for private consumption). Live samples were put in polyethylene bags and transported to the laboratory. Then, for a 48-h period, the mollusks were kept in filtered and continuously aerated seawater (collected at the corresponding stations) to allow the elimination of the particulate matter residues presented in the mantle cavity and digestive tract (Conti and Cecchetti 2003). Mollusks collected at different sampling plots were put in different tanks, and additionally, *H. trunculus* and *T. decussatus* were also separated because the marine snail is a predator of the bivalve.

When purified, specimens were prepared in a microwave by boiling them in ultrapure deionized water (18 MΩ·cm) for 30 s. After that, the soft parts were carefully extracted with a plastic spatula, dried at 80°C (Chiu et al. 2000), and ground to a fine powder in a mill. The powder obtained was mineralized as follows (Ybáñez et al. 1991): 12.5 mL of HNO_3 50% *v*/*v*+2.5 of coadjuvant agent ($Mg(NO_3)_2 \cdot 6H_2O$ 20% *w*/*v*+MgO 2% *w*/*v*) were added to a porcelain crucible containing 0.6 g of ground dried sample. The crucibles were put in a sand bath until total dryness at a maximum temperature of 130°C. The coadjuvant agent accelerates the oxidation of the organic matter and prevents the volatilization of As in the form of chlorides and oxichlorides (Damkröger et al. 1997).

The pellet obtained was calcined in a muffle furnace following a temperature ramp to a maximum of 450°C. This was maintained for 12 h in order to obtain a white ash which was dissolved in concentrated nitric acid and diluted with 0.6 N nitric acid to a determined volume. Afterwards, the samples were filtered through an Albet 145 ashless filter paper (7–11 μm pore diameter) and then Cu, Mn, Zn, Cd, Pb, and As contents were measured with an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500A).

2.3 Sediments Sampling and Analysis

In each plot, four subsamples of sediment were taken (top 10 cm), put in polyethylene bags, and homogenized to obtain a composite sample per plot. The samples were transported to the laboratory, air-dried, and sieved to 2 mm in order to remove coarse fragments. Particle size distribution was determined by the Robinson's pipette method, following organic matter oxidation with H_2O_2 30% and dispersion with sodium hexametaphosphate (Calgon). The sediment organic matter was determined by weight lost upon ignition of dry sediment in a muffle furnace at 450°C for 6 h (LOI) (Beaudoin 2003; Heiri et al. 2001; Marín-Guirao et al. 2005a).

A portion of each sieved sample was ground in an automatic Agatha mortar to obtain a fine powder and total calcium carbonate was determined by Bernard's calcimeter method. Total metal contents were determined in triplicate according to method 3052, proposed by the United States Environmental Protection Agency (USEPA 1996): a portion of the ground sediments (0.25 g) was put in perfluoroalkoxy polymer vessels, and 9 mL of pro-analysis HNO_3 69% and 3 mL of pro-analysis HF 40% were added. The vessels were closed, placed into a microwave, and heated for 5 min to reach 180°C, then kept at this temperature for 10 min and finally allowed to cool for 60 min. Afterwards, the samples were filtered through an Albet 145 ashless paper which was then washed with a determined volume of H_3BO_3 5%. Metals and As were measured in the same equipment as in the case of the mollusks.

2.4 Analytical Quality Control

The accuracy of measurements was compared with European Reference Material ERM-CE278 (mussel tissue) and CRM027-050 (sediment). The results were in agreement with certified values, and the standard deviations (SDs) were low, showing good repeatability (Tables 1 and 2).

Table 1 Analysis of certified reference material (mussel tissue): ERM-CE278 (means±1 SD)

Metal	Certified	Found
As	6.07±0.13	5.42±0.40
Cd	0.348±0.007	0.354±0.178
Cu	9.45±0.13	10.87±1.55
Mn	7.69±0.23	8.09±0.61
Pb	2.00±0.04	2.28±0.42
Zn	83.1±1.7	75.6±7.6

Data are expressed in milligrams per kilogram dry weight (n=5)

Table 2 Analysis of certified reference material (sediment): CRM027-050 (means±1 SD)

Metal	Certified	Found
As	12.4±2.80	13.92±2.84
Cd	12.0±1.74	10.04±1.27
Cu	9.87±1.46	11.56±1.38
Mn	259±21.8	299.3±13.8
Pb	51.9±7.28	40.27±7.95
Zn	51.3±7.76	51.13±4.64

Data are expressed in milligrams per kilogram dry weight (n=5)

Solutions of HNO_3 without samples were used as blanks to detect possible contamination. One sample of reference material and blanks were included in each analytical batch. Volumetric polyethylene material was used for all the laboratory work.

2.5 Statistical Analysis

Statistics were computed with SPSS 13.0 (SPSS, USA). Nonparametric correlations (Spearman) were done to test the relationships among the characteristics of the sediments and metal concentrations in the mollusks. Nonparametric comparisons (Kruskal–Wallis test) were applied to test the differences between mollusk metal concentrations in different stations.

3 Results

3.1 Sediments

The sediments had sand contents higher than 80% and fine particles (silt+clay) lower than 15% (Fig. 2). Plots 3, 4, 6, 8, and 9 had the lowest contents in fine sediments (4.47±0.65% silt+clay), whereas plots 2, 5, and 10 had the highest contents (15.2±0.68% silt+clay). The contents in calcium carbonate varied between 769.3 g kg^{-1} (plot 11) and 270.3 g kg^{-1} (plot 13, the nearest to the mouth of the Rambla del Beal). No clear pattern for variation of particle size or carbonates was detected along the coast line between plots 1 and 16 (Fig. 2).

The organic matter content (as determined by LOI) was very variable and showed a relationship with the zone in which the plots were located (Fig. 2). The lowest contents were obtained in plots 7 (9.8 g kg^{-1}), 8 (6.6 g kg^{-1}), and 9 (7.0 g kg^{-1}) at the beaches of Los Urrutias village, which are regularly cleaned for

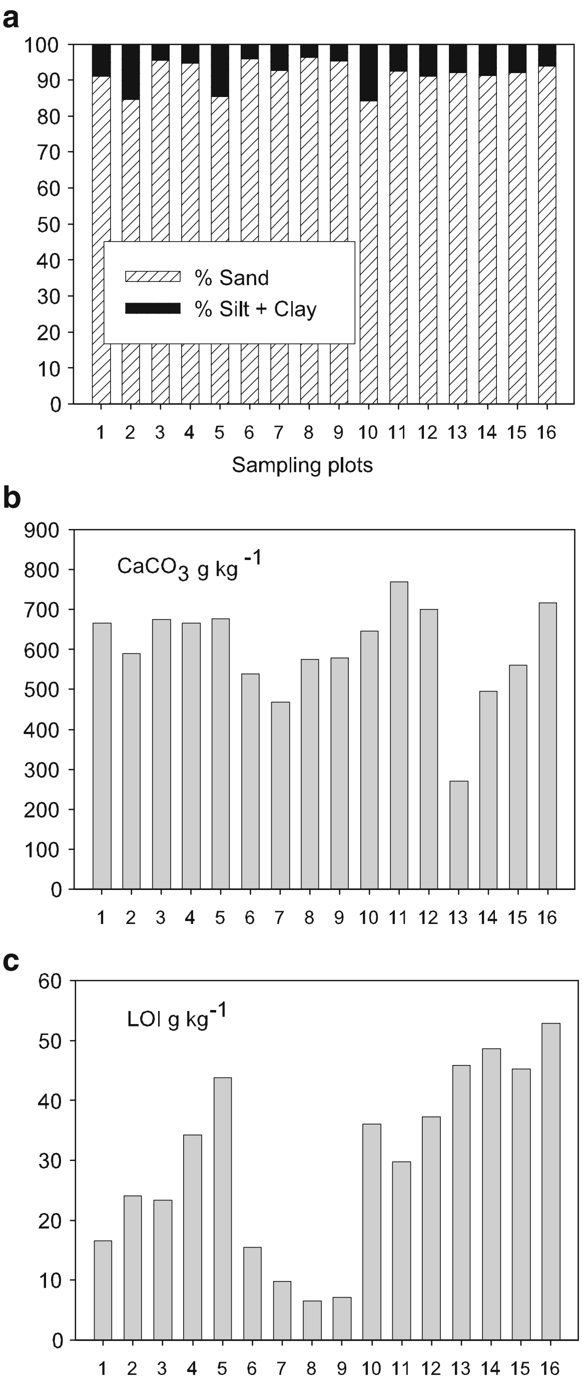

Fig. 2 Particle size distribution (**a**), total calcium carbonate (**b**), and total organic matter as determined by LOI (**c**) in sediment samples taken in 16 sampling plots in the Mar Menor lagoon

recreational purposes. Plots located at the beaches of La Marina del Carmolí and Lo Poyo salt marshes had organic matter contents higher than 10 g kg^{-1}, reaching a maximum of 52.9 g kg^{-1} in plot 16.

