

# Bioavailability of metals along a contamination gradient in San Diego Bay (California, USA)

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## Abstract

San Diego Bay is heavily contaminated with metals, but little is known about their biological availability to local marine organisms. This study on 15 elements showed that concentrations of metals associated with sediment increased from the mouth to the back of the Bay while metals in seawater particulates were similar throughout the Bay. Metal bioavailability was assessed over 8 weeks following transplant of the local brittlestar, *Ophiothrix spiculata* (Ophuroidea, Echinodermata), from outside to inside the Bay. Despite a gradient of contamination, brittlestars accumulated similar levels of metals throughout the Bay, suggesting that metal contamination occurred through dissolved metals as well as through the diet. Sediment transplanted in dialysis tubing in the Bay accumulated metals only when placed on the sea-floor bottom, indicating greater metal bioavailability near the bottom; the level of accumulation was similar between the mouth and the back of the Bay. The results are consistent with a circulation pattern in which a bottom layer of seawater, enriched with metals, drains from the back to the mouth of the Bay. There was a positive correlation between metal concentration in brittlestars and tidal range, suggesting increased metal exposure due to bay-ocean water exchange. For brittlestar arms the correlation was higher at the mouth than the back of the Bay, indicating greater metal accumulation in arms from dissolved metals in seawater than from ingestion of metal contaminated diet. In contrast, for brittlestar disks the correlation was higher at the back of the Bay, indicative of metal accumulation mainly through the diet. The results highlight the importance of considering bioavailability and physical processes in environmental quality assessments.

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## 1. Introduction

San Diego Bay is a semi-enclosed body of water that is the site of intense economic and public activities. It

has long been known that, as with other bays acting as harbors and surrounded by a metropolis, San Diego Bay is subject to contamination by various hazardous substances (State Department of Public Health, 1951; Torres, 1988; Environmental Health Services, 1990). Amongst them, metals are some of the most important contaminants, originating from multiple recurring past and present sources mainly linked to military and recreational boating, such as contamination from antifouling

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paint, fuel combustion, and shipyard activities. Contamination of the Bay was particularly intense until 1963, when completion of the San Diego Metropolitan Sewage System allowed a large percentage of municipal and industrial waste to be discharged offshore instead of directly in the Bay (State Department of Public Health, 1951; Torres, 1988; Environmental Health Services, 1990). Today, San Diego Bay harbors many ships and shipyards, the largest component being military. Recreational boating is significant as well, accounting for thousands of vessels, some of them densely packed in marinas around the Bay. All these boats are potential sources of pollution, especially by metals (Federal Water Pollution Control Administration, 1969; Young et al., 1979; Nichols, 1988; Schiff et al., 2004).

San Diego Bay has often been subject to monitoring surveys over the years, with the Bay divided into a northern part (the mouth of the Bay) that opens to the Pacific Ocean and is frequently flushed, and a southern part (the back of the Bay), which is the closed and poorly flushed dead-end of the Bay. Considering also that most shipyard activity takes place towards the back of the Bay, it is not surprising that there is an increasing gradient of metal total concentration in sediment from the mouth to the back of the Bay (Anderson et al., 1996; Fairey et al., 1998). This does not necessarily mean, however, that these metals are biologically available to the local fauna. In fact, only a few studies have assessed the bioavailability of metals in the Bay, then using seawater-filtering organisms as experimental models. Mussels transplanted into sub-surface Bay waters accumulate metals, an indication that metals are bioavailable from seawater and/or seawater particulates (Martin, 1985; Steinert et al., 1998). Yet, there is a lack of knowledge on the actual amount of bioavailable metals in the Bay, as based on the ratio between environmental levels of metals and the quantity actually taken up by organisms. Thus, it is not known to what extent metals present in the Bay can be transferred to the local biota.

Bioavailability depends on multiple environmental factors that affect metal complex formation, including physico-chemical parameters like salinity and the concentration of organic material soluble in the environment, and on biological and/or physiological characteristics of the organism that is exposed to the metal, including digestion and uptake efficiency, storage, and excretion capacities. Bioavailability therefore is a difficult parameter to assess because of the many environmental and species-specific factors that can affect metal uptake and/or accumulation (Luoma, 1983, 1989; Campbell, 1995; Rainbow, 2002).

Brittlestars live in sandy/muddy areas where metals are preferentially concentrated (Clark, 1997), and are thus directly exposed to contamination from dissolved metals in seawater and from metals concentrated in their

diet, which consists of sediment and/or organic detritus. For this reason brittlestars, as well as other echinoderms such as seastars and sea urchins, are excellent bioindicators of environmental quality because they can uptake bioavailable contaminants from the surrounding environment (Warnau et al., 1995; Newton and McKenzie, 1998).

This study measured environmental levels of fifteen metals in the marine ecosystem of San Diego Bay and assessed their bioavailability following metal accumulation in brittlestars transplanted from outside to inside the Bay.

## 2. Materials and methods

### 2.1. Environmental parameters

This study was performed from June, 14 to August, 9 2001 at four sites (A–D) distributed along the San Diego Bay (Fig. 1); water depth was 16 m for site A, 15 m for site B, and 11 m for both sites C and D. At each site, water temperature, salinity, and turbidity were measured once a week during 7 weeks (week 0, 1, 2, 3, 4, 6, and 8), in parallel with the collection of samples involved in the transplant experiment (Section 2.2). Water temperature was measured from the seafloor bottom using an Oceanic dive computer and from the surface with a standard ASTM certified mercury thermometer (Fisher Scientific) immersed for several minutes before reading. Salinity was measured from subsurface seawater samples using a portable refractometer (Fisher Scientific), while turbidity was estimated from Secchi disk measurements of water clarity. Seawater renewal in the Bay due to tidal mixing and exchange with the open Ocean was estimated for each week of the experiment (except for week 0)



Fig. 1. Location of sites of investigation in San Diego Bay, California. The sites were located near navigation buoys R8 (site A), R16A (site B), G1 (site C) and R34 (site D).

using as a proxy the tidal range for the whole week preceding each collection time. The tidal range therefore was defined as the greatest difference in water level height that occurred during that week (maximum values of Mean Higher High Water—minimum value of Mean Lower Low Water). The tidal range was calculated using NOAA tide data (<http://co-ops.nos.noaa.gov>) from tidal benchmark station #9410170, located between sites B and C.

## 2.2. Metal partitioning and bioavailability

Partitioning of metals in the environment was assessed from samples of sediment and seawater particulates; endemic benthic macro-invertebrates were not considered because of the Bay's paucity of macro-benthic life; only a few specimens of macro-invertebrates were found over the entire experimental period.

Metal partitioning was also experimentally assessed using sediment samples in dialysis tubing transplanted to the studied sites at the seafloor bottom versus the mid-water column. Assuming that the tubing allowed passage of only the free ionic and dissolved form of metals, this experiment served to monitor any change in metal concentrations in sediment due to exchange of only the non-particulate form of metals present in the surrounding environment (Eimers et al., 2001; Solbra et al., 2004).

## 2.3. Collection and sample preparation

All samples were collected in situ by SCUBA using acid washed containers. Bay sediment samples were collected from the upper surface layer (3 cm) of sediment at each site by using a 50 ml polypropylene Falcon<sup>®</sup> tube as a mini-corer. Back in the laboratory the sample was divided into sediment from the top, middle, and bottom section of the collecting tube. Bay seawater samples were collected at mid-depth using a 1 l polycarbonate jar. For logistical reasons, water samples could not be processed immediately and were frozen upon return to the laboratory. Thawed samples were thoroughly mixed by hand before being divided into three 200 ml fractions to allow triplicate analysis. Each fraction was vacuum filtered separately onto acid digestible Nitrocellulose Millipore HAWP filters (0.45  $\mu\text{m}$  pore size). In phytoplankton and other organisms, metals are associated with membranes and intracellular ligands (Ettajani et al., 2001; Rijstenbil and Gerringa, 2002). After freezing and thawing, metals are not expected to be released in free ionic form. Thus the material retained on filters is considered to contain most of the metals representative of seawater particulates.

