

Biomonitoring of heavy metals in the coastal waters of two industrialised bays in southern Spain using the barnacle *Balanus amphitrite*

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ABSTRACT

This paper evaluates the bioavailability of As, Cd, Cu, Mn, Hg, Ni and Zn in the coastal waters of the two most important bays in southern Spain (Cádiz and Algeciras). Concentrations of these metals were measured in the bodies of *Balanus amphitrite* barnacles in 2005 and 2006. Seawater samples were collected from the same sites as the barnacles to assess metal concentrations and to gain additional information on the environmental conditions. Analysis of variance (ANOVA) showed significant geographic differences in the local bioavailability of metals to the barnacles, as reflected in the concentrations of accumulated metals. *Balanus amphitrite* accumulates large amounts of metals, with high concentration factors for Zn, Cd, Cu and Mn, in relation to the concentrations in marine waters.

Keywords: biomonitoring, metal pollution, barnacles, *Balanus amphitrite*, Cádiz Bay, Algeciras Bay

1. INTRODUCTION

Biomonitoring of heavy metals has become an important tool for assessing the degree of pollution in coastal waters (Ruelas-Inzunza and Paez-Osuna, 1998). The use of selected organisms as biomonitors of metal bioavailability in coastal waters facilitates comparisons over space and time, biomonitors offering easily integrated measures of the ecologically significant fraction of ambient metal in the habitat (Rainbow and Blackmore, 2001).

Barnacles have been used to assess the bioavailability of heavy metals in the coastal waters of many locations (Ruelas-Inzunza and Paez-Osuna, 1998; Rainbow and Blackmore, 2001; Rainbow *et al.*, 2004). We can assume that higher metal bioavailability in seawater leads to a greater accumulation of metal in barnacles (Paez-Osuna *et al.*, 1999). Barnacles are sessile and exist in most salinities and shore types and with varying degrees of exposure to wave action. In the two bays studied in this research (Cádiz and Algeciras), the barnacle *Balanus amphitrite* is present in different sites

where contrasting activities exist and other groups of organisms do not occur. It lives attached to surfaces found in the intertidal zone, such as rocks, piles, piers, *etc.* and is easy to collect at low tide.

This paper looks at the geographic variation in the bioavailability of seven metals in the two most important bays in southern Spain (Cádiz and Algeciras) using the barnacle *B. amphitrite* as biomonitor. The study also involves the analysis of metal levels in the waters of the two bays, comparing them with the metal concentrations in *B. amphitrite*.

2. MATERIALS AND METHODS

Study area

Cádiz and Algeciras bays are part of the coastline in Cádiz province in southern Spain (Figure 1). The former is a cove about 10 km wide located on the south-east Spanish Atlantic coast (Gulf of Cádiz) between the municipality of Rota and the city of Cádiz. The Guadalete River empties into it. Cádiz Bay consists of two clearly differentiated zones

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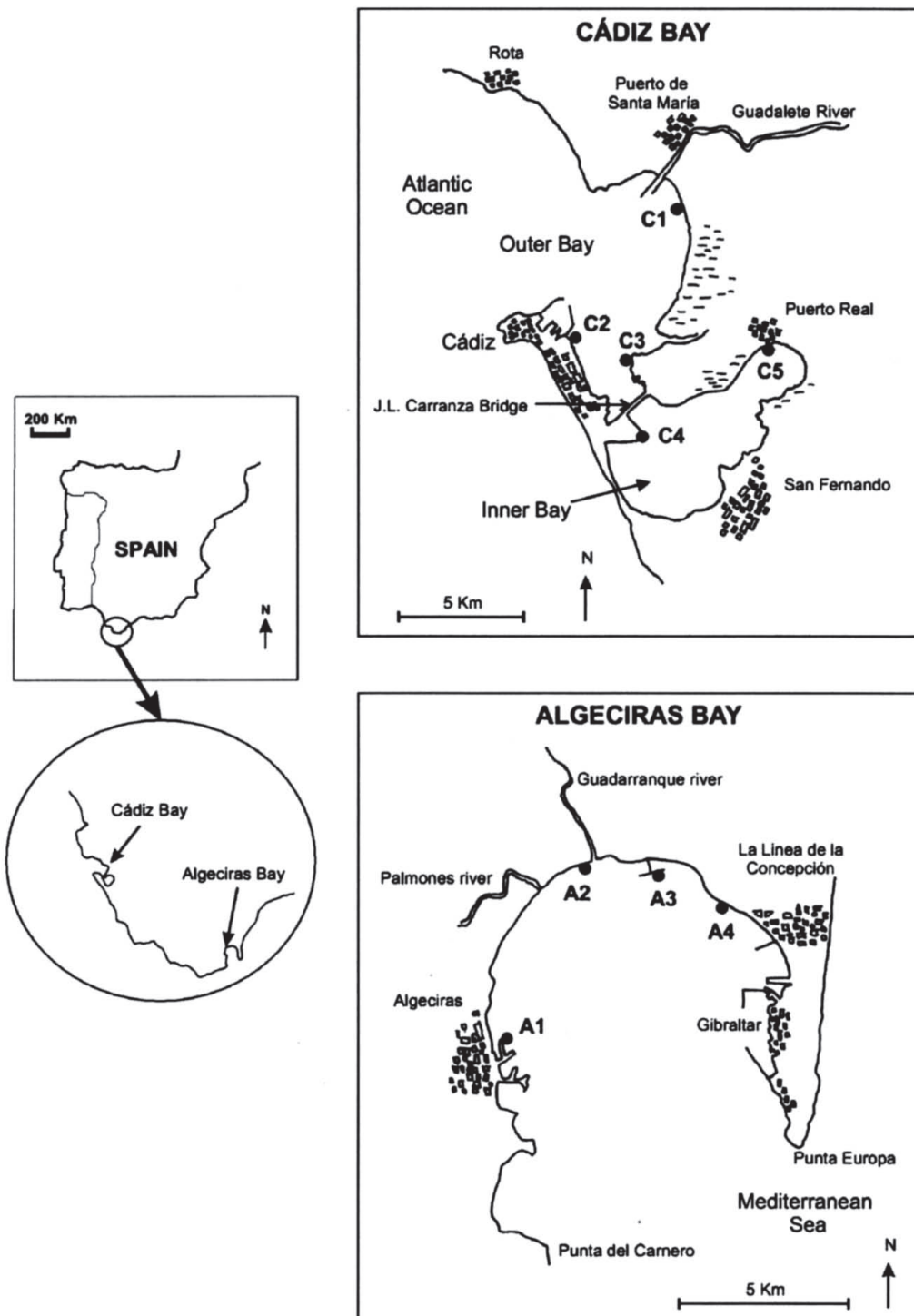