The concentrations of As and metals in the sediments followed the order Zn>≈Pb>Mn≫As≫Cu>Cd with a clear increase toward the southern part of the study zone (Fig. 3), i.e., plots 10 to 16 next to the beach of Lo Poyo salt marsh. Plot 13, located just in the mouth of the Rambla del Beal, had the maximum levels of Zn (7,132 mg kg^{-1} dry weight [d.w.]), Pb (6,975 mg kg^{-1} d.w.), Mn (5,039 mg kg^{-1} d.w.), As (501 mg kg^{-1} d.w.), and Cu (74 mg kg^{-1} d.w.), while the highest sediment Cd content (9.1 mg kg^{-1} d.w.) was obtained in plot 14.

3.2 Mollusks

H. trunculus could be collected at all the sampling plots. However, the collection of specimens of *T. decussatus* was very difficult in the Lo Poyo salt marsh area and it was not possible to find enough bivalves to do the analyses from plots 9 to 16. An interesting finding was that the average size of the *H. trunculus* specimens collected from plots 10 to 16 (5.1±0.36 cm) was significantly larger than those collected from the other plots (4.1±0.23 cm) ($p<0.05$, nonparametric Kruskal–Wallis test). Since *T. decussatus* was not collected from plots 9 to 16, a comparison of the sizes could not be made for this species.

The two species of mollusks (one gastropod and one bivalve) had different amounts of metals in their soft tissues, but in both cases, the accumulation order was Zn≫As>Pb>Mn>Cu>Cd (Fig. 4).

H. trunculus accumulated higher metal content by far, and an important variability in the concentrations (mg kg^{-1} d.w.) was observed among different plots, mainly for Cd (between undetectable [plots 1 to 4] and 8.4 [plot 12]), Pb (between 0.09 [plot 6] and 222 [plot 12]), and Zn (between 883 [plot 1] and 3,130 [plot 15]). Cu concentrations (mg kg^{-1} d.w.) ranged between 17.7 (plot 7) and 47.2 (plot 14); As between 144 (plot 1) and 418 (plot 12); and Mn between 7.6 (plot 8) and 17.7 (plot 14).

The metal concentrations in *T. decussatus* varied as follows (mg kg^{-1} d.w.): Zn between 73 (plot 7) and 109 (plot 5); As between 42 (plot 3) and 78 (plot 6); Pb between undetectable (plots 1 to 3) and 15 (plot 5); Mn between 5.6 (plot 7) and 9.2 (plot 5); and Cu between 0.3 (plot 6) and 4.2 (plot 5). As previously indicated, this species was not collected from plots 9 to 16 and hence no data for metal contents were obtained here while Cd was below detection limits in the specimens collected from all of the plots.

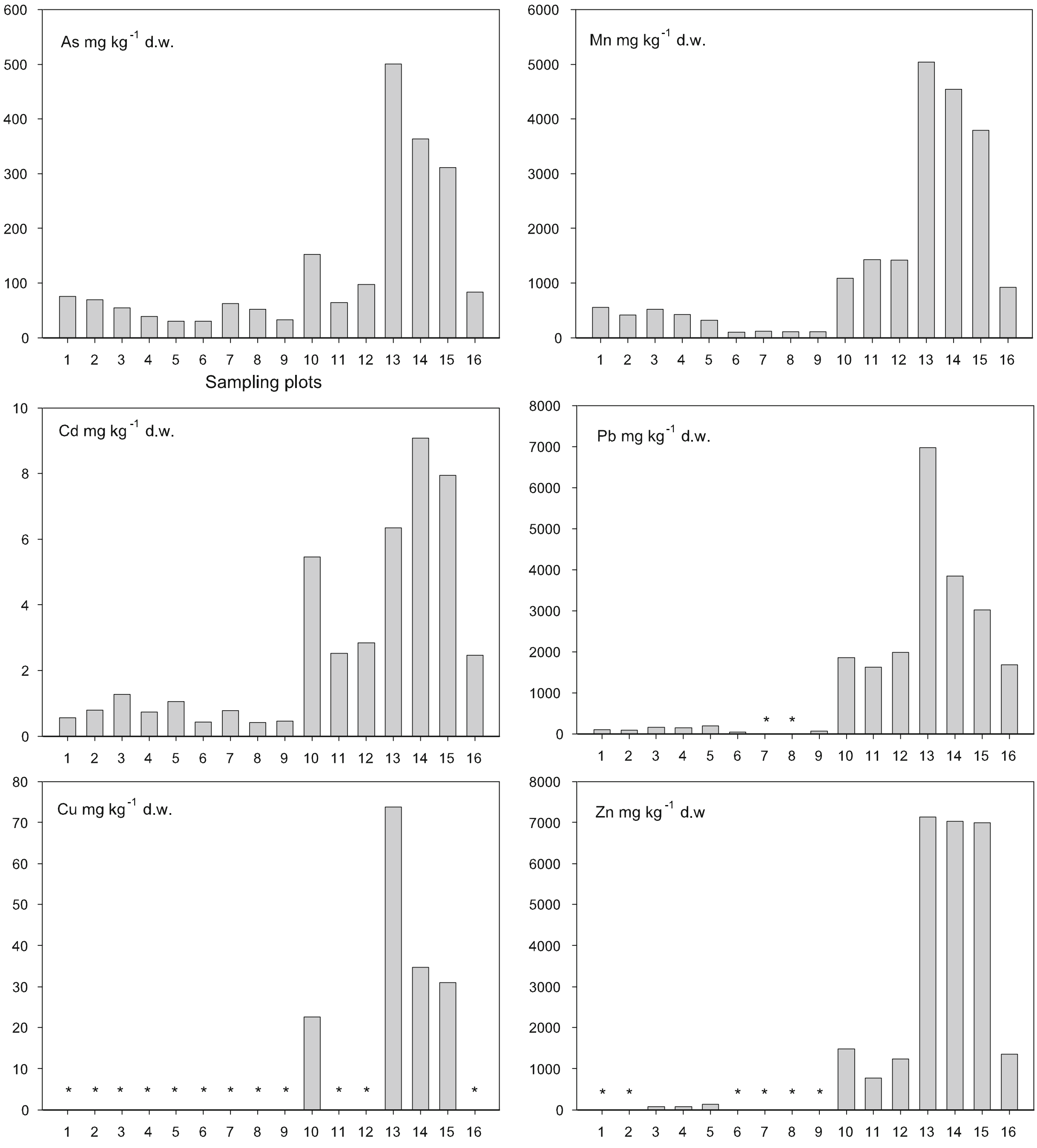

Fig. 3 Total As and metal contents in sediment samples taken from 16 sampling plots in the Mar Menor lagoon (in milligrams per kilogram dry weight). *Asterisks* indicate below detection limits

4 Discussion

4.1 Characteristics of the Sediments

The predominance of coarse particles in the samples was in agreement with the sandy texture of these sediments (Calvín-Calvo et al. 1999). Local variations in sediment composition, such as those found in plots 2, 5, and 10, may be a consequence of changes induced by human activities, which include dredging and sand deposition to create new beaches in residential zones of the Mar Menor (Calvín-Calvo