For use in transplant experiments, seafloor surface sediment and brittlestars were collected by SCUBA between 12 and 15 m depth in the La Jolla Cove Under-

water Reserve (considered a non-contaminated reference), about 15 miles north of San Diego Bay. The sediment was oven-dried for 3 weeks immediately after collection, and placed in 33 cellulose ester dialysis tubing (300 kDa cut off; Spectra/Por<sup>®</sup>); each tubing contained about 10 g of sediment and was sealed at both ends by a knot and a plastic clip closure. Dialysis tubing was then placed in running seawater for 6 weeks to allow degradation of the original marine biofilm and organic material that coated the sediment grains prior to oven drying (Schorer and Eisele, 1997; Petersen et al., 1998). Sediment from 3 of the 33 tubing was then collected and directly processed for metal analysis. From the remaining 30 tubing, 12 tubing were placed in triplicate in four cages to be transplanted at the seafloor bottom of each studied site in the Bay. Another 12 tubing were placed at those sites in triplicate in a large mesh bag in suspension in the mid-water column (3–6 m above bottom seafloor, depending on the site) using a sub-surface buoy attached to a permanent anchor. Another 3 tubing were used as negative control of contamination and left in running seawater at the SIO Experimental Aquarium facility. The last 3 tubing were used as positive control of contamination, being exposed to 2 l of seawater contaminated with  $10^{-7}$  M Cu ( $\text{CuCl}_2$ , Sigma) that was renewed every other day. The 30 experimental tubing were left 2 weeks in their respective condition, whether in the field or in the aquarium, before being collected and returned to the laboratory. Each tubing was then sliced open along its length using a scalpel, and sediment from mid-length of the tubing processed for metal analysis.

Metal bioavailability was assessed following field transplant of brittlestars from outside to inside the Bay; brittlestars are target species for exposure to elevated metal concentrations, with possible metal uptake and accumulation, because they live in contact with, and feed on, marine sediments and seawater particulates.

The brittlestar *Ophiothrix spiculata* (Ophiuroidea, Echinodermata) is a common species along the southern California coast and was chosen as the transplant organism in this study. Brittlestars were collected from kelp holdfasts and maintained in open-circuit seawater conditions at the SIO experimental aquarium facility until transplant. Individuals of similar size (1.9–2.1 mm disk diameter) and with five intact arms were transplanted in the Bay in cages, each containing three individuals and three mesh balls that provided substrate for the brittlestars. Once collected from the Bay, the brittlestars were removed from their cage, anesthetized in 3.5%  $\text{MgCl}_2$  solution in artificial seawater as commonly used in neuro-physiological studies involving Echinoderms, and measured under a stereo microscope for disk diameter and arm length. The arms were separated from the disk using a micro-scalpel, and the disk cut open in two; each body part was then quickly rinsed in artificial

seawater to remove mineral particles before being placed in a glass tube for metal processing.

Transplant was done using round cages made of PVC pipe sections (20 cm diameter  $\times$  3 cm height) with each open side of the section pipe covered with mesh (710  $\mu\text{m}$ ). The mesh was sealed on the seafloor-facing side using silicone glue, while the water column-facing side was tightly attached using two 50 cm-long cable ties, after the brittlestars and mesh balls were put inside the cage. This took place one day prior to the transplant in the Bay, and the cages were kept overnight in circulating seawater before deployment at each site; three individuals were also collected from the aquarium and directly processed for metal analysis (week 0 of the experiment). A total of 24 cages were distributed among the four sites, allowing the collection of one cage at each time point of the experiment for each site. Cages were attached to an external frame to avoid flipping due to current, and the frame was positioned on the sea floor with mesh of cages side down to allow sediment to enter inside; the whole structure was attached to a permanent anchor.

Samples of seafloor sediment, seawater and brittlestars were hand-collected by SCUBA diving at the four underwater sites (Fig. 1) during the 8-week experiment; transplanted sediment was deployed at each site at week 6, and collected for analysis at week 8. The samples were collected in individual acid washed Falcon<sup>®</sup> tubes for sediment, polycarbonate jars for seawater, and Zip-lock<sup>®</sup> plastic bags for transplanted brittlestars and sediment dialysis tubing, and transported to the laboratory where they were processed for metal analysis. The sampling protocol was the same at each time point and for each site, and the collected samples, once prepared in the laboratory for metal processing (which included sediment extraction from tubing and brittlestar dissection), were stored dried (sediment and brittlestar disks and arms) or frozen (seawater) until the start of metal analysis.

All manipulations were done under controlled conditions to avoid metal contamination, using metal free solutions, nitric acid washed plastic-ware and filtration apparatus, stainless steel dissecting tools, and disposable polypropylene or high-density polyethylene supplies, including forceps. Glass was used only as jars to store highly concentrated nitric acid, and as nitric acid washed Pyrex test tubes to hold samples during drying. Trace Metal Grade nitric acid (Fisher Scientific) was used in sample preparation with concentrations  $<10^{-7}$  mg g<sup>-1</sup> for each metal, thus having a negligible effect on sample metal concentrations.

#### 2.4. Extraction and analysis of metals

For sediment, the amount of metals extracted is related to the strength of acid treatment, with weak acid

treatment extracting mainly leachable and particle bound metals while strong acid treatment also dissolves constitutive metals from the mineral matrix (Katz and Kaplan, 1981; Cook et al., 1997). Metals that are potentially available to organisms can be leached from the particle's surface. Therefore, although there is no defined protocol to measure bioavailable metals associated with sediment (Luoma, 1989), typically the amount of metals extracted from sediment samples following relatively mild acid treatment (i.e., leachable metals) is used as a proxy of bioavailable metals (Snape et al., 2004; Deheyn et al., 2005). The use of mild acid treatment ensures complete extraction of leachable metals while limited extraction of constitutive metals and limited dissolution of the matrix of the mineral particles (Cook et al., 1997). In this study, 45% nitric acid in Milli-Q water (7 N HNO<sub>3</sub>) was used; the relatively mild acid treatment completely digested organic matter to release all associated metals, extracting metals adsorbed to sediment particles but not constitutive metals from the mineral structure. Thus, in this study total metal concentrations of sediment (leachable portion plus constitutive metals) were not assessed.

For brittlestar disk and arm tissue and seawater particulates, the 45% nitric acid solution resulted in full digestion of the samples, thus extracting the entire load of metal from the samples except, on occasion, a few mineral particles (identified as fine sediment) that were not digested.

Seawater particulates, sediment, and brittlestar disk and arm tissue were dried, weighed using a high precision microscale (Sartorius, R160P), and digested in nitric acid. After 1 d at room temperature, samples were oven dried at 65 °C for 3–5 d to complete the digestion process and allow for acid evaporation. A known quantity (determined using the microscale) of 0.7% nitric acid solution (0.1 N HNO<sub>3</sub>) in Milli-Q water was then used to resolubilize the digested material.

A total of 15 elements (Ag, Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sn, Sr, Ti, Zn) was analyzed simultaneously using an Induced Coupled Plasma Atomic Emission Spectrum (ICP-AES) spectrometer (Optima 3000 XL, Perkin Elmer), with detection limits from 0.05 to  $4 \times 10^{-6}$  mg g<sup>-1</sup> depending on the element (Perkin Elmer, 2000). The instrument was calibrated before every run by successive dilution of a 100 ppm Multi-Element Instrument Calibration Standard solution (Fisher Scientific). The samples were run in a random order of site and week. Approximately 100 samples were run at one time and for all metals, the intra-run reading variation of a 1 ppm multi-element Quality Insurance standard analyzed every 20 samples over the course of the run was  $102.6 \pm 0.32\%$  (mean  $\pm$  one standard error), with a range of 95.4–112.8%. Internal blanks were used to assess any background contamination originating from sample manipulation

and preparation. Blanks were processed exactly as regular samples but contained only nitric acid. Blank readings were always low indicating minimal contamination during sample processing, and they were subtracted from sample readings to give final values of metal concentration.

### 2.5. Quality assurance

Accuracy of sample processing was checked using samples of DOLT-2 (marine organism tissue) Matrix Certified Reference Materials with known concentrations of specified metals (National Research Council, Canada). The recoverability factor of the expected concentration had a median value of 102.6%, the variation ranging from 30.4% to 150.2% depending on the metal (Table 1). Variability of the instrument readings, represented by the coefficient of variation, was different among metals, ranging from 1.0% to 18.5%. This variability was due to the simultaneous measurement of the 15 elements from each sample. Although low recoverability could reflect loss of metals by evaporation during processing and/or adsorption to the vial, as well as instrument reading variability, recoverability >100% was strictly related to instrument variability and was not sign of contamination as the blanks were always low in metals.