Figure 1 Study area showing the location of the sampling sites.

delimited by the José León de Carranza Bridge. The first zone is the interior of the bay, a shallow area rarely as much as five metres deep, with a silty bottom and a low water turnover rate, which favours the accumulation of pollutants discharged into the sea. The outer bay is deeper and water turnover is faster. Surrounding the bay are large

urban centres such as Rota, El Puerto de Santa María, Puerto Real, San Fernando and Cádiz. The area is also a major industrial zone with companies in the metalworking sector (shipbuilding, manufacture of automobile parts, *etc.*), food processing and food derivatives (alcohol, tobacco, *etc.*). Maritime traffic is also heavy, which pollutes the water from

“accidental” spills or discharges occurring during loading and unloading of goods. Finally, another important factor is the additional pollution coming from the Guadalete River, which picks up sewage from large towns like Jerez and Arcos and effluents from wine making, sugar refineries, agriculture, etc.

Algeciras Bay, located on the south-western Spanish Mediterranean coast, is delimited by Punta del Carnero (near the city of Algeciras) and Punta Europa (in Gibraltar) and has an 8-km mouth and a 10-km interior. Large amounts of pollutants are discharged into the bay from major towns (Algeciras, La Línea de la Concepción and Gibraltar) and the surrounding industrial area, one of the largest in Andalusia, with a significant number of petrochemical plants and refineries, in addition to the production of steel, paper and power (thermal power plants). The Palmones and Guadarranque rivers empty into the bay, and it is also polluted by heavy maritime traffic; Algeciras port is one of the largest in Spain. In contrast to Cádiz Bay, the water has a high turnover because of its proximity to the Strait of Gibraltar, where the Mediterranean Sea and the Atlantic Ocean meet, and the strong currents that predominate in the area. These factors, together with its deep waters, mean that pollutants are dispersed in a large body of water and their effects are considerably diminished.

Collection and analysis of *B. amphitrite*

Balanus amphitrite was sampled at nine stations (Figure 1): five in Cádiz Bay (C1, C2, C3, C4 and C5) and four in Algeciras Bay (A1, A2, A3 and A4). Sampling took place twice, once in 2005 and once in 2006. Sixty organisms of the same size (9–12 mm rostro-carinal axis) were selected and collected from rocks and piers at each station in the intertidal area and transported in closed, refrigerated containers. In the laboratory, the soft parts were separated and pooled to form 6 replicates of 10 bodies each from each of sites. Each replicate sample was weighed and then dried to constant weight at 60°C and weighed again. We noted that among the replicate samples there were no significant differences in the mean dry weight of individuals of the 10 pooled bodies, which means that the effect of body size on accumulated trace metals can be ruled out.

The samples were digested in an automatic microwave digestion system because of the advantages of this technique, which include speed of digestion and

less possibility of contamination during the process. A portion of the dry, finely powdered solid tissue was accurately weighed in a dry, pre-cleaned Teflon digestion vessel. 5 mL of 65% HNO₃ were added to each vessel. The vessels were sealed and placed in the microwave chamber at 300 W for 5 min and at 600 W for another 5 min. The vessels were allowed to cool for a few minutes before carefully adding 1 mL of 30% H₂O₂, and digestion continued for a further 5 min at 300 W and 5 min at 600 W. The contents of each microwave vessel were allowed to cool before transferring carefully into 50-mL volumetric flasks and diluted to volume with deionised water. The digestions were performed in batches of 12 samples (the microwave has a rotating 12-position sample carousel).

Following acid digestion, all the samples were analysed for seven elements by atomic absorption spectrometry (AAS). A Perkin-Elmer 4110 ZL atomic absorption spectrometer with longitudinal Zeeman background correction equipped with a THGA graphite furnace (transverse heated graphite atomiser) and AS-70 autosampler was used to determine Cd and Ni. Cu, Mn and Zn were determined using an air-acetylene flame (Perkin-Elmer 2380 with a double beam and deuterium background corrector). The working parameters and matrix modifiers employed were those recommended by the unit's manual.