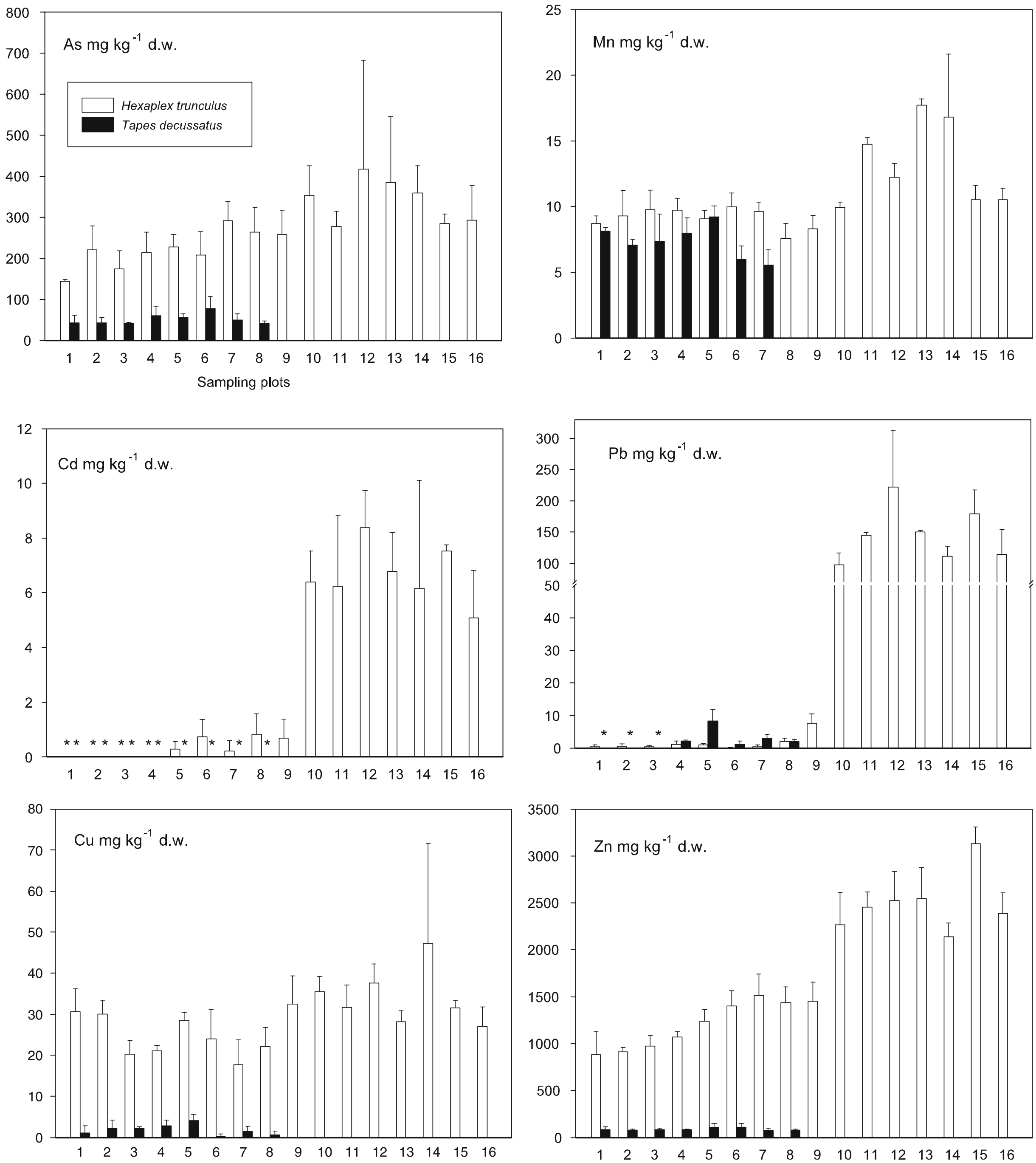

Fig. 4 Total As and metal contents in mollusks collected from 16 sampling plots in the Mar Menor lagoon (in milligrams per kilogram dry weight). *Lines over the bars* indicate 1 SD (n=3). *Asterisk* indicates below detection limits. *T. decussatus* was not found from plots 9 to 16

et al. 1999; Pérez-Ruzafa et al. 1991). Calcium carbonate content was not correlated (p>0.05) with any other variable, and the abundance of this component was not surprising due to the oolitic nature of these sands and the high content of mollusk shell pieces that appeared in them.

As previous authors have pointed out (Marín-Guirao et al. 2005a; Pérez-Ruzafa et al. 1991), a significant positive correlation between fine particles and organic matter (r=0.4794, p<0.05) was found. Plots with finer sediments were those with higher LOI, which can be attributable to the formation of mineral–organic complexes.

The concentrations of all the metals measured were correlated (p<0.05) and metal contents increased strongly in the area of influence of the Rambla del Beal in accordance with the transport of metal mine wastes by this watercourse (Álvarez-Rogel et al. 2004). The results were similar to those obtained by other researchers during the last 35 years (De Leon et al. 1982; Marín-Guirao et al. 2005a, b; Rodríguez-Puente et al. 2001; Salas et al. 2005; Simonneau 1973) and indicated the persistence of the pollution in this site for more than three decades. Similar levels of trace elements were found in an area of The Coeur d'Alene (CDA) River in northern Idaho (USA), also polluted by mine wastes (Farag et al. 1998). According to the classification proposed by Long et al. (1995), the sediments between the mouth of the Rambla del Albujón and Los Urrutias village (plots 1 to 9) can be considered moderately contaminated for As and Pb and hardly contaminated for Cd, Cu, and Zn. However, near the mouth of the Rambla del Beal (plots 10 to 16), the contamination was high for As, Pb, and Zn, moderate for Cd, and moderate to low for Cu.

A positive significant correlation between metals concentrations and organic matter was found, which could be explained as a consequence of the high productivity of the seagrass beds dominated by *Cymodocea nodosa* (Ucria) Ascherson in contaminated sites (Marín-Guirao et al. 2005a). The latter authors found a significant increase in leaf productivity of this species in sampling stations next to the mouth of the Rambla del Beal, compared to the productivity of noncontaminated sites in the Mar Menor. A higher growth rate of biological communities living in polluted sites is a common phenomenon in nature because the low number of species capable of tolerating the most stressful conditions reduces competition and facilitates access to the resources by the adapted organisms (Margalef 1991). The larger size of *H. trunculus* specimens collected in the polluted plots and the difficulty of collecting *T. decussatus* in these sites seem to be in agreement with this finding.

4.2 Metal Content in Mollusks and its Relationship with Metal Content in Sediments

Since it was not possible to find *T. decussatus* in the polluted plots, metal and As contents in organisms and sediments could only be compared for *H. trunculus*. The order of accumulation obtained was according to the sequence Zn>Cu>Cd, which was in agreement with the results of Wagner and Boman (2004). The variance in both marine mollusks was generally higher in the plots where the mollusks accumulated higher levels of metals. Similar behavior has been observed in other studies (Langston and Spence 1995; Lobel et al. 1982; Lobel and Wright 1982; Taylor and Maher 2003).

Spearman rank correlations indicated a significant positive correlation (r>0.653, p<0.01) between the concentrations of metals in *H. trunculus* and in the sediments, except for Cu (r=0.402, p>0.05). In fact, relatively high concentrations of Cu were detected in *H. trunculus* collected from zones where Cu concentrations in the sediments were negligible. This can be related to the capacity of this species to accumulate high Cu content (Catsiki and Arnous 1987) due to the formation of accumulator granules in the interstitial cells (Nott and Nicolaidou 1989). However, the levels of this metal obtained in the soft tissues of the marine snail were lower than those found by other authors, such as Rodríguez-Puente et al. (2001) and Roméo et al. (2006), and did not increase when the levels of Cu in the sediments increased (corresponding to the area of Lo Poyo salt marsh). The latter may be explained by a physiological mechanism where exposure to high Zn concentrations in sediments, such as those obtained in this study in the area of Lo Poyo salt marsh, can lead to displacement of the Cu by competition with cellular ligands (Kaland et al. 1993; Viarengo 1989). The Kruskal–Wallis test applied to the levels of Cu in the marine snail showed that there were no significant differences between stations (p<0.01). This situation can also be partly explained by a regulatory mechanism of the levels of Cu by *H. trunculus* since the levels of this metal in the sediments are low enough to make it possible (Bryan et al. 1983). Hence, the trend of Cu in soft tissues of *H. trunculus* can be explained both by competition with Zn in the most polluted plots and by a regulatory mechanism of the levels of the metal in this marine snail.