The accuracy of processing was not assessed for sediment because certified values of metals for commercially available sediment reference materials are always obtained using strong acid treatment (National Research

Council, 2000) to dissolve the particle matrix and release constitutive as well as adsorbed metals. There are no certified values for the weak acid treatment used in this study (Cantillo and Calder, 1990).

### 2.6. Statistical analysis

Analysis of Variance (single or multiple factor ANOVA) and post-hoc multiple comparison of means (Fisher's PLSD) were used to test significance of differences between metal concentrations considering  $\log(x + 1)$  transformed data set to comply with heteroscedasticity (Zar, 1996). Correlations between metal concentrations in disk and arm tissue of brittlestar and between metal concentration and environmental parameters were tested using the Spearman ranked correlation coefficient ( $\rho$ ).

Tidal range was used to represent input of ocean water into the Bay; it is thus associated with movement of Bay water and changes in local physico-chemical parameters that can affect metal partitioning and bioavailability in the environment. The relationship between metal concentration in brittlestars and tidal range was tested for significance using least-squares fit of the simple exponential model  $y = a * \exp(b * x)$ , where "a" was the initial metal concentration in brittlestar before transplant, and "b" the angular coefficient of the variation, thus representing the rate at which metal concentration changed with tidal range. We anticipate that the greater the tidal range, the greater the movement of metal contaminated Bay seawater particu-

Table 1

Metal concentrations (mean  $\pm$  standard error of dry weight) measured from DOLT-2 marine organism tissue matrix reference material, compared to certified values

Element	Certified value ( $\mu\text{g g}^{-1}$ d.w.)	Observed value ( $\mu\text{g g}^{-1}$ d.w.)	Coefficient of variation (%)	% Recoverability
Ag	0.608 $\pm$ 0.032	0.784		128.9
Al	25.2 $\pm$ 2.4	20.10 $\pm$ 0.48	2.4	79.8
As	16.6 $\pm$ 1.1	5.05 $\pm$ 0.74	14.7	30.4
Cd	20.8 $\pm$ 0.5	22.45 $\pm$ 0.73	3.3	107.9
Cr	0.37 $\pm$ 0.08	0.27 $\pm$ 0.05	18.5	73.0
Cu	25.8 $\pm$ 1.1	29.56 $\pm$ 1.74	5.9	114.6
Fe <sup>a</sup>	1.1 $\pm$ 0.05	1.07 $\pm$ 0.011	1.0	97.3
Mn	6.88 $\pm$ 0.56	5.20 $\pm$ 0.17	3.3	75.6
Ni	–	–		
Pb	–	–		
Se	6.06 $\pm$ 0.49	9.1 $\pm$ 0.59	6.5	150.2
Sr	–	–		
Ti	–	–		
Sn	–	–		
Zn	85.8 $\pm$ 2.5	101.67 $\pm$ 1.19	1.2	118.5

Percent recoverability is the ratio of observed value to certified value  $\times$  100, while the coefficient of variation is the ratio of standard error to the mean of observed values  $\times$  100. Number of replicates for observed values ranges from 1 to 6.

– Not analyzed.

<sup>a</sup> in  $\text{mg g}^{-1}$  dry weight.

lates, and the greater the metal exposure to brittlestars, and thus the greater the concentration in their tissues. Model correlation coefficient  $r$  was tested for significance using an upper-tailed Student's  $t$ -test (Zar, 1996).

Metal bioavailability was assessed using as a proxy the metal bioconcentration factor for brittlestar disk and arm tissue. Bioconcentration factor was calculated using the ratio of the median concentration in disk and arm tissue to the concentration of leachable metals associated with sediment, including all weeks except week 0.

All statistical analyses were performed using Statview<sup>®</sup> 5.0 software (SAS Institute, Inc.), with significance based on an  $\alpha$  of 0.05 for two-tailed comparisons. Unless otherwise stated, values represent means with one standard error, and stated  $P$  values are the highest encountered among all comparisons. Descriptive statistics were based only on samples with concentration values higher than the spectrometer detection limit while any other statistical analysis included the values below detection threshold by replacing them with the limit threshold value of the instrument (Perkin Elmer, 2000).

### 3. Results

Environmental characteristics were different between the mouth of the Bay (sites A and B), which showed low turbidity and a stratified water column, and the back of the Bay (sites C and D) with high turbidity and a homogeneous water column. Metal concentrations associated with sediment were lower at the mouth of the Bay, and higher in the back, while metal concentrations in transplanted brittlestars were similar throughout the Bay. At each site, metal concentrations were generally highest when associated with sediment, with successively lower concentrations in brittlestar disk and arm tissue, and seawater particulates. The order of metals ranked by decreasing median concentration was  $\text{Fe} > \text{Al} > \text{Sn} > \text{Ti} > \text{Mn} > \text{Zn} > \text{Sr} > \text{Cu} > \text{Pb} > \text{Cr} > \text{Ni} > \text{As} > \text{Cd} > \text{Ag} > \text{Se}$  when associated with sediment,  $\text{Fe} > \text{Al} > \text{Sn} > \text{Ti} > \text{Sr} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Cr} > \text{Pb} > \text{Ni} > \text{As} > \text{Ag} > \text{Cd} > \text{Se}$  in seawater particulates,  $\text{Sr} > \text{Fe} > \text{Al} > \text{Zn} > \text{Sn} > \text{Ni} > \text{Pb} > \text{Mn} > \text{As} > \text{Se} > \text{Ti} > \text{Cu} > \text{Ag} > \text{Cr} > \text{Cd}$  in brittlestar disk tissue and  $\text{Sr} > \text{Fe} > \text{Zn} > \text{Al} > \text{Sn} > \text{Mn} > \text{Pb} > \text{As} > \text{Cu} > \text{Ni} > \text{Ag} > \text{Ti} > \text{Se} > \text{Cd} > \text{Cr}$  in brittlestar arm tissue. In order to optimize data presentation, only major trends among the metals Al, Cd, Cu, Fe Mn, and Zn are presented in this study. These metals were selected because they have known toxic effects on marine organisms and they represent the full range of metal concentrations in association with sediment seafloor in the Bay. Results for the complete dataset of 15 metals are available elsewhere (Deheyn and Latz, 2005).

#### 3.1. Environmental parameters

Both bottom and surface seawater temperatures increased from sites A to D, being sometimes similar between sites C and D, and showed no particular variation pattern with week (Fig. 2). Bottom and surface temperatures were significantly different for sites A and B only ( $P \leq 0.0007$ ), with higher surface temperatures at those sites. Surface seawater salinity was similar among sites and weeks, with a median value of 35 PSU (range 34–36.7 PSU) for all sites (Fig. 2). Secchi depth was significantly different and greater at site A compared to the other sites ( $P \leq 0.0001$ ) indicating greater clarity of the water column at the mouth of the Bay (Fig. 2). For all sites there was a significant increase of Secchi depth with time of the experiment ( $P \leq 0.0001$ ), except for a significant decrease at week 2 for sites A and D ( $P \leq 0.004$ ), and at week 6 for site A ( $P \leq 0.0053$ ) (Fig. 2). Tidal range for the entire Bay peaked at weeks 2 and 6, indicating greater tidal currents and water exchange between the Bay and Ocean at that time. See Deheyn and Latz (2005) for additional

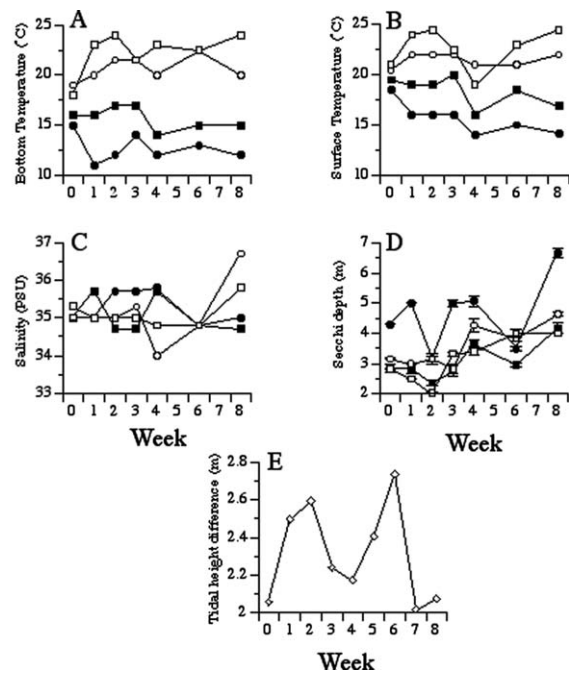


Fig. 2. Environmental parameters over the course of the experiment (June 14–August 9, 2001). (A) Bottom seawater temperature; (B) seawater surface temperature; (C) sub-surface salinity; (D) Secchi depth as a measure of water column clarity; (E) greatest difference in tidal height for the week prior the sampling time measuring outside-inside Bay water exchange for all sites. Symbols for sites: (A–D) ( $\diamond$ ); A ( $\bullet$ ), B ( $\blacksquare$ ), C ( $\circ$ ) and D ( $\square$ ).

information on time of collection and environmental conditions.