The hydride-generation technique was used to determine As, employing a Perkin-Elmer MSH-10 connected to a Perkin-Elmer 2380 spectrophotometer. An aliquot (1–10 mL) of the resulting analyte solution was added to 5 mL of 35% HCl and 5 mL of a solution containing 5% potassium iodide and 5% ascorbic acid in a PE flask for pre-reduction of As (V). Heating to 50°C for 1 h ensured complete reaction to As (III). The vessels were then filled to 25 mL, and the final solutions were analysed for As by hydride generation, using a solution of 3% NaBH₄ in 1% NaOH under the conditions recommended in the unit's manual.

The cold-vapour technique was used for the analysis of Hg, employing a Perkin-Elmer MSH-10 connected to a Perkin-Elmer 2380 spectrophotometer. The solution was analysed using sodium borohydride as reducing agent (3% NaBH₄ in 1% NaOH) in the conditions recommended in the unit's manual.

All the analyses were performed within the laboratory's updated rigorous quality control system (International Standards Organization ISO/IEC

17025, 1999), which guarantees the reliability of all the results.

The accuracy of the metal-testing results was evaluated with both a certified reference material (CRM 278R, Community Bureau of Reference) and matrix spikes. The test results with the reference material showed that the accuracy was satisfactory. The recovery percentages were 90% for As, 91% for Hg, 98% for Mn, 102% for Cd, 104% for Zn and 106% for Cu. One sample and one acid blank in every batch digestion were spiked with a known amount of metal spike standard (added to the microwave vessel prior to digestion). The analysis of spike recoveries showed that the sample digestion was complete and matrix interference did not occur. The matrix spike recoveries for As, Cd, Cu, Mn, Hg, Ni and Zn were usually in the range of 90 to 105%. These results met our laboratory's acceptance criteria for results, which require that the percent recovery for a matrix spike must fall between 90 and 110%.

Precision was verified by analysing a replicate sample in every batch digestion. Most of the replicates agreed within 10% for the relative percent difference and were therefore considered satisfactory.

The metal pollution sources were evaluated analysing one acid blank in every batch. The results of the blanks were always below the method's detection limit. The detection limits were calculated using the procedure recommended by the EPA (US Environmental Protection Agency, Method 1631, 1999). This procedure involves spiking seven replicate aliquots of reference matrix or the sample matrix with the analytes of interest at a concentration within one to five times the estimated detection limit. The seven aliquots were carried through the entire analytical process, and the standard deviation of the seven replicate determinations was calculated. The standard deviation was multiplied by 3.14 (the Student's *t* value at 6 degrees of freedom) to give the MDL (Method Detection Limit).

Water collection and analysis

Water samples were taken eight times in 2005 and 2006 (once every three months) from the same sampling sites as the organisms using one-litre acid-leached polyethylene bottles. All the samples were taken at low tide. The recommendations in "Standard Methods for the Examination of Water

and Wastewater" (APHA, 1998) were the basis for collecting and storing the samples. The analyses were made using filtered samples. The samples were filtered at the time of collection using a preconditioned vacuum filtering device and passed through a pre-washed ungridded membrane filter with a pore diameter of 0.45 μm (Whatman, cellulose nitrate). The filter and filter device were preconditioned in 1 M HNO_3 and rinsed with deionised water. The metal analyses were carried out by means of atomic absorption spectrophotometry (AAS) using a double-beam Perkin-Elmer 2380 AAS with deuterium background correction. With the exception of As and Hg, the metals were analysed with the procedure contained in "Standard Methods for the Examination of Water and Wastewater" (APHA, 1998): 3111C (Extraction Air-Acetylene Flame Method).

The hydride-generation technique was used to determine As, employing a Perkin-Elmer MSH-10 connected to a Perkin-Elmer 2380 spectrophotometer. The procedure described in ISO standard 11969 (International Standards Organization, ISO 11969, 1996) was used for preparing and analysing the samples.

The cold-vapour technique was used to test for Hg, employing a Perkin-Elmer MSH-10 connected to a Perkin-Elmer 2380 spectrophotometer. The procedure described in European Standard EN 1483 (European Standard EN 1483, 1997) was followed, using a solution of 3% NaBH_4 in 1% NaOH as a reducing agent.

Our laboratory is accredited by the Spanish National Accreditation Association (accreditation no. 248/LE499, www.enac.es) to determine metals in water and take samples of surface water. This organisation has signed multilateral mutual recognition agreements with numerous international accreditation bodies, such as European Cooperation for Accreditation (EA), International Laboratories Accreditation Cooperation (ILAC) and the International Accreditation Forum (IAF). Accreditation is a formal recognition that a laboratory is qualified to carry out specific calibrations or tests, or specific types of calibrations or tests.

The accuracy of the results for metals was evaluated with both a certified reference material (CASS-4, National Research Council, Canada) and matrix spikes. The certified reference material (CRM) was analysed with the 3111C method (APHA, 1998). Two aliquots of the CRM were spiked with a known amount of metal spike stan-

dard, and were analysed with the 3111C method. The CRM and one spike were also analysed according to ISO 11969 for As and EN 1483 for Hg. The metal recoveries were usually in the range of 90 to 105%, indicating that accuracy was acceptable.

Precision was verified by replicate analysis. Values within 10% were obtained for the relative percent difference and were therefore considered acceptable.

The sources of metal pollution were evaluated by analysing blanks, and the results were always below the methods' detection limits.