The existence of mechanisms for regulating the concentrations of biologically essential elements, such as Cu and Zn, in marine mollusks for a limited range of concentrations in the environment has been broadly described (Amiard-Triquet et al. 1986; Bryan et al. 1983; Phillips 1995; Rainbow 1995; Soto et al. 1997; Wilson 1982). The results show that the specimens of *H. trunculus* collected from sites with Zn concentrations in the sediments ≈7,000 mg kg^{-1} d.w. (plots 13, 14, and 15) did not have a significantly higher Zn content in their soft tissues than those collected from sites with Zn concentrations in the sediments ≈1,000 mg kg^{-1} d.w. (plots 10, 11, 12, and 16) (Fig. 5). This seems to indicate the absence of a regulatory mechanism in these adverse environmental conditions. It might be possible that, in the most Zn-polluted plots (13, 14, and 15), *H. trunculus* had reached the maximum permissible level (between 2,500 and 3,000 mg kg^{-1} d.w.) that did not cause their death. That can be the reason why it did not have a significantly higher Zn content than those collected from sites with sediment Zn concentrations ≈1,000 mg kg^{-1} d.w. (plots 10, 11, 12, and 16).

As regards nonessential elements (As, Pb, and Cd), whose concentrations are not supposed to be regulated by the organisms (Wagner and Boman 2004), the results were in accordance with the latter finding since the As, Pb, and Cd concentrations in soft tissues increased in polluted sediments.

The As and Zn concentrations in *H. trunculus* were of a similar range to those obtained by Rodríguez-Puente et al. (2001) in two sampling stations located in the Northeast coast of the Mar Menor lagoon. However, Pb concentrations in the current research were up to tenfold higher than those in the study of the latter authors while Cd was slightly lower.

Although Roméo et al. (2006) indicated that *H. trunculus* is not a good bioindicator, these results support the idea that this marine snail can be an acceptable sentinel organism to biomonitor Zn, As, Cd, Mn, and Pb in the Mar Menor. The linear regressions between the concentrations of the four last trace elements in the sediments and in the mollusks were significant (Fig. 6), the slope of the function suggesting the relationship between the concentration of a metal in the sediment and its bioavailability. Slopes for As and Cd were approximately unity (1.08 and 0.72, respectively) which indicates that their accumulation increased proportionally when sediment concentration increased. However, slopes higher than unity were obtained principally for Mn (459.64) but also for Pb (17.4), hence indicating that the concentrations of these two metals in *H. trunculus* increased disproportionally with the degree of enrichment in the sediments.

H. trunculus satisfies all the requirements to be a sentinel organism: it is sedentary; abundant and representative of the study site; hardy and tolerant of high levels of metals and salinity (Anderson 1958); easy to identify and collect; its dimensions are suitable and it accumulates metals to a satisfactory degree (Conti and Cecchetti 2003; Orescanin et al. 2006; Phillips 1990; Taylor and Maher 2003). However, to use this species as a sentinel organism, factors such as age, size, and weight of the specimens

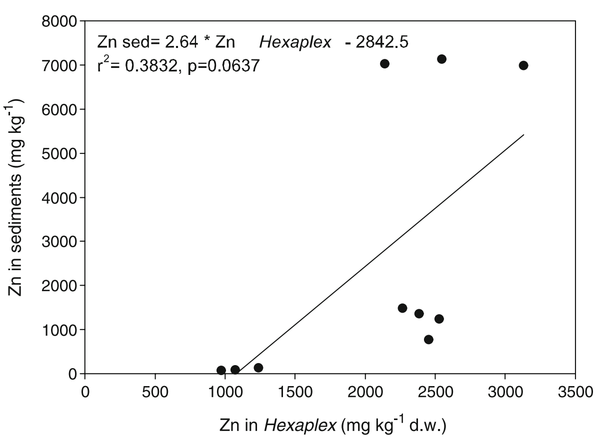

Fig. 5 Linear regression between Zn content in soft tissues of *H. trunculus* and Zn content in the sediment

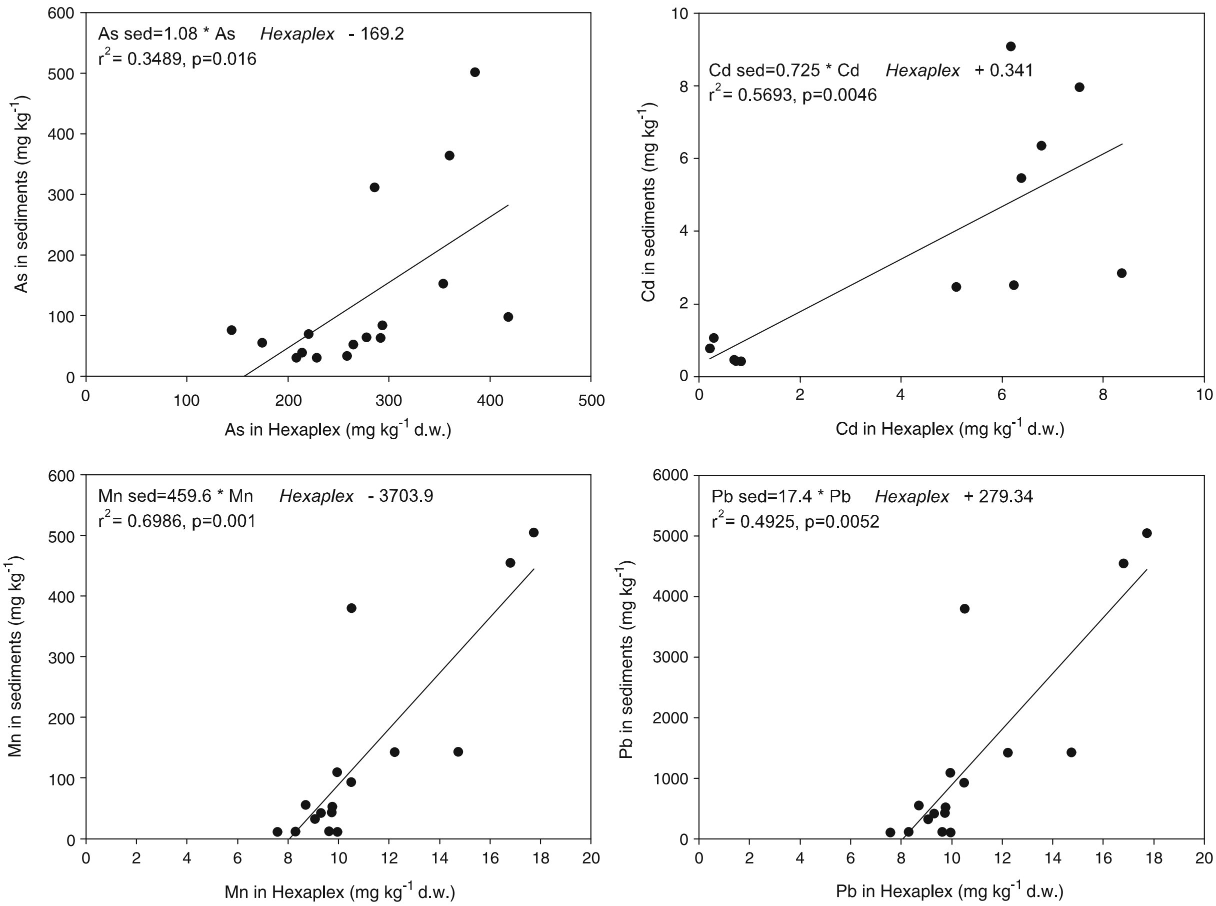

Fig. 6 Linear regressions between As, Cd, Mn, and Pb contents in soft tissues of *H. trunculus* and the contents in corresponding sediments

should be as homogeneous as possible in order to correctly interpret the results obtained (Conti and Cecchetti 2003; Cubbada et al. 2001; Roméo et al. 2006).

The absence of data for *T. decussatus* in the most polluted zone was in agreement with the results obtained by Marín-Guirao et al. (2005a, also in the Mar Menor) and with the findings of Morillo et al. (2005) in mine-polluted zones of the Huelva estuary (SW Spain). Morillo et al. (2005) indicated that bivalve mollusks do not survive in extreme conditions, e.g., in a badly polluted environment (Paulson et al. 2003), and in those conditions, they cannot be used for biomonitoring. A comparison of the concentrations of Zn, Cd, and Pb in the sediment samples with the toxicity levels established by USEPA (Zn=410 mg kg^{-1} d.w.; Cd=9.6 mg kg^{-1} d.w.; Pb=220 mg kg^{-1} d.w.; O'Connor and Paul 2000) showed that the critical levels were largely surpassed in the zone of the Rambla del Beal (Fig. 3), and probably this was the reason why *T. decussatus* did not appear there.