### 3.2. Metal partitioning in the environment

Metals ranked by decreasing median concentration value followed the same order in samples of sediment and seawater particulates, being  $\text{Fe} > \text{Al} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Cd}$  (Fig. 3). Metal concentrations associated with sediment ranged from (minimum–maximum values, in  $\text{mg g}^{-1}$ ): 5.7–38.1 for Fe; 4.2–29.7 for Al; 0.055–0.62 for Mn; 0.015–1.24 for Zn; 0.0026–0.155 for Cu; and 0.00017–0.00123 for Cd. Overall, concentrations of leachable metals were about 20 times greater in sediment samples than in seawater particulates (based on median values of the sediment/particulates ratio of concentration for the same element), with the ratio ranging from 11.6 (Cd) to 36.6 (Fe).

Concentrations of leachable metals associated with sediment were lower at sites A and B, and higher at sites

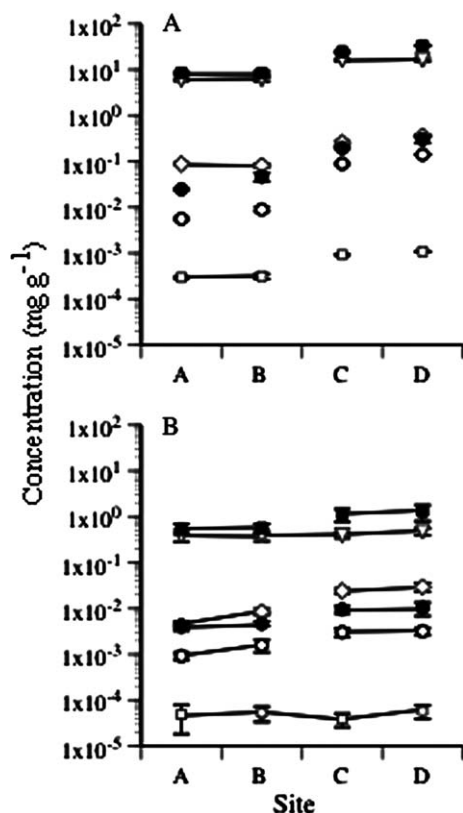


Fig. 3. Concentrations (mean  $\pm$  SE) of metals (A) associated with sediment and (B) in seawater particulates, for each site. Because of limited variation of concentrations among weeks, presented data included all weeks together ( $N = 21$ ). Al ( $\nabla$ ); Cd ( $\square$ ); Cu ( $\circ$ ); Fe ( $\blacksquare$ ); Mn ( $\diamond$ ); Zn ( $\bullet$ ). For each metal symbols linked by a line represent sites with concentrations that are not significantly different ( $\alpha > 0.05$ ).

C and D. Generally, metal concentrations were not statistically different between sites A and B ( $P \geq 0.3842$ ), while they were significantly different ( $P \leq 0.0001$ ) and gradually higher at sites C, and D (Fig. 3). Exceptions to this pattern were Al, whose concentrations were not significantly different between sites C and D ( $P = 0.6754$ ), and Cu and Zn with significant differences of concentrations among all sites ( $P \leq 0.0213$ ) (Fig. 3). Variability of metal concentrations among samples was mostly due to the site factor, which explained 89.3% of the observed variation, with values ranging from 69.7% for Mn to 97.2% for Cu (Table 2). Metal concentration also changed significantly from week to week (except for Zn), yet time variation explained only 1.3% of the overall observed variability among samples, ranging from 0.2% for Cu to 4.8% for Mn. The interaction factor between site and week was also significant (except for Al and Zn), accounting for 5.6% of the observed variation, ranging from 2.2% for Cu to 22.6% for Mn.

Metal concentrations in seawater particulates were similar among all sites for Al and Cd ( $P \geq 0.4916$ ), and for Cu, Fe, Mn, and Zn, similar between sites A and B ( $P \geq 0.066$ ), and between sites C and D ( $P \geq 0.1958$ ), the later being significantly different and higher than the former ( $P \leq 0.0156$ ) (Fig. 3). The factor site was significant and explained 13.6% of the observed variation of metal concentrations among samples based on the median value for all metals, ranging from 5.4% for Fe to 33.3% for Mn (Table 2). The factor week was significant for all metals and explained 30.9% of the observed variation, ranging from 10.6% for Al to 46.7% for Cd. With the exception of Cd the interaction factor between site and week was significant, with a median value of 26.1% among metals, indicating that metal concentrations of particulates in the water column changed over time and within a particular site.

### 3.3. Metals associated with transplanted sediment

The dialysis tubing with transplanted sediment was placed in the mid-water column where they became covered by epifaunal organisms and at the bottom seafloor where they did not show any sign of epifaunal growth. The epifaunal coverage was much greater on the tubing placed at site D than on those at site B.

Overall, concentrations of metals associated with bottom-transplanted sediment were significantly different ( $P \leq 0.0470$ ) and higher after the 2-week transplant, indicative of adsorption of dissolved elements from bottom seawater (Fig. 4). The increase in metal concentrations was similar among sites, but always lower at site D for all metals, and also at site C for Al. In contrast, for mid-water transplanted samples there was no significant difference of metal concentrations after transplant ( $P > 0.0982$ ), the exceptions being Al ( $P = 0.0115$ ) and Cu ( $P = 0.0458$ ) with higher concentrations after the

Table 2  
Factors influencing metal concentration associated with sediment and in seawater particulates from the San Diego Bay

Metal	Factor	DF	Sample			
			Sediment		Seawater particulates	
			%	<i>P</i> -value	%	<i>P</i> -value
Al	Site	3	72.8	<.0001	0.5	0.8899
	Week	6	4.0	<b>0.0342</b>	10.6	<b>0.0424</b>
	Site * Week	18	8.0	0.0814	46.6	<b>0.0002</b>
	Residual	56	15.2		42.3	
Cd	Site	3	89.1	<.0001	1.7	0.4916
	Week	6	1.3	<b>0.0176</b>	46.7	<.0001
	Site * Week	18	5.4	<.0001	11.9	0.5452
	Residual	56	4.2		39.7	
Cu	Site	3	97.2	<.0001	17.4	<.0001
	Week	6	0.2	<.0001	31.4	<.0001
	Site * Week	18	2.2	<.0001	26.7	<b>0.0002</b>
	Residual	56	0.4		24.5	
Fe	Site	3	89.4	<.0001	5.4	<b>0.001</b>
	Week	6	1.2	<b>0.0108</b>	45.4	<.0001
	Site * Week	18	5.7	<.0001	32.8	<.0001
	Residual	56	3.7		16.3	
Mn	Site	3	69.7	<.0001	33.3	<.0001
	Week	6	4.8	<.0001	16.7	<.0001
	Site * Week	18	22.6	<.0001	16.7	<b>0.0481</b>
	Residual	56	2.9		33.3	
Zn	Site	3	90.4	<.0001	9.7	<b>0.0029</b>
	Week	6	0.8	0.4863	30.3	<.0001
	Site * Week	18	2.9	0.1572	25.5	<b>0.0094</b>
	Residual	56	5.9		34.6	

For each metal, the considered factors include the site (Site), and the time of collection (Week), and the interaction between those two factors (Site \* Week). Other sources of variation were included in the factor residual. The degree of freedom (DF), the percentage of variation due to a given factor (%), and the associated statistical significance (*P*-value, except for the residual factor) are shown. *P*-values in bold represent statistical significance ( $\alpha < 0.05$ ).

2-week transplant for all sites. Other exceptions with significantly different and higher concentrations, but only at specific sites, were Cd ( $P = 0.0175$ ) at site A, and Mn ( $P = 0.0213$ ) at site D (Fig. 4).