Reagents

The nitric acid and hydrogen peroxide used for barnacle digestion were Suprapur (Merck). All other reagents used for analysing barnacles and water were of analytical reagent grade (Merck). Stock solutions (Merck) of 1000 mg L^{-1} of the different elements analysed were used to prepare the calibration standards and spike solutions. Working mercury standard solutions were prepared just before use by appropriately diluting the stock standard solution with a stabilising solution consisting of potassium permanganate/nitric acid/sodium chloride hydroxylamine hydrochloride. The reducing solution used for hydride generation and mercury analysis (NaBH_4 in NaOH solution) was obtained by dissolving NaOH pellets (for analysis, Merck) and NaBH_4 (for analysis, Merck) in deionised water. This solution was freshly prepared prior to use. The deionised water used for dilutions and reagents was obtained with a Millipore-Q system. All glassware was soaked in nitric acid and rinsed with deionised water before use.

Statistical analysis

Statistical evaluation of differences between metal concentrations was performed using analysis of variance (ANOVA) and Student's *t*-test. In the tests, the hypotheses of variance normality and homogeneity (tested with the Shapiro-Wilk, Lilliefors (Kolmogorov-Smirnov, and Levene tests) were successful. After assessing the significance of the averages, multiple comparison tests were carried out to assess which averages differed from the others and to accurately assess which sites accumulated metals differently from the others. The multiple comparison tests performed were LSD and Tukey. *P* values < 0.05 were considered significant.

3. RESULTS AND DISCUSSION

Metal in waters

A comparison of the metal concentrations in the two bays (Table 1) showed that Cádiz Bay presented higher Cu and Zn concentrations than Algeciras Bay ($P < 0.05$). In Cádiz Bay the highest metal content was found at point C5 ($P < 0.05$) with maximum concentrations of $2.1 \mu\text{g L}^{-1}$ for Cu, $7.9 \mu\text{g L}^{-1}$ for Mn and $18 \mu\text{g L}^{-1}$ for Zn in 2005 and $2.7 \mu\text{g L}^{-1}$ for Cu, $4.6 \mu\text{g L}^{-1}$ for Mn and $30 \mu\text{g L}^{-1}$ for Zn in 2006. This is not surprising, considering that this is the innermost point in the bay and therefore the water has a lower turnover rate. In addition, the nearby city of Puerto Real is one of the most highly industrialised zones in the bay. To the contrary, the lowest concentrations of the elements mentioned above were found at point C1, located in the outermost part of the bay and, consequently, subject to higher water turnover. In Algeciras Bay the highest levels of Cu, Mn and Zn ($P < 0.05$) came from point A1, located close to the bay's largest city, Algeciras. The lowest levels of these metals were obtained from point A4, the least affected by urban and industrial waste. With regard to As, Cd and Ni, we found no significant differences between the two bays or between the different sampling points in each bay. The Hg values obtained were always below the detection limit of the analytical method used ($0.1 \mu\text{g L}^{-1}$).

Of the elements studied, Zn was the most abundant and showed mean values of $15 \mu\text{g L}^{-1}$ in Cádiz Bay and $8.8 \mu\text{g L}^{-1}$ in Algeciras Bay. These concentrations were considerably higher than those usually found in the open sea, as a consequence of metals coming from the rivers and inputs from human activity in the two bays. For example, Cotté-Krief *et al.* (2000) found Zn levels between 0.06 and $1.3 \mu\text{g L}^{-1}$ in Portuguese coastal waters, and Kremling and Streu (2001) reported maximum Zn values close to $1 \mu\text{g L}^{-1}$ in the open Atlantic. Zn was followed in order of importance by Mn, with a mean value of $3.2 \mu\text{g L}^{-1}$ in Cádiz Bay and $1.9 \mu\text{g L}^{-1}$ in Algeciras Bay. These concentrations are higher than the $0.5 \mu\text{g L}^{-1}$ of Mn reported by Millward *et al.* (1998) in surface waters from the North Sea. In the Celtic Sea and the Hebridean Sea, Le Gall *et al.* (1999) found a maximum Mn concentration of $0.3 \mu\text{g L}^{-1}$, also much lower than the values we found in the two bays studied.

Table 1 Metal concentrations ($\mu\text{g L}^{-1}$) in the Cádiz Bay and Algeciras Bay waters