Although the seawater was not analyzed, previous studies indicated that the soluble metals concentrations in the Mar Menor were very low because of the basic pH and high salinity (Auernheimer et al. 1984, 1996; Lloret et al. 2005; Marín-Guirao 2007; Salas et al. 2005; Velasco et al. 2006). Therefore, bivalve mollusks living in the Mar Menor, such as *T. decussatus*, which are filter-feeder organisms (suspension-feeder organisms), probably absorbed the metals associated with suspended particles (Huang et al. 2006; Phillips 1977). However, *H. trunculus* is an opportunistic feeder, exhibiting both carnivorous and scavenging behavior (Zavodnik and Simunovic 1997). In fact, this marine snail is a predator of *T. decussatus* and other marine mollusks (Peharda and Morton 2006). In our study, its levels of metals and As were higher than in the bivalve. So, we can say that, at least in these two steps of the trophic chain, there is a biomagnification of these trace elements.

Marín-Guirao (2007) confirmed that only during the strong rainfalls the concentrations of dissolved metals on the Mar Menor coast corresponding to the mouths of the ramblas are high. Water becomes toxic although only for a few days.

4.3 Risks for Human Consumption

In order to assess to which degree the mollusks studied in this zone were contaminated by trace elements, the metal concentrations in the species analyzed were compared with the specific standards. These were the European legislation for bivalve mollusks (European Communities 2001, 2002) and the international standards in mollusk/shellfish compiled by the Food and Agricultural Organization (FAO) of the United Nations (Wagner and Boman 2004), both expressed in wet weight (w.w.).

The European legislation establishes the maximum permissible concentration in bivalves for Pb= 1.5 mg kg^{-1} w.w. and for Cd=1.0 mg kg^{-1} w.w. Regarding Cd, the levels in *T. decussatus* were always below the maximum permissible concentration (even below the detection limit), whereas for Pb, only the sample located in plot 5, which had a value of 2.23 mg kg^{-1} w.w., was above the maximum permissible concentration.

The standards compiled by FAO for the other trace elements (Cu, As, and Zn) were used both for *T. decussatus* and *H. trunculus* and for Cd and Pb in the marine snail (Fig. 7). Cu concentrations in soft tissues of the two species were always below the different standards according to FAO, except for plot 14 in *H. trunculus* with a value of 11.88 mg kg^{-1} w.w., which was slightly higher than the lowest limit (10 mg kg^{-1} w.w.).

Regarding Zn, all the samples of the bivalve mollusk were below the standards, whereas the Zn levels in *H. trunculus* were always much higher than the highest standard of 100 mg kg^{-1} w.w., reaching a maximum of 707 mg kg^{-1} w.w. in plot 15.

Concentrations of Pb in *H. trunculus* collected in the southern zone (plots 10 to 16) largely surpassed the highest level of 6 mg kg^{-1} w.w. admissible by FAO, though sampling plots 1 to 9 had levels below the standards. The Cd limit established by FAO (2 mg kg^{-1} w.w.) was not surpassed in any plot of *H. trunculus*.

In spite of the fact that the first view seems to show a very worrying picture, it should be taken into account that such standards are expressed as total concentrations of metals and not as metal concentrations that are potentially bioavailable to human consumption (Bragigand et al. 2004). Although until recently only metals present in the soluble fraction of mollusks had been considered as bioavailable to the consumer, some authors have also studied the bioavailability of metals present in insoluble fractions (Bragigand et al. 2004; Reinfelder and Fisher 1994; Wallace et al. 1998; Wallace and Luoma 2003). All these authors emphasized that only a portion of the metals present in insoluble fractions of mollusks, such as cell walls and granules, is bioavailable, and hence, the estimation of the total quantities of metals will lead to an overestimation of the quantities likely to be available to a consumer. Additionally consideration of only the metals present in the soluble fractions (proteins and cytosol) will lead to an undervaluation (Bragigand et al. 2004). The necessity of a better evaluation of the risks of toxic metal transfer in food chains led Wallace and Luoma (2003) to introduce the term trophically available metal in which metal associated with metallothionein, “enzymes,” and organelle fractions is collected together.

Although As concentrations were higher than the different FAO limits (0.1–5 mg kg^{-1} w.w.) in all the sampling plots and for both species of mollusks analyzed, it must be taken into account that the main percentage of total As in marine animals is represented by arsenobetaine (Borak and Hosgood 2007; Francesconi et al. 1998; Goessler et al. 1997; Prohaska and Stingeder 2005), an organic arsenical compound considered relatively nontoxic (Ochsenkühn-Petropulu et al. 1997). Percentages of arsenobetaine of about 95% were found in *H. trunculus* (Ochsenkühn-Petropulu et al. 1997) and in other carnivorous gastropods (Francesconi et al. 1998).

In order to estimate the metal intake by human consumption of *H. trunculus* and *T. decussatus*, the total metal contents were calculated by taking into account the fresh weight and metal concentrations and calculating the corresponding dose from ten specimens (about 32 g of fresh soft tissue) for the marine snail and other 15 samples (about 24 g of fresh soft tissue) for the bivalve. The results obtained were compared with the provisional maximum tolerable daily intake (PMTDI) for Cu and Zn and with the