Metal concentrations of sediment transplant negative controls in aquarium conditions (sediment in tubing in seawater) were similar at the end of the experimental period ( $P \geq 0.6288$ ), except for Zn that was significantly different ( $P \leq 0.0001$ ) and lower (Fig. 4). For the sediment transplant positive control in aquarium (sediment in tubing in Cu-contaminated seawater), concentrations of Cu increased during the time of the experiment but the difference was not significant from initial concentrations ( $P = 0.6901$ ) (Fig. 4).

#### 3.4. Metals in transplanted brittlestars

Transplanted brittlestars were recovered alive and with no sign of arm autotomy, except for missing indi-

viduals for week 8 at sites A and D. Overall, brittlestars accumulated metals in disk and arm tissue when transplanted in the Bay, and not when kept under control aquarium conditions. Indeed, metal concentrations in disk and arm tissue of Bay transplanted individuals were three to four orders of magnitude greater than those in tissues of aquarium controls (Fig. 5). Some metals showed a less extensive tissue accumulation, including Cd, with concentrations 3–6 times greater than in aquarium for both disk and arm tissue; Cu, with concentrations 1–10 times greater than in aquarium for arm tissue; and Fe, with concentrations 15–40 times greater than in aquarium for arm tissue (Fig. 5).

Metal accumulation was significantly different ( $P \leq 0.05$ ) and greater in disk than arm tissue for all metals. Based on median concentration ratio, the metal concentration was 5.8 times greater in disk than arm tissue for Al, 1.3 times for Cd, 2.9 times for Cu, 5.1 times for Fe, 1.7 times for Mn, and 1.3 times for Zn. The order



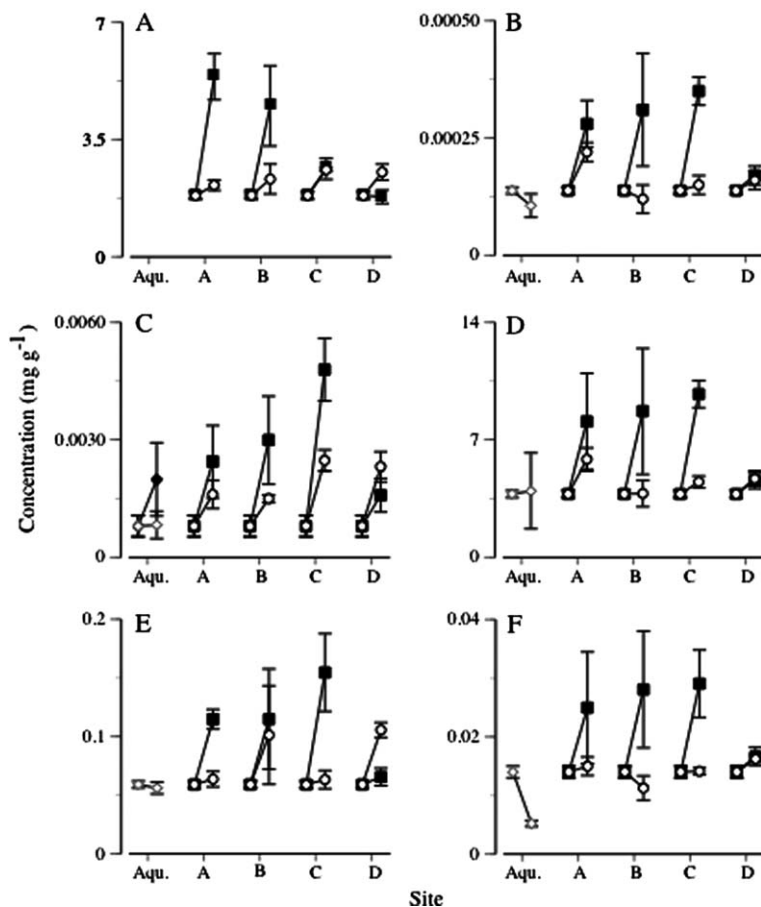


Fig. 4. Metal concentrations (mean  $\pm$  SE) in transplanted sediment in dialysis tubing from time 6 to 8 weeks. Sediment was kept in aquarium condition as a negative control (Aqu., open diamond) or transplanted at the seafloor bottom (filled square) or in the mid-water column (open circles) at sites A, B, C and D, and analyzed for (A) Al; (B) Cd; (C) Cu; (D) Fe; (E) Mn; and (F) Zn. For the aquarium positive control (C), the filled diamond represents exposure to  $10^{-7}$  M Cu. No data are available for Al in aquarium controls.

of metals ranked by decreasing median concentration value was Fe > Al > Zn > Mn > Cu > Cd for disk tissue, and Fe > Zn > Al > Mn > Cu > Cd for arm tissue.

Accumulation in disk and arm tissue usually occurred within the first week for all metals, yet the accumulation did not gradually increase with week because no cumulative pattern was observed. Instead, concentrations varied from 1 week to another for the same site, a pattern not observed for aquarium controls (Fig. 5). The effect of the factor week on accumulation was significant for every metal ( $P \leq 0.0071$ ), and variation in metal concentrations due to the factor week ranged from 16.7% to 33.2% in disk tissue, and 19.3% to 47.3% in arm tissue; Cd in disk tissue was the only metal for which the factor week was not significant ( $P = 0.0922$ ) (Table 3).

In general for disk tissue of transplanted brittlestars, metal concentrations were higher at weeks 2, 3 and 6, with the exception of Mn that was higher at week 1.

However, the ranking of the sites by increasing metal concentration differed with week. For example, at week 3, concentrations in disk tissue followed the ranking  $C > D > A > B$  for Al;  $A > B > C > D$  for Cd;  $D > A > C > B$  for Cu;  $C > A > D > B$  for Fe;  $D > C > A > B$  for Mn; and  $A > B > D > C$  for Zn. The observed differences were not repeated for other weeks. At week 6, concentrations in disk tissue followed the ranking  $B > A > D > C$  for Al;  $A > C > D > B$  for Cd;  $B > C > A > D$  for Cu;  $B > C > D > A$  for Fe;  $B > D > A > C$  for Mn; and  $A > C > D > B$  for Zn. The site difference, however, was not significant for most weeks and metals. Accordingly, the factor site did not significantly affect metal accumulation in disk tissue ( $P \geq 0.0526$ ), the extent of this factor ranging from 3.6% to 8.8%. The only exceptions were Cu and Mn, with a significant factor site ranging from 12.0% to 16.7% ( $P \leq 0.0008$ ) (Table 3). Interaction between the