		As	Cd	Cu	Mn	Hg	Ni	Zn
Cádiz Bay								
2005	C1	1.7 ± 0.1	0.03 ± 0.03	0.8 ± 0.2	1.9 ± 1.0	ND	0.5 ± 0.1	7.3 ± 1.5
	C2	1.7 ± 0.1	0.03 ± 0.04	1.3 ± 0.4	3.4 ± 2.2	ND	0.5 ± 0.1	9.0 ± 3.2
	C3	1.8 ± 0.2	0.02 ± 0.03	1.5 ± 0.5	4.6 ± 2.1	ND	0.8 ± 0.4	12 ± 1.9
	C4	1.6 ± 0.2	0.02 ± 0.02	1.4 ± 0.2	5.1 ± 1.7	ND	0.8 ± 0.2	12 ± 1.8
	C5	1.7 ± 0.3	0.02 ± 0.01	2.1 ± 0.3	7.9 ± 1.8	ND	0.9 ± 0.3	18 ± 3.5
2006	C1	1.6 ± 0.2	0.03 ± 0.02	1.5 ± 0.3	1.4 ± 0.6	ND	0.8 ± 0.2	10 ± 3.5
	C2	1.8 ± 0.2	0.02 ± 0.02	1.9 ± 0.6	1.7 ± 0.7	ND	0.7 ± 0.2	14 ± 4.1
	C3	1.9 ± 0.5	0.02 ± 0.01	2.3 ± 0.2	2.5 ± 1.4	ND	0.8 ± 0.2	16 ± 5.7
	C4	2.2 ± 0.5	0.03 ± 0.02	2.1 ± 0.3	2.8 ± 1.3	ND	0.7 ± 0.3	18 ± 5.0
	C5	1.8 ± 0.2	0.02 ± 0.01	2.7 ± 0.3	4.6 ± 1.6	ND	0.9 ± 0.5	30 ± 5.8
Mean		1.8 ± 0.2	0.02 ± 0.01	1.8 ± 0.6	3.2 ± 2.0	ND	0.7 ± 0.1	15 ± 6.5
Algeciras Bay								
2005	A1	1.6 ± 0.4	0.03 ± 0.01	2.2 ± 0.4	4.9 ± 1.2	ND	0.8 ± 0.4	12 ± 3.8
	A2	1.7 ± 0.3	0.02 ± 0.01	0.8 ± 0.2	1.4 ± 0.6	ND	0.8 ± 0.2	7.3 ± 2.5
	A3	1.4 ± 0.2	0.03 ± 0.02	0.8 ± 0.2	1.9 ± 0.6	ND	1.1 ± 0.4	7.4 ± 2.6
	A4	1.5 ± 0.2	0.02 ± 0.01	0.7 ± 0.3	1.2 ± 0.4	ND	0.7 ± 0.3	4.8 ± 1.5
2006	A1	1.7 ± 0.3	0.02 ± 0.01	1.5 ± 0.4	3.6 ± 0.6	ND	0.8 ± 0.1	16.1 ± 4.2
	A2	1.7 ± 0.3	0.01 ± 0.005	1.0 ± 0.3	0.8 ± 0.5	ND	0.9 ± 0.1	8.0 ± 2.8
	A3	1.6 ± 0.2	0.01 ± 0.005	0.8 ± 0.3	0.9 ± 0.5	ND	0.8 ± 0.3	9.2 ± 3.3
	A4	1.8 ± 0.4	0.02 ± 0.01	0.7 ± 0.2	0.7 ± 0.3	ND	0.6 ± 0.2	5.2 ± 2.4
Mean		1.6 ± 0.1	0.02 ± 0.01	1.1 ± 0.5	1.9 ± 1.5		0.8 ± 0.1	8.8 ± 3.7

Mean ± standard deviation (four sample sets per year).

ND = not detected.

The Cu concentrations – between 0.7 and 2.7 $\mu\text{g L}^{-1}$ in both bays – were higher than those reported by Van Geen *et al.* (1991) in coastal waters in the Gulf of Cádiz, who obtained values between 0.37 and 0.42 $\mu\text{g L}^{-1}$. Usero *et al.* (2003) reported similar Cu levels in the salt marshes of Cádiz Bay (1.6 and 2.2 $\mu\text{g/L}$). Values for As were slightly less than 2 $\mu\text{g L}^{-1}$, similar to those reported by Usero *et al.* (2000) in the coastal waters of Cádiz. Ni presented mean concentrations of less than 1 $\mu\text{g L}^{-1}$ in the two bays studied. Morales *et al.* (1999) also found Ni levels of less than one (0.70 $\mu\text{g L}^{-1}$) in the Gulf of Valencia (western Mediterranean, Spain). Cd was the metal found in the lowest concentrations – between 0.01 and 0.03 $\mu\text{g L}^{-1}$. These values are similar to those reported by Van Geen *et al.* (1991) in the Gulf of Cádiz coastal waters.

Metal concentrations in *B. amphitrite*

Similar to the findings in the water, the *B. amphitrite* collected in Cádiz Bay presented higher concentrations of Cu and Zn ($P < 0.05$) than those from Algeciras Bay (Table 2). The highest levels ($P < 0.05$) of these metals in Cádiz Bay were found

at point C5, with maximums of 294 mg kg^{-1} for Cu, 245 mg kg^{-1} for Mn and 5,760 mg kg^{-1} for Zn in 2005 and 403 mg kg^{-1} for Cu, 169 mg kg^{-1} for Mn and 7,860 mg kg^{-1} for Zn in 2006. These levels indicate that this species is a good accumulator of these metals and that the site has high bioavailability of Cu, Mn and Zn, which is related to the enrichment of the metals in the water. The minimum levels came from point C1 in the outermost part of the bay. In Algeciras Bay there were significant differences in the bioavailability of Cu, Mn and Zn. The highest bioavailability of these metals was found at point A1, near the city of Algeciras. We also found the highest concentrations of Cu, Zn and Mn in the water at this point.

As in the water, the concentrations of the rest of the metals in this organism showed no significant differences between the two bays or among the different sampling points, with the exception of Ni, which has a considerably higher concentration at point A2 (31 mg kg^{-1} in 2005 and 32 mg kg^{-1} in 2006) than at the other points. This very high maximum was not seen in the water; the cause may be that a steel manufacturing plant that uses Ni in its alloys is

Table 2 Metal concentrations (mg kg⁻¹, dry mass) in *B. amphitrite* from Cádiz Bay and Algeciras Bay