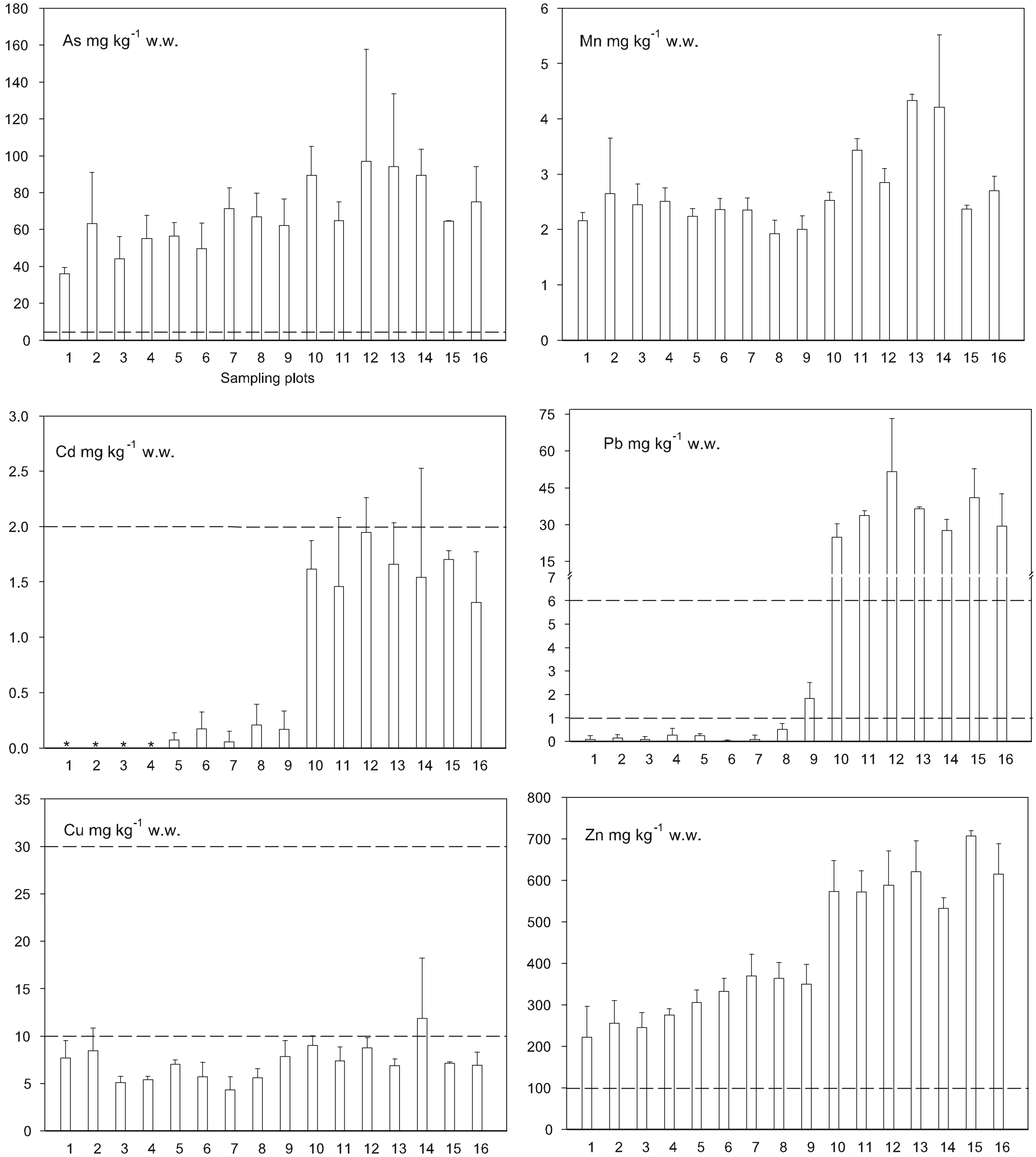

Fig. 7 Total As and metal contents in *Hexaplex* collected from 16 sampling plots in the Mar Menor lagoon (in milligrams per kilogram wet weight) compared to the standards compiled by FAO (*dashed lines*): As, 0.1–5; Cd, 2; Cu, 10–30; Pb, 1–6; Zn, 40–100. *Lines over the bars* indicate 1 SD (n=3)

provisional tolerable weekly intake (PTWI) for Pb, Cd, and inorganic As (Codex Alimentarius Commission 2007) (Table 3), taking the reference as an adult human of 70 kg of body weight. Obviously, the comparisons are not conclusive because the total metal contents were calculated, not the bioavailable contents. With this important caution, it can be stated that PTWI was only surpassed by As, though it must

Table 3 Comparison between the provisional tolerable weekly intake (PTWI) for As, Cd, and Pb and the provisional maximum tolerable daily intake (PMTDI) for Cu and Zn proposed by the Codex Alimentarius Commission (2007) and our data

Trace element	Toxicological term	Value	Our data converted to toxicological terms					
			Hexaplex trunculus			*Tapes decussatus*		
			Mean±SD	Maximum	Minimum	Mean±SD	Maximum	Minimum
As	PTWI	0.015[a]	0.031±0.008	0.044	0.016	0.0026±0.00065	0.0039	0.0019
Cd	PTWI	0.007	0.00034±0.00036	0.00089	BDL	BDL	BDL	BDL
Cu	PMTDI	0.05–0.5	0.0033±0.0008	0.0054	0.002	0.0001±0.000067	0.00022	0.000017
Pb	PTWI	0.025	0.0071±0.0085	0.024	0.00001	0.00019±0.00026	0.0008	BDL
Zn	PMTDI	0.3–1	0.198±0.074	0.323	0.102	0.0044±0.0008	0.0056	0.0035

For PTWI, data are expressed in milligrams per kilogram body weight per week. For PMTDI, data are expressed in milligrams per kilogram body weight per day. For PMTDI (Cu and Zn), the lowest level represent the level of essentiality and the highest level represent the PMTDI

BDL below detection limits

[a] Value for inorganic As

be taken into account that the PTWI is for inorganic As whereas the total arsenic content was used. Moreover, as has been said before, in *H. trunculus*, the percentage of inorganic As is very low, so it is to be expected that the PTWI for As will also be much lower. As for Mn, the World Health Organization (WHO) (1996) were unable to set a range of safe population intakes since low-manganese diets did not result in conclusive or marked effects on the health of adults and the toxicity threshold was unknown (UK Food Standars Agency 2002).

5 Conclusions

The results show that today the contamination by As, Pb, Zn, Mn, Cd, and Cu detected in the submerged sediments in the zone influenced by the mouth of Rambla del Beal during the last 35 years still continues to exist.

H. trunculus can be considered a good bioindicator of pollution by As, Mn, Pb, Cd, and Zn in the submerged sediments of the Mar Menor. Cu concentration in the latter snail seems to be regulated by a mechanism that reduces its accumulation, and hence, the analyses of soft tissues of this species were not correlated to the concentrations of this metal in the sediments. Although the absence of *T. decussatus* from the most polluted sites can be considered as evidence of contamination by trace elements in the sediments, other reasons, such as depredation by *Hexaplex* and/or physiological or food requirements, that were not studied could cause this absence. Hence, it cannot be stated conclusively to which degree the lack of this species indicates a high content of heavy metals in the sediments.

The concentrations of As, Pb, and Zn in *H. trunculus* were much above the international standards in mollusks/shellfish compiled by FAO. Due to the fact that this marine snail is normally consumed by people in the zone, it can be concluded that a risk of toxicity exists, though it must be taken into account that the values of the different toxicological levels proposed by the Codex Alimentarius Commission were not surpassed. Specific studies are necessary in order to evaluate in more detail the risk of transference of metals to higher trophic networks.

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References

Álvarez-Rogel, J., Ramos, M. J., Delgado, M. J., & Arnaldos, R. (2004). Metals in soils and above-ground biomass of plants from a salt marsh polluted by mine wastes in the coast of the Mar Menor lagoon, SE Spain. *Fresenius Environmental Bulletin*, *13*, 274–278.

Amiard-Triquet, C., Amiard, J. C., Ferrand, R., Andersen, A. C., & Dubois, M. P. (1986). Disturbance of a met-enkephalin-like hormone in the hepatopancreas of crabs contaminated by metals. *Ecotoxicology and Environmental Safety*, *11*, 198–209.

Anderson, H. (1958). The gastropod genus *Bembicium philippi*. *Australian Journal of Marine and Freshwater Research*, *9*, 546–572. doi:10.1071/MF9580546.

Auernheimer, C., Llavador, F., & Pina, J. A. (1984). Chemical minority elements in bivalve shells. A natural model (Mar Menor, Spain). *Archives Des Sciences Genève*, *37*, 317–331.

Auernheimer, C., Chinchón, S., & Pina, J. A. (1996). Lead pollution in bivalve shells. Mar Menor, Spain. *Archives Des Sciences Genève*, *49*, 87–98.

Beaudoin, A. (2003). A comparison of two methods for estimating the organic content of sediments. *Journal of Paleolimnology*, *29*, 387–390. doi:10.1023/A:1023972116573.

Borak, J., & Hosgood, H. D. (2007). Seafood arsenic: Implications for human risk assessment. *Regulatory Toxicology and Pharmacology*, *47*, 204–212.

Bragigand, V., Berthet, B., Amiard, J. C., & Rainbow, P. S. (2004). Estimates of trace metal bioavailability to humans ingesting contaminated oysters. *Food and Chemical Toxicology*, *42*, 1893–1902. doi:10.1016/j.fct.2004.07.011.

Bryan, G. W., Langston, W. J., Hummerstone, L. G., Burt, G. R., & Ho, Y. B. (1983). An assessment of the gastropod *Littorina littorea* as an indicator of heavy-metal contamination in UK estuaries. *Journal of the Marine Biological Association of the United Kingdom*, *63*, 327–345.

Catsiki, A. V., & Arnous, A. (1987). Etude de la Variabilité des Teneurs en Hg, Cu, Zn et Pb de Trois Espèces de Mollusques de l'Étang du Berre (France). *Marine Environmental Research*, *21*, 175–187. doi:10.1016/0141-1136(87)90064-X.

Calvín-Calvo, J. C., Belmonte, A., Franco-Navarro, I., Martínez-Inglés, A. M., Marín, A., Ruíz, J. M., et al. (1999). *El litoral sumergido de la Región de Murcia. Cartografía bionómica y valores ambientales. Dirección General del Medio Natural*. Región de Murcia: Consejería de Medio Ambiente, Agricultura y Agua.

Chiu, S. T., Lam, F. S., Tze, W. L., Chau, C. W., & Ye, D. Y. (2000). Trace metals in mussel from mariculture zones, Hong Kong. *Chemosphere*, *41*, 101–108. doi:10.1016/S0045-6535(99)00395-1.

Codex Alimentarius Commission. (2007). Joint FAO/WHO Food Standards Programme, Codex Committee on Contaminants in Foods, 1st Session. Working document for information and use in discussions related to contaminants and toxins of the GSCTF. CX/CF 07/1/6. Beijing, China. Retrieved from ftp://ftp.fao.org/Codex/cccf1/cf0106ae.pdf.