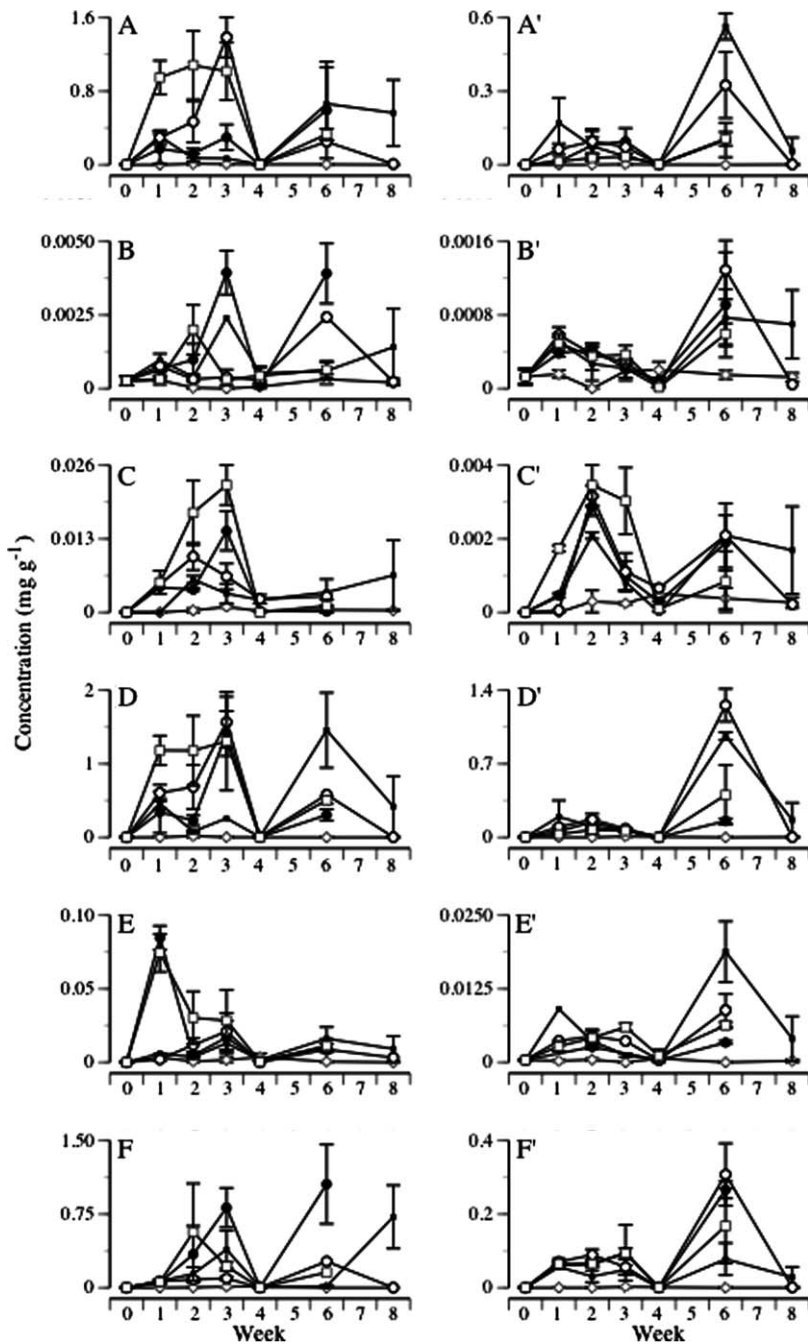


Fig. 5. *Ophiothrix spiculata*. Variation with week of metal concentration (mean  $\pm$  SE) in (A–F) disk and (A'–F') arm tissue of brittlestars in aquarium controls ( $\diamond$ ) and field transplanted at sites A ( $\bullet$ ), B ( $\blacksquare$ ), C ( $\circ$ ), and D ( $\square$ ). Metal analysis for (A–A') Al; (B–B') Cd; (C–C') Cu; (D–D') Fe; (E–E') Mn; and (F–F') Zn.

factor site and week was significant only when both individual factors were significant as well, such as for Cu and Mn, explaining 33.3% to 52.0% of the variation in metal concentration in disk tissue (Table 3).

The pattern of accumulation was different for arm tissue, with metal concentrations usually higher at week

6, with the exception of Cu that also had higher concentrations at weeks 2 and 3 (Fig. 5). The week factor was significant for all metals, ranging from 19.3% to 47.3%, while the site factor had no significant effect on metal concentration in arm tissue ( $P \geq 0.1447$ ), ranging from 1.8% to 5.3%, except for Cu that was significant with

Table 3  
Factors influencing metal concentration in disk and arm tissue of brittlestars transplanted in the San Diego Bay

Metal	Factor	DF	Sample			
			Disk tissue		Arm tissue	
			%	P-value	%	P-value
Al	Site	3	8.3	0.0683	5.3	0.1999
	Week	5	22.3	<b>0.0037</b>	32.2	<b>0.0002</b>
	Site * Week	15	18.1	0.3746	13.2	0.6294
	Residual	47	51.3		49.3	
Cd	Site	3	9.4	0.0526	1.8	0.5609
	Week	5	11.4	0.0922	47.3	<.0001
	Site * Week	15	26.1	0.1301	9.4	0.7604
	Residual	47	53.1		41.4	
Cu	Site	3	12.0	<.0001	10.3	<.0001
	Week	5	20.0	<.0001	19.3	<.0001
	Site * Week	15	52.0	<.0001	53.7	<.0001
	Residual	47	16.0		16.7	
Fe	Site	3	3.6	0.2578	2.7	0.4210
	Week	5	33.2	<.0001	44.6	<.0001
	Site * Week	15	23.0	0.0651	9.5	0.7771
	Residual	47	40.2		43.2	
Mn	Site	3	16.7	<b>0.0008</b>	4.9	0.1447
	Week	5	16.7	<b>0.0011</b>	38.0	<.0001
	Site * Week	15	33.3	<b>0.0071</b>	16.6	0.2511
	Residual	47	33.3		40.5	
Zn	Site	3	8.8	0.0591	2.8	0.5077
	Week	5	20.6	<b>0.0065</b>	34.9	<b>0.0003</b>
	Site * Week	15	18.4	0.3778	6.4	0.9795
	Residual	47	52.2		56.0	

Week 8 was not included because of missing samples. See Table 2 for details of the analysis.

10.3% of explained variation ( $P \leq 0.0001$ ). The interaction between the factors site and week was significant only for Cu, explaining 53.7% of the variation in metal concentration in arm tissue (Table 3). Metals that were found in higher concentration in arm tissue were not necessarily found in higher concentration in disk tissue. Accordingly, tissue metal concentrations were significantly correlated between disk and arm tissue only for Cu (Spearman ranked correlation  $\rho = 0.509$ ;  $P = 0.0053$ ), Fe ( $\rho = 0.400$ ;  $P = 0.0047$ ), and Mn ( $\rho = 0.343$ ;  $P = 0.0427$ ). For those elements a positive correlation coefficient  $\rho$  indicated an increase of metal in the disk tissue was associated with a proportional increase in the arm tissue.

### 3.5. Metal bioconcentration factors

Metal bioconcentration factor, the ratio of median concentration values of brittlestar tissue to median concentration values associated with sediment at that site, was highest for Cd and Zn, followed by Cu and Mn, and Fe and Al, for both disk and arm tissue. The fac-

tors, however, were always 2–5 times lower for arm tissue, except for Cd and Zn that showed similar values

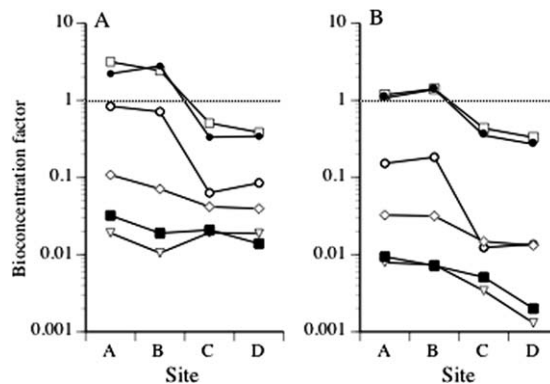


Fig. 6. *Ophiothrix spiculata*. Bioconcentration factor for each metal at each site. The factor is the ratio of median brittlestar metal concentration to median sediment leachable metal concentration for (A) disk tissue and (B) arm tissue of transplanted brittlestars. Al ( $\nabla$ ); Cd ( $\square$ ); Cu ( $\circ$ ); Fe ( $\blacksquare$ ); Mn ( $\diamond$ ); Zn ( $\bullet$ ).

between disk and arm tissue at sites C and D (Fig. 6). Bioconcentration factors for aquarium controls were 10–1000 times lower than those found for field transplanted brittlestars, except for Cd in disk and arm tissue and Cu in arm tissue for which the factor was similar to the ones found at sites C and D (data not shown).

The bioconcentration factors were usually <1, except for sites A and B for Cd and Zn in disk tissue (up to 3.2) and arm tissue (up to 1.3). They were approximately 1 (0.8) for Cu in disk tissue at sites A and B, and otherwise <0.2, being lowest for Al and Fe with values around 0.02 in disk tissue, and ranging from 0.0015 to 0.008 in arm tissue (Fig. 6).

Metal bioconcentration factors were generally greater for sites A and B than for sites C and D. Indeed, the factors at sites A and B were 2–10 times greater than those at sites C and D in disk tissue, and 3–12 times greater in the case of arm tissue; the exception came from Al in disk tissue that showed no particular variation among sites (Fig. 6).