		As	Cd	Cu	Mn	Hg	Ni	Zn
Cádiz Bay								
2005	C1	7.2 ± 2.6	6.4 ± 1.9	174 ± 34	139 ± 37	0.17 ± 0.08	4.9 ± 1.2	2200 ± 320
	C2	6.8 ± 2.8	7.7 ± 2.5	185 ± 41	151 ± 25	0.16 ± 0.09	4.6 ± 1.5	2600 ± 420
	C3	10 ± 3.5	7.3 ± 1.5	244 ± 42	172 ± 36	0.31 ± 0.12	4.8 ± 1.0	4230 ± 628
	C4	9.0 ± 2.1	5.2 ± 2.4	236 ± 37	178 ± 24	0.23 ± 0.17	5.9 ± 2.0	3860 ± 529
	C5	11 ± 2.9	4.6 ± 1.8	294 ± 51	245 ± 51	0.35 ± 0.21	6.1 ± 1.9	5760 ± 985
2006	C1	12 ± 3.1	3.2 ± 0.9	163 ± 26	105 ± 22	0.18 ± 0.10	4.2 ± 0.9	3220 ± 525
	C2	9.1 ± 1.8	3.3 ± 0.7	195 ± 42	112 ± 19	0.16 ± 0.08	3.7 ± 0.9	3780 ± 658
	C3	11 ± 2.1	3.2 ± 0.8	357 ± 28	115 ± 28	0.26 ± 0.13	5.3 ± 1.4	5480 ± 896
	C4	12 ± 1.9	3.2 ± 0.9	294 ± 47	136 ± 21	0.15 ± 0.11	5.2 ± 1.5	6470 ± 728
	C5	13 ± 1.8	3.1 ± 0.6	403 ± 38	169 ± 25	0.37 ± 0.22	5.1 ± 1.6	7860 ± 1253
Mean	10.1 ± 2.1	4.7 ± 1.8	254 ± 81	152 ± 42	0.23 ± 0.08	5.0 ± 0.7	4550 ± 1800	
Algeciras Bay								
2005	A1	10 ± 3.2	4.7 ± 1.2	280 ± 55	235 ± 43	0.10 ± 0.08	8.1 ± 2.8	3080 ± 569
	A2	9.8 ± 1.9	6.4 ± 2.1	130 ± 45	109 ± 21	0.20 ± 0.15	31 ± 6.2	1500 ± 358
	A3	10 ± 1.5	5.0 ± 1.0	108 ± 34	173 ± 29	0.25 ± 0.12	11 ± 3.9	1100 ± 315
	A4	9.3 ± 2.2	4.6 ± 1.3	86 ± 19	98 ± 15	0.20 ± 0.10	6.2 ± 2.5	480 ± 158
2006	A1	9.6 ± 2.3	2.5 ± 1.5	248 ± 43	192 ± 45	0.12 ± 0.05	6.6 ± 2.8	5220 ± 852
	A2	12 ± 2.4	3.2 ± 1.2	128 ± 23	98 ± 36	0.30 ± 0.20	32 ± 5.5	1860 ± 452
	A3	10 ± 1.2	2.0 ± 0.8	116 ± 39	142 ± 31	0.25 ± 0.15	9.7 ± 3.6	1380 ± 425
	A4	12 ± 2.7	3.4 ± 0.7	95 ± 22	85 ± 19	0.26 ± 0.12	5.1 ± 2.9	1030 ± 463
Mean	10.3 ± 1.1	4.0 ± 1.5	149 ± 73	141 ± 54	0.21 ± 0.07	14 ± 11	1960 ± 1520	

Mean ± standard deviation (six replicate samples).

located near this point. Intermittent discharges of wastewater from this plant may not be detected in spot analyses of the water, although Ni may be present in *B. amphitrite*. This highlights the importance of supplementing water analyses with the use of living organisms.

Zn was the most abundant metal in *B. amphitrite*, undoubtedly because it is the majority element in the water and because the organism has a high capacity for accumulating this metal (Walker *et al.*, 1975; Blackmore, 1999). A high level of Zn in comparison to other elements is typical of barnacles (Fialkowsky and Newman, 1998; Páez-Osuna *et al.*, 1999) and other animal species commonly used as pollution biomonitors (Kang *et al.*, 1999; Castro *et al.*, 1999; Wong *et al.*, 2000). Cu concentrations were the second highest, especially in Cádiz Bay, with a mean value of 254 mg kg⁻¹. Algeciras Bay had lower Cu concentrations (mean value 149 mg kg⁻¹). These Cu levels were higher than those found in other barnacle studies. For example, Rainbow *et al.* (2000) reported Cu values between 42.5 and 86.2 mg kg⁻¹ in *Balanus improvisus* in the Gulf of Gdansk (Baltic Sea, Poland). Similarly, Blackmore (2001) obtained a considerably lower

Cu concentration (57.8 mg kg⁻¹) in *Balanus amphitrite* than we found in this work.

Mn mean concentrations were 152 mg kg⁻¹ in Cádiz Bay and 141 mg kg⁻¹ in Algeciras Bay. These Mn levels are also higher than usually found in barnacle studies (Blackmore *et al.*, 1998; Blackmore, 1999; Páez-Osuna *et al.*, 1999). However, Rainbow *et al.* (2000) obtained Mn concentrations between 187 and 307 mg kg⁻¹ in *Balanus improvisus* from the Gulf of Gdansk, similar to the values in our study. No significant differences were seen in As concentrations in the *B. amphitrite* from the two bays, which presented mean levels around 10 mg kg⁻¹. Cd concentrations were also similar for both bays, with mean values between 4 and 5 mg kg⁻¹. The lowest concentrations were those for Hg, with values around 0.2 mg kg⁻¹ in the two bays.