Conesa, H. M. (2005). Restauración/estabilización de suelos contaminados por metales pesados como consecuencia de actividades mineras en la zona de Cartagena y La Unión. Ph. D. Thesis, Universidad Politécnica de Cartagena, España.

Conesa, H., & Jiménez-Cárceles, F. J. (2007). The Mar Menor lagoon (SE Spain): a singular natural ecosystem threatened by human activities. *Marine Pollution Bulletin*, *54*, 839–849. doi:10.1016/j.marpolbul.2007.05.007.

Conesa, H. M., Faz, A., & Arnaldos, R. (2006). Heavy metal accumulation and tolerance in plants from mine tailings of the semiarid Cartagena–La Unión mining district (SE Spain). *The Science of the Total Environment*, *366*, 1–11. doi:10.1016/j.scitotenv.2005.12.008.

Conti, M. E., & Cecchetti, G. (2003). A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. *Environmental Research*, *93*, 99–112. doi:10.1016/S0013-9351(03)00012-4.

Cravo, A., Bebianno, M. J., & Foster, P. (2004). Partitioning of trace metals between soft tissues and shells of *Patella aspera*. *Environment International*, *30*, 87–98. doi:10.1016/S0160-4120(03)00154-5.

Cubbada, F., Conti, M. E., & Campanella, L. (2001). Size-dependent concentrations of trace metals in four Mediterranean gastropods. *Chemosphere*, *45*, 561–569. doi:10.1016/S0045-6535(01)00013-3.

Damkröger, G., Grote, M., & Janben, E. (1997). Comparison of sample digestion procedures for the determination of arsenic in certified marine samples using the FI-HG-AAS-technique. *Fresenius' Journal of Analytical Chemistry*, *357*, 817–821. doi:10.1007/s002160050255.

De Leon, A. R., Guerrero, J., & Faraco, F. (1982). Evolution of the pollution of the coastal lagoon of Mar Menor. VI Journées Étud. Pollutions. C.I.E.S.M., Cannes.

El-Sikaily, A., Khaled, A., & El Nemr, A. (2004). Heavy metals monitoring using bivalves from Mediterranean Sea and Red Sea. *Environmental Monitoring and Assessment*, *98*, 41–58. doi:10.1023/B:EMAS.0000038178.98985.5d.

European Communities (2001). Commission regulation (EC) no. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities, Brussels, L77, pp. 1–13.

European Communities (2002). Commission regulation (EC) no. 221/2002 of 6 February modifying Commission regulation (EC) no. 466/2001. Official Journal of the European Communities, Brussels, L37, pp. 4–6.

Farag, A. M., Woodward, D. F., Goldstein, J. N., Brumbaugh, W., & Meyer, J. S. (1998). Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River Basin, Idaho. *Archives of Environmental Contamination and Toxicology*, *34*, 119–127. doi:10.1007/s002449900295.

Francesconi, K. A., Goessler, W., Panutrakul, S., & Irgolic, K. J. (1998). A novel arsenic containing riboside (arsenosugar) in three species of gastropod. *The Science of the Total Environment*, *221*, 139–148. doi:10.1016/S0048-9697(98)00272-1.

Gilabert, J. (2001). Seasonal plankton dynamics in a Mediterranean hypersaline coastal lagoon: the Mar Menor. *Journal of Plankton Research*, *23*, 207–217. doi:10.1093/plankt/23.2.207.

Goessler, W., Maher, W., Irgolic, K. J., Kuehnelt, D., Schlagenhaufen, C., & Kaise, T. (1997). Conversion of arsenic compunds in a marine food chain. *Fresenius Journal of Analytical Chemistry*, *359*, 434–437.

Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology*, *25*, 101–110. doi:10.1023/A:1008119611481.

Huang, H., Wu, J. Y., & Wu, J. H. (2006). Heavy metal monitoring using bivalved shellfish from Zhejiang coastal waters, East China Sea. *Environmental Monitoring and Assessment*, *129*, 315–320. doi:10.1007/s10661-006-9364-9.

Jiménez-Cárceles, F. J., Egea, C., Rodríguez-Caparrós, A. B., Barbosa, O. A., Delgado, M. J., Ortiz, R., & Álvarez-Rogel, J. (2006). Contents of nitrogen, ammonium, phosphorus, pesticides and heavy metals, in a salt marsh in the coast of the Mar Menor lagoon (SE Spain). *Fresenius Environmental Bulletin*, *15*, 370–378.

Kaland, T., Andersen, T., & Hylland, K. (1993). Accumulation and subcellular distribution of metals in the marine gastropod *Nassarius reticulatus* L. In R. Dallinger, & P. S. Rainbow (Eds.), *Ecotoxicology of metals in invertebrates* (pp. 37–53). Boca Raton, FL: Lewis.

Langston, W. J., & Spence, S. K. (1995). Biological factors involved in metal concentrations observed in aquatic organisms. In A. Tessier, & D. R. Turner (Eds.), *Metal speciation and bioavailability* (pp. 407–478). New York: Wiley.

Lloret, J., Marin, A., Marin-Guirao, L., & Velasco, J. (2005). Changes in macrophytes distribution in a hypersaline coastal lagoon associated with the development of intensively irrigated agriculture. *Ocean and Coastal Management*, *48*, 828–842. doi:10.1016/j.ocecoaman.2005.07.002.

Lobel, P. B., & Wright, D. A. (1982). Gonadal and nongonadal zinc concentrations in mussels. *Marine Pollution Bulletin*, *13*, 320–323.

Lobel, P. B., Mogie, P., Wright, D. A., & Wu, B. L. (1982). Metal accumulation in four molluscs. *Marine Pollution Bulletin*, *13*, 170–174.

Long, E. R., MacDonald, D. D., Smith, S. L., & Calder, F. D. (1995). Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management*, *19*, 81–97. doi:10.1007/BF02472006.

Margalef, R. (1991). *Ecología*. Barcelona: Omega, S. A.

Marín-Guirao, L. (2007). Aproximación ecotoxicológica a la contaminación por metales pesados en la laguna costera del Mar Menor. Ph.D. Thesis, Universidad de Murcia, Murcia, España. Retrieved from http://www.tesisenred.net/TESIS_UM/AVAILABLE/TDR-0529107-101746.

Marín-Guirao, L., Marín-Atucha, A., Lloret, J., Martínez-López, E., & García-Fernández, A. J. (2005a). Effects of mining wastes on a seagrass ecosystem: metal accumulation and bioavailability, seagrass dynamics and associated community structure. *Marine Environmental Research*, *60*, 317–337. doi:10.1016/j.marenvres.2004.11.002.

Marín-Guirao, L., César, A., Marín, A., Lloret, J., & Vita, R. (2005b). Establishing the ecological quality status of soft-bottom mining-impacted coastal water bodies in the scope of the Water Framework Directive. *Marine Pollution Bulletin*, *50*, 374–387. doi:10.1016/j.marpolbul.2004.11.019.

Morillo, J., Usero, J., & Gracia, I. (2005). Biomonitoring of trace metals in a mine-polluted estuarine system (Spain). *Chemosphere*, *58*, 1421–1430. doi:10.1016/j.chemosphere.2004.09.093.

Nott, J. A., & Nicolaidou, A. (1989). Metals in gastropods—metabolism and bioreduction. *Marine Environmental Research*, *28*, 201–205. doi:10.1016/0141-1136(89)90225-0.

O'Connor, T. P., & Paul, J. F. (2000). Misfit between sediment toxicity and chemistry. *Marine Pollution Bulletin*, *40*, 59–64. doi:10.1016/S0025-326X(99)00153-8.

Ochsenkühn-Petropulu, M., Varsamis, J., & Parissakis, G. (1997). Speciation of arsenobetaine in marine organisms using a selective leaching/digestion procedure and hydride generation atomic absorption spectrometry. *Analytica Chimica Acta*, *337*, 323–327. doi:10.1016/S0003-2670(96)00411-4.

Oén, I. S., Fernández, J. C., & Manteca, J. I. (1975). The lead–zinc and associated ores of La Unión–Sierra de Cartagena, Spain. *Economic Geology and the Bulletin of the Society of Economic Geologists*, *70*, 1259–1278.