### 3.6. Correlation with environmental parameters

The only significant correlation between metal concentration in brittlestars and environmental parameters in the Bay was with the tidal range ( $P \leq 0.05$ ). Overall, there was a positive correlation between metal concentration in brittlestar tissue and tidal mixing; incidentally, weeks of low tidal mixing, such as weeks 0, 4, and 8, coincided with lower metal concentrations in brittlestar tissue (compare Fig. 2E and Fig. 5). The correlations were greater for arm than disk tissue, and were different across sites, being greater for sites B and C (Table 4). Median values of  $R^2$  for the sites were:  $A = 0.10$ ,  $B = 0.42$ ,  $C = 0.50$ ,  $D = 0.15$  for disk tissue, and  $A = 0.46$ ,  $B = 0.55$ ,  $C = 0.67$ ,  $D = 0.50$  for arm tissue. The correlation was different with the metal considered and was the greatest (based on median  $R^2$  values) for Fe (0.44) and Mn (0.40) for disk tissue, and Fe (0.65) and Mn (0.64) for arm tissue. In general, metal concentrations in brittlestar tissue increased with tidal range

Table 4  
Model parameters for the relationship between tissue metal concentration and tidal range

Metal	Site	Model parameters							
		Disk				Arm			
		<i>N</i>	<i>a</i>	<i>b</i>	$R^2$	<i>N</i>	<i>a</i>	<i>b</i>	$R^2$
Al	A	3	NA	NA	NA	5	$7.45 \times 10^{-9}$	5.94	0.42
	B	6	$1.63 \times 10^{-8}$	6.23	<b>0.66</b>	6	$4.26 \times 10^{-7}$	4.75	0.41
	C	6	$5.69 \times 10^{-7}$	5.16	0.36	6	$2.04 \times 10^{-10}$	7.71	<b>0.69</b>
	D	5	$6.59 \times 10^{-10}$	7.82	0.39	5	$3.39 \times 10^{-7}$	4.4	<b>0.59</b>
Cd	A	5	$1.97 \times 10^{-5}$	1.41	0.06	5	$1.06 \times 10^{-6}$	2.26	<b>0.66</b>
	B	6	$1.17 \times 10^{-4}$	0.65	0.04	6	$2.23 \times 10^{-6}$	1.99	0.13
	C	6	$8.41 \times 10^{-7}$	2.69	<b>0.67</b>	6	$1.51 \times 10^{-9}$	5.00	<b>0.82</b>
	D	5	$1.94 \times 10^{-4}$	0.28	0.14	5	$1.28 \times 10^{-9}$	4.90	0.58
Cu	A	5	$7.53 \times 10^{-2}$	-1.59	0.04	5	$4.23 \times 10^{-6}$	2.20	0.33
	B	5	$1.37 \times 10^{-2}$	-0.57	0.08	5	$5.50 \times 10^{-4}$	0.33	0.02
	C	4	$1.32 \times 10^{-3}$	0.53	0.04	6	$3.81 \times 10^{-6}$	2.14	0.14
	D	3	NA	NA	NA	4	$4.09 \times 10^{-9}$	4.89	0.57
Fe	A	5	$1.53 \times 10^{-5}$	3.77	0.14	5	$9.14 \times 10^{-10}$	7.15	<b>0.65</b>
	B	6	$2.31 \times 10^{-9}$	7.30	<b>0.61</b>	6	$2.77 \times 10^{-11}$	8.67	<b>0.64</b>
	C	6	$4.84 \times 10^{-9}$	7.22	0.50	6	$1.80 \times 10^{-11}$	8.88	<b>0.72</b>
	D	5	$9.37 \times 10^{-10}$	7.67	0.37	5	$1.13 \times 10^{-11}$	8.66	0.49
Mn	A	5	$4.58 \times 10^{-7}$	3.89	0.36	3	NA	NA	NA
	B	6	$6.23 \times 10^{-6}$	2.73	0.43	6	$8.77 \times 10^{-10}$	6.16	<b>0.90</b>
	C	6	$9.79 \times 10^{-10}$	5.99	0.18	5	$1.63 \times 10^{-7}$	3.99	0.64
	D	5	$3.92 \times 10^{-8}$	5.07	0.43	5	$5.64 \times 10^{-5}$	1.70	0.33
Zn	A	5	$2.30 \times 10^{-9}$	7.01	0.32	5	$1.02 \times 10^{-8}$	5.86	0.49
	B	6	$3.48 \times 10^1$	-2.60	0.13	6	$2.80 \times 10^{-10}$	7.30	<b>0.58</b>
	C	6	$1.43 \times 10^{-11}$	8.77	<b>0.68</b>	6	$2.56 \times 10^{-11}$	8.55	<b>0.74</b>
	D	5	$2.98 \times 10^{-8}$	5.76	0.34	5	$4.24 \times 10^{-8}$	5.46	0.35

The relationship is described by the exponential model “ $y = a * \exp(b * x)$ ”.  $R^2$  is the coefficient of determination, in bold for  $P \leq 0.05$ , and *N* is the number of samples used to calculate the regression. NA: not available (when  $N = 3$ ).

(positive correlation), except for Cu and Zn for disk tissue at sites A and/or B (see negative “*b*” values in Table 4). Considering only the positive correlations, the “*b*” values increased gradually with sites for disk tissue (median “*b*” values for site *A* = 2.39, *B* = 2.73, *C* = 4.28, *D* = 5.07) while they were lower for sites A and C for arm tissue (median “*b*” values for site *A* = 3.00, *B* = 4.49, *C* = 3.99, *D* = 4.59); overall, the “*b*” values at sites A and B were greater for arm than disk tissues, while the contrary was observed for sites C and D. The “*b*” values were also different with the metal considered, the greatest being (median “*b*” values) Fe (7.26) for disk tissue, and Fe (8.67) for arm tissue, and the lowest for Cd with values of 1.03 for disk tissue, and 3.6 for arm tissue (Table 4).

#### 4. Discussion

This study showed that metal concentrations associated with seafloor sediment increased from the mouth to the back of San Diego Bay. Unexpectedly, however, brittlestars transplanted in the Bay accumulated metals in similar levels throughout the Bay, indicating that metal bioavailability was greater at the mouth compared to the back of the Bay. Based on the increase in metal concentrations in sediment transplanted in dialysis tubing, metals were mainly available closer to the seafloor bottom than in the mid-water column. Coupled with the circulation pattern of the Bay, this suggests that water movement in the Bay, especially along the bottom, plays a key role in the transport, fate, and bioavailability of metals.

##### 4.1. Metal concentration in seafloor sediment is related to seawater residence time in the Bay

San Diego Bay is known to be a highly contaminated place where metals are found in much greater concentrations than the local background (Katz and Kaplan, 1981; Anderson et al., 1996; Fairey et al., 1998). Metals originated from past industrial activities (mostly fishing and canning industries) and raw sewage discharge, which took place until 1963 in the Bay, with most of the metals probably still trapped today in the seafloor sediment. Sources of metals also include storm water runoff and current anthropogenic activities related to shipyard industry and to extensive use of the Bay as a protected navigational area. The Bay harbors many ships, the largest component being military, and many recreational boats, launched on a daily basis or densely packed in marinas around the Bay (Federal Water Pollution Control Administration, 1969; Young et al., 1979; Schiff et al., 2003, 2004). All these boats represent potential sources of metals, with copper, originating from

antifouling ship paint, being by far the metal that has drawn the most interest (Zirino et al., 1978, 1998; Blake et al., 2004; Chadwick et al., 2004).

Contamination in the Bay is often considered originating from non-point sources because of the multiple and chronic nature of individual sources spread around and in the Bay (i.e. boats, marinas, and shipyards), but also because of the complex water circulation of the Bay waters sustained mainly by tidal forces, as well as by vertical mixing due to wind and/or day/night temperature difference of water masses, especially in the shallower parts of the back of the Bay (Chadwick et al., 1994; Esser and Volpe, 2002; Volpe and Esser, 2002). Based on a model for copper, metals would most likely reach the Bay water in dissolved form before being transformed into the particulate form that will sink into the seafloor sediment (Zirino et al., 1978; Chadwick et al., 2004). Because the Bay has only one opening to the Pacific Ocean, the residence time of the water mass in the Bay is driven by the extent of the tidal flushing, and ranges from 1 d at the mouth to 27 d at the back of the Bay (Chadwick and Largier, 1999a,b; Chadwick et al., 2004). Thus, according to this scenario, deposition of metal enriched particulate material would be greater on the seafloor sediment at the back compared to the mouth of the Bay, resulting in higher metal concentrations associated with sediment at the back of the Bay (Chadwick et al., 2004).

This study supports the above model because all the studied metals increased in concentration in sediment from the mouth to the back of the Bay, even though metal concentrations in seawater particulates were similar throughout the Bay, mainly showing variability among weeks. This study showed for most metals similar concentrations in sediment between sites A and B, and between sites C and D, which is consistent with the seawater residence time for each site, being closer between sites A and B, 1 d and 7 d, respectively, and closer between sites C and D, 17 d and 23 d, respectively (Chadwick et al., 2004). Turbidity was lower at site A compared to the other sites, thus implying, if considering only the model that metals associated with seafloor sediment originate from sinking seawater particulates, that metal concentrations associated with sediment would be different between sites A and B, which was not observed. Yet, sites A and B have similar sediment characteristics, which could play a role in their similar metal content. Sites A and B have medium/coarse grain size sediment (sand) and lower organic content while sites C and D have fine grain size sediment (mixture of sand, silt and clay) and higher organic content (Newman, 1958; Anderson et al., 1996). Metal bioavailability relies, among other biological and environmental factors, on the adsorption process that binds the metal to its support matrix. The strength of this adsorption bond

depends on the composition of the matrix (inorganic/organic), chemical speciation of the metal, and surface area per unit mass of the matrix, all of which varies with grain size. In general, finer sediment particles exhibit greater binding site density, and thus lower bioavailability (Luoma, 1990). Therefore, the difference in sediment properties in San Diego Bay supports lower metal concentrations associated with sediment closer to the Bay mouth and greater concentrations towards the Bay back, indicating that additional factors other than the water residence time may also affect metal concentrations in the Bay seafloor sediment.