Bioconcentrations and relation between metals in organisms and water

Balanus amphitrite has a high capacity for accumulating metals, as shown in Figure 2, which gives the accumulation factors for the elements studied as defined from the ratio of the metal concentration in

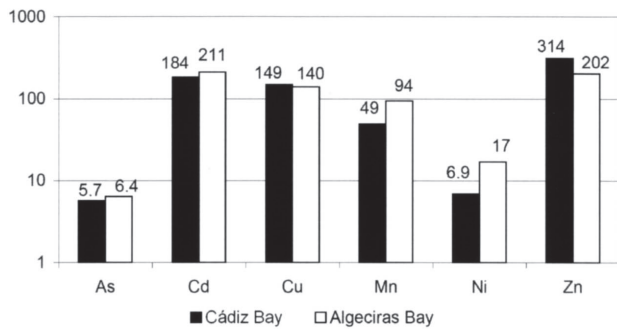


Figure 2 Mean values of accumulation factors (metal in *B. amphitrite*/metal in water).

the organism to that in the water. Zn, Cd, Cu and Mn were the metals accumulated in the highest proportions by this organism. It is noteworthy that the Zn accumulation factor for Cádiz Bay (314) was much higher than for Algeciras Bay (202). The accumulation factor for Cu was also higher in Cádiz Bay than in Algeciras Bay. The opposite was true for the rest of the elements studied.

There was a significant correlation ($P < 0.05$) for concentrations of Cu, Mn and Zn in *B. amphitrite* relative to their concentrations in water in the two bays (Table 3). The other metals (As, Cd and Ni)

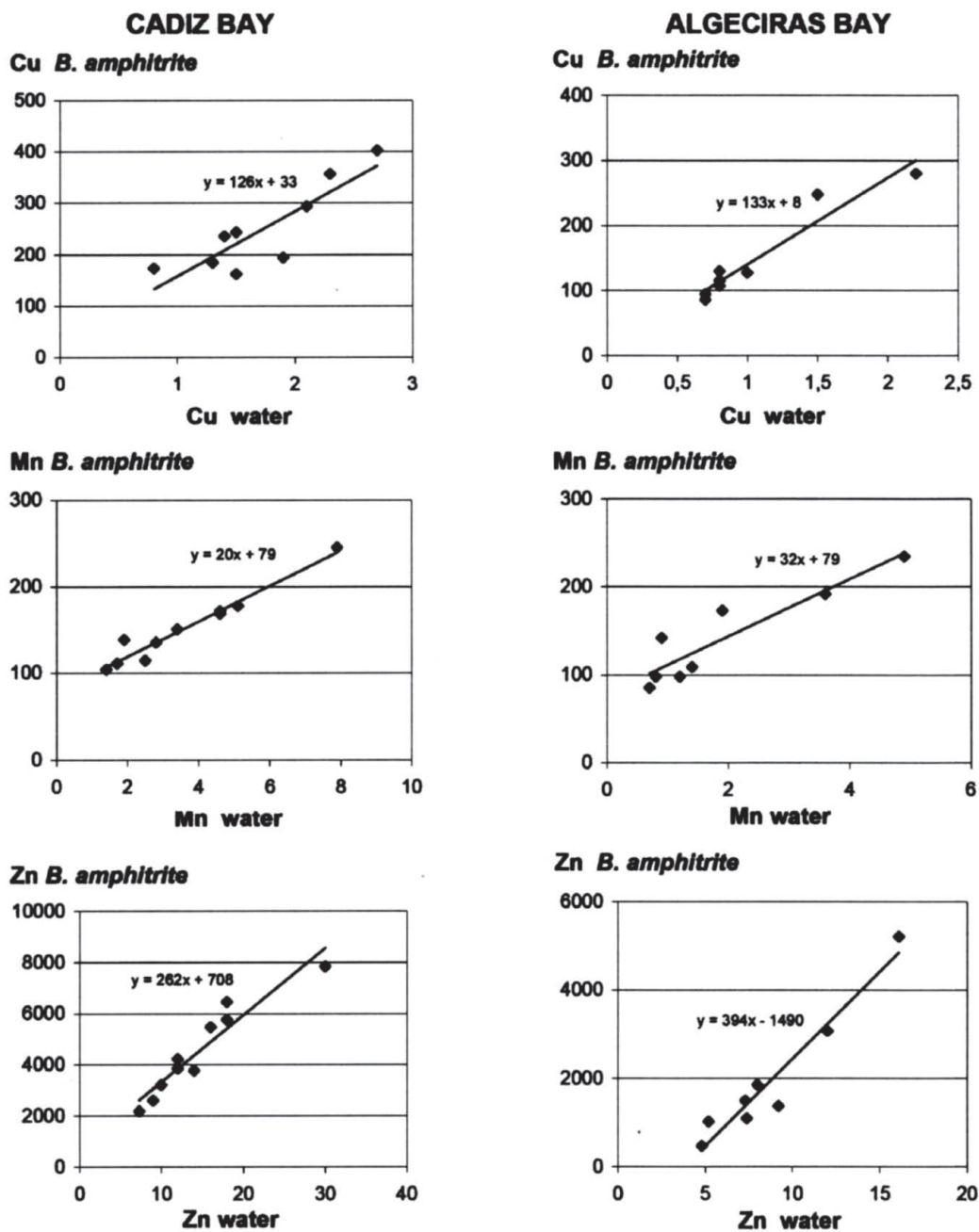


Figure 3 Linear regression between metals in *B. amphitrite* (mg kg^{-1} dry mass) and water ($\mu\text{g L}^{-1}$).