Orescanin, V., Lovrencic, I., Mikelic, L., Barisic, D., Matasin, Z., Lulic, S., et al. (2006). Biomonitoring of heavy metals and arsenic on the east coast of the Middle Adriatic Sea using *Mytilus galloprovincialis*. *Nuclear Instruments & Methods in Physics Research. Section B, Beam Interactions with Materials and Atoms*, *245*, 495–500. doi:10.1016/j.nimb.2005.11.050.

Paulson, A. J., Sharack, B., & Zdanowicz, V. (2003). Trace metals in ribbed mussels from Arthur Kill, New York/New Jersey, USA. *Marine Pollution Bulletin*, *46*, 139–152. doi:10.1016/S0025-326X(02)00312-0.

Peharda, M., & Morton, B. (2006). Experimental prey species preferences of *Hexaplex trunculus* (Gastropoda: Muricidae) and predator–prey interactions with the black mussel *Mytilus galloprovincialis* (Bivalvia: Mytilidae). *Marine Biology (Berlin)*, *148*, 1011–1019. doi:10.1007/s00227-005-0148-5.

Pérez-Ruzafa, A., Marcos, C., & Ros, J. D. (1991). Environmental and biological changes related to recent human activities in the Mar Menor (SE of Spain). *Marine Pollution Bulletin*, *23*, 747–751. doi:10.1016/0025-326X(91)90774-M.

Phillips, D. J. H. (1977). The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments—a review. *Environmental Pollution*, *13*, 281–311. doi:10.1016/0013-9327(77)90047-7.

Phillips, D. J. H. (1990). Use of macroalgae and invertebrates as monitors of metal levels in estuaries and coastal waters. In R. W. Furness, & P. S. Rainbow (Eds.), *Heavy metals in the marine environment* (pp. 81–99). Boca Raton, FL: CRC.

Phillips, D. J. H. (1995). The chemistries and environmental fates of trace metals and organochlorines in aquatic ecosystems. *Marine Pollution Bulletin*, *31*, 193–200. doi:10.1016/0025-326X(95)00194-R.

Prohaska, T., & Stingeder, G. (2005). Arsenic and arsenic species in environment and human nutrition. In R. Cornelis, J. Caruso, H. Crews, & K. Heumann (Eds.), *Handbook of elemental speciation II—species in the environment, food, medicine and occupational health* (pp. 69–85). Chichester, UK: Wiley.

Rainbow, P. S. (1995). Biomonitoring of heavy metal availability in the marine environment. *Marine Pollution Bulletin*, *31*, 183–192. doi:10.1016/0025-326X(95)00116-5.

Rainbow, P. S., & Philips, D. J. H. (1993). Cosmopolitan bioindicators of trace metals. *Marine Pollution Bulletin*, *26*, 593–601. doi:10.1016/0025-326X(93)90497-8.

Reinfelder, J. R., & Fisher, N. S. (1994). The assimilation of elements ingested by marine planktonic bivalve larvae. *Limnology and Oceanography*, *39*, 12–20.

Robles-Arenas, V. M., Rodríguez, R., García, C., Manteca, J. I., & Candela, L. (2006). Sulphide-mining impacts in the physical environment: Sierra de Cartagena–La Unión (SE Spain) case study. *Environmental Geology*, *18*, 47–64. doi:10.1007/s00254-006-0303-4.

Rodríguez-Puente, C., Guerrero, J., García, I. M., & Jornet, A. (2001). *Estudio piloto sobre niveles y efectos del tributilo de estaño (TBT) y metales pesados en el Mar Menor. Instituto Español de Oceanografía*. España: Informe Interno de la Consejería de Agricultura, Agua y Medio Ambiente de la Región de Murcia.

Roméo, M., Frasila, C., Gnassia-Barelli, M., Damiens, G., Micu, D., & Mustata, G. (2005). Biomonitoring of trace metals in the Black Sea (Romania) using mussels *Mytilus galloprovincialis*. *Water Research*, *39*, 596–604. doi:10.1016/j.watres.2004.09.026.

Roméo, M., Gharbi-Bouraoui, S., Gnassia-Barelli, M., Dellali, M., & Aïssa, P. (2006). Responses of *Hexaplex (murex) trunculus* to selected pollutants. *The Science of the Total Environment*, *359*, 135–144. doi:10.1016/j.scitotenv.2005.09.071.

Sanchiz, C., García-Carrascosa, A. M., & Pastor, A. (2000). Heavy metal contents in soft-bottom marine macrophytes and sediments along the Mediterranean coast of Spain. *Marine Ecology (Berlin)*, *21*, 1–16. doi:10.1046/j.1439-0485.2000.00642.x.

Salas, F., Marcos, C., Pérez-Ruzafa, A., & Marques, J. C. (2005). Application of the exergy index as ecological indicator of organically enrichment areas in the Mar Menor lagoon (South-Eastern Spain). *Energy*, *30*, 2505–2522. doi:10.1016/j.energy.2005.01.005.

Simonneau, J. (1973). Mar Menor: Evolution Sedimentologique et Geochimique recente du remplissage. Ph.D. Thesis, Université de Toulouse, France.

Soto, M., Ireland, M. P., & Marigómez, I. (1997). The contribution of metal/shell-weight index in target-tissues to metal body burden in sentinel marine molluscs. 1. *Littorina littorea*. *The Science of the Total Environment*, *198*, 135–147. doi:10.1016/S0048-9697(97)05452-1.

Szefer, P. (1986). Some metals in benthic invertebrates in Gdansk Bay. *Marine Pollution Bulletin*, *17*, 503–507. doi:10.1016/0025-326X(86)90639-9.

Taylor, A., & Maher, W. (2003). The use of two marine gastropods, *Austrocochlea constricta* and *Bembicium auratum* as Biomonitors of zinc, cadmium and copper exposure: effect of mass, within and between site variability and net accumulation relative to environmental exposure. *Journal of Coastal Research*, *19*, 541–549.

United Kingdom Food Standars Agency. (2002). Review of manganese, revised version. Expert Group on Minerals, London, United Kingdom. Retrieved from http://www.foodstandards.gov.uk/multimedia/pdfs/evm9922p.pdf.

United States Environmental Protection Agency (USEPA) Method 3052 (1996). Microwave assisted acid digestion of siliceous and organically based matrices. USEPA, Washington, DC. Retrieved from http://www.epa.gov/SW-846/pdfs/3052.pdf.

Viarengo, A. (1989). Heavy metals in marine invertebrates: mechanisms of regulation and toxicity at the cellular level. *Aquatic Sciences*, *1*, 295–317.

Viaroli, P., Lasserre, P., & Campostrini, P. (2007). Lagoons and coastal wetlands. *Hydrobiologia*, *577*, 1–3. doi:10.1007/s10750-006-0412-9.

Velasco, J., Lloret, L., Millán, A., Barahona, J., Abellán, P., & Sánchez-Fernández, D. (2006). Nutrient and particulate inputs into the MarMenor lagoon (SE Spain) from an intensive agricultural watershed. *Water, Air, and Soil Pollution*, *176*, 37–56. doi:10.1007/s11270-006-2859-8.

Wagner, A., & Boman, J. (2004). Biomonitoring of trace elements in Vietnamese freshwater mussels. *Spectrochimica Acta Part B*, *59*, 1125–1132. doi:10.1016/j.sab.2003.11.009.

Wallace, W. G., & Luoma, S. N. (2003). Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically available metal (TAM). *Marine Ecology Progress Series*, *257*, 125–137. doi:10.3354/meps257125.

Wallace, W. G., Lopez, G. R., & Levinton, J. S. (1998). Cd resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. *Marine Ecology Progress Series*, *172*, 225–237. doi:10.3354/meps172225.

Wilson, J. G. (1982). Heavy metals in Littorina rudis along a copper contamination treatment. *Journal of Life Sciences Royal Dublin Society*, *4*, 27–35.

World Health Organization (WHO) (1996). *Trace elements in human nutrition and health*. Geneva: WHO.

Ybáñez, N., Cervera, M. L., Montoro, R., & De la Guardia, M. (1991). Comparison of dry mineralization and microwave-oven digestion for the determination of arsenic in mussel products by platform in furnace Zeeman-effect atomic absorption spectrometry. *Journal of Analytical Atomic Spectrometry*, *6*, 379–384. doi:10.1039/ja9910600379.

Zavodnik, D., & Simunovic, A. (1997). *Beskraljesnjaci morskog dna Jadrana*. Sarajevo: IP Svjetlost.