#### 4.2. Bottom seawater shows greater contamination because of Bay-Ocean circulation

The water column at sites A and B is stratified while it is more homogeneous at sites C and D (Zirino et al., 1978; Chadwick and Largier, 1999a). At sites C and D evaporation during the day and cooling during the night produce denser and cooler seawater that progressively flows towards the Bay mouth along the seafloor bottom (Zirino et al., 1978; Chadwick et al., 1994). The bottom seawater layer at sites A and B therefore consists of water coming from the Bay back, with a long residence time in the Bay and thus a long time of exposure to metal contamination, therefore having a greater load of metals (Fig. 7). Accordingly, dissolved Cu and Zn are found in greater concentrations near the seafloor bottom at the mouth of the Bay (Zirino et al., 1978).

This study showed that metal concentrations increased in sediment samples when transplanted at the seafloor bottom of the Bay, but not in the mid-water column, consistent with the occurrence of a bottom seawater

layer throughout the Bay with higher concentrations of dissolved metals. This layer would be minimal at site D, where samples showed similar behavior whether bottom or mid-water transplanted, and well established at sites A, B and C, where the water column appears to be stratified with surface ocean water of short residence time in the Bay, and metal enriched bottom water from the back of the Bay. Therefore, for dissolved metals in seawater there is a gradient of contamination from the back to the mouth of the Bay driven by the Ocean-Bay water circulation, opposite to the gradient for the concentration of metals associated with sediment. Lower contamination in the tubing placed in the mid-water column (whether at the mouth or the back of the Bay) supports previous observations that the predominant forms of metals in surface water of San Diego Bay are metal complexes and aggregates formed with organic ligands mainly produced by microalgae and microbes; the complexing capacity then increases from the mouth to the back of the Bay, thus emphasizing the essential role of physical processes in metal speciation and distribution in the Bay (Zirino et al., 1998; Blake et al., 2004; Rivera-Duarte and Zirino, 2004; Shafer et al., 2004).

#### 4.3. Metal bioavailability is increased at the mouth of the Bay

This study used brittlestars transplanted in the field to assess bioavailability of metals in San Diego Bay. Brittlestars can become contaminated with metals through direct exposure to dissolved metals in seawater (Gounin et al., 1995; Hutchins et al., 1996), but also, as in other Echinoderms, through ingestion and digestion of metal contaminated food items. In the latter case, contaminants can be actively taken up from the food by the digestive tissue before being distributed to other tissues for detoxification, excretion, and/or storage (Warnau et al., 1995, 1996). In this study, brittlestars accumulated metals in similar levels among sites in the Bay, thus indicating uptake of metals from ingested sediment material and/or seawater in similar extent throughout the Bay. It was shown that the further back in the Bay the greater the metal concentrations associated with sediment and the lower the metal concentrations dissolved in seawater; thus towards the back of the Bay the predominant route of contamination for brittlestars would be through the diet. Alternatively, towards the mouth of the Bay the bottom seawater layer would constitute a significant source of metals other than the metals acquired from ingested material, because concentrations of metals associated with sediment are low and dissolved metal concentrations are high in the metal enriched bottom seawater layer. Such combined sources of metals support the results that brittlestars showed similar levels of contamination throughout the

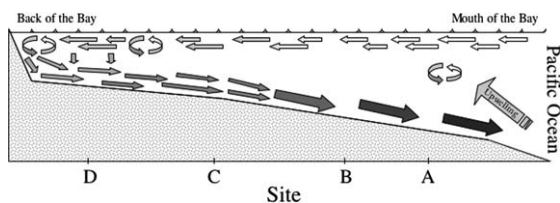


Fig. 7. Schematic representation of water movement and metal enrichment process in San Diego Bay. Surface and/or upwelled water enter the Bay by tidal movement. The upper layer of water gets progressively more contaminated from surface input as it is moves further into the Bay. Following evaporation and cooling at the back of the Bay, the surface water becomes denser, sinks, and drains on the seafloor bottom towards the opening of the Bay. During its seaward movement, the bottom layer of water becomes enriched with various contaminants, including dissolved elements, thus being more concentrated with metals at the mouth than the back of the Bay. Increasing shades of grey represent increasing concentrations of dissolved contaminants in seawater.

Bay, despite an increasing gradient of metals associated with sediment from the mouth to the back of the Bay.

#### 4.4. Dissolved metals as primary source of contamination for brittlestars in San Diego Bay

In this study, metal concentration in brittlestars changed with week. Temporal changes in tissue concentrations most likely reflect the efficient accumulation of dissolved metals (Gounin et al., 1995; D'Andrea et al., 1996; Hutchins et al., 1996) although variation in other physiological processes such as excretion cannot be ruled out. Metal concentrations in brittlestars were low when tidal range, used as a proxy for Bay-ocean mixing, was low, and vice versa, suggesting that local physico-chemical characteristics affecting metal bioavailability change with water mixing. Specifically, the organic complexing capacity driving metal speciation and bioavailability in the Bay (Zirino et al., 1998; Blake et al., 2004; Rivera-Duarte and Zirino, 2004; Shafer et al., 2004) can be locally decreased by dilution due to mixing with water of different physico-chemical characteristics and lower complexing capacity. The correlation between metal concentrations in brittlestars and tidal range was greater for arm than disk tissue, indicating that metal contamination in arm tissue reflects better than disk tissue the variation of dissolved bioavailable metals in seawater. The relationship was weak in the case of Cu, especially for site B which was considered under immediate Cu exposure due to its proximity to the Shelter Island marina, known to be a point-source for dissolved Cu and other metals (Zirino et al., 1998; Salazar and Salazar, 2003; Blake et al., 2004; Chadwick et al., 2004; Shafer et al., 2004; Ignacio et al., 2005; Rosen et al., 2005). Increased Cu and Mn concentrations in mid-water transplanted sediment also provided evidence that Shelter Island is a significant source of dissolved metals in the water column. Accordingly, the Cu, Cd, and Zn bioconcentration factors in brittlestar arms were the greatest at site B compared to other sites.

In general, metals were accumulated in greater concentrations in disk than arm tissue, suggesting a contamination process through the diet, with ingestion of metal contaminated sediment and/or particulates. However, the elevated concentrations in disk tissue could also result from direct contamination through dissolved metals in seawater because the digestive tissue can uptake and accumulate metals no matter the form of exposure (Warnau et al., 1995, 1996, 1997). The weekly pattern in metal concentrations in disk, which was similar to the pattern for that in arm tissue, suggested a similar contamination pathway, involving exposure to dissolved metals. Nevertheless, in both disk and arm tissue metals accumulate in the soft tissue from where they can be spread to the entire organisms via the circulatory system and be either excreted or included in the skeleton (ossi-

cles) where they can remain trapped as a route of detoxification.

#### 4.5. Possible role of upwelling in metal supply at the mouth of the Bay

Near-shore upwelling is common along Southern California and can represent a significant source of dissolved metals for marine organisms (Lares et al., 2002). With respect to this study, upwelling has been identified just off the coast from San Diego Bay (Segovia-Zavala et al., 1998; Pringle and Riser, 2003) and at the mouth of the bay (Roughan et al., 2005), increasing the supply of dissolved and particulate metals, and also affecting seawater physico-chemical properties, and thus metal bioavailability, particularly at the mouth of the Bay.

This study showed that bioconcentration factors for Cd and Zn in disk and arm tissue were above 1 at the mouth of the Bay, indicating an increased bioavailability and/or supply for those metals. This was not observed for the back of the bay where Cd and Zn bioconcentration factors were lower than 1 despite greater metal concentrations associated with sediment, and were similar between disk and arm tissue, indicating that most of the metals was accumulated from the dissolved form in seawater