Table 3 Correlation coefficients between metal concentrations in waters and in *B. amphitrite*

Zone	As	Cd	Cu	Mn	Ni	Zn
Cádiz Bay	0.37	0.19	0.87**	0.98**	0.40	0.95**
Algeciras Bay	0.57	0.58	0.96**	0.92**	0.31	0.97**

$P < 0.05$; ** $P < 0.01$.

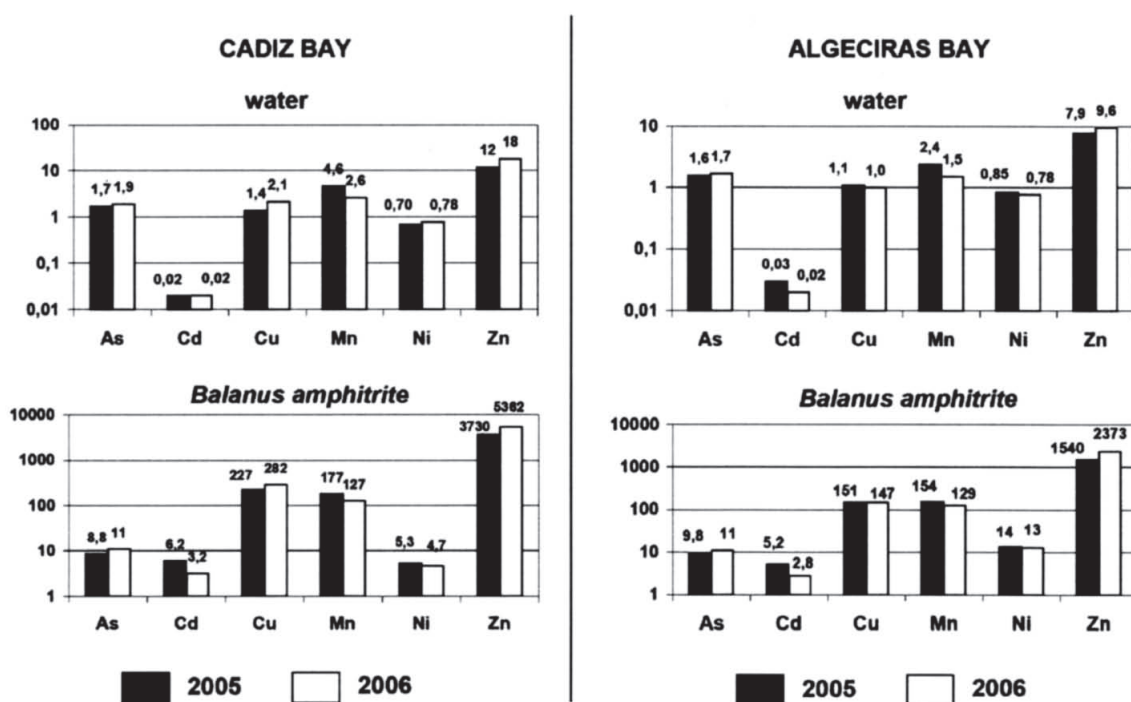


Figure 4 Mean metal concentrations in water ($\mu\text{g L}^{-1}$) and in *B. amphitrite* (mg kg^{-1} dry mass).

showed positive correlations but the level of confidence was not significant. Several studies (Wang *et al.*, 1999a,b) have evidenced that barnacles obtain most metal from their diet (suspended metals). Therefore, the correlation between the dissolved metal concentration and the accumulated metal concentration in barnacles is indirect.

Figure 3 shows the relation between metal concentrations in *B. amphitrite* and water based on a linear regression model. The elements can be ordered as follows in terms of the regression line slopes: $\text{Zn} > \text{Cu} > \text{Mn}$. A greater slope means a higher increase in a metal's bioavailability, parallel to the increase in its concentration in the medium (Szefer *et al.*, 1999). If we compare the two bays we see that the slope for these metals is greater in Algeciras Bay. If one metal has a greater slope than another, then, given the same increase in their concentrations in the water, there will be a greater increase of that metal in *B. amphitrite*.

Comparing the mean concentrations obtained in the water and *B. amphitrite* in the two years studied, we observe a similar evolution (Figure 4). The water and *B. amphitrite* in Cádiz Bay showed the highest mean concentrations of As, Cu and Zn and the lowest of Mn in 2006. In the same year the Algeciras Bay concentrations were higher for As and Zn and lower for Cd, Cu and Ni.

4. CONCLUSIONS

The results show that there are differences between these two bays. Cádiz Bay has higher Cu and Zn levels in the water and in *B. amphitrite* than found in Algeciras Bay. We also found significant differences in bioavailability among the different points in the two bays. The highest bioavailability of Cu, Zn and Mn in Cádiz Bay is found at the innermost point near the city of Puerto Real, one of the most highly industrialised zones in the bay. The lowest metal

availability is in the outermost part of the bay, and this area can serve as a useful baseline site for estimating the degree of pollution in other parts of the bay. The highest bioavailability of Cu Zn and Mn in Algeciras Bay is near the city of Algeciras. Ni bioavailability is high in an area near a steel manufacturing plant that uses Ni in its alloys. Based on the accumulation factors (metal in *B. amphitrite*/metal in water), we can conclude that *B. amphitrite* has a strong tendency to accumulate metals, especially Zn, Cd, Cu and Mn.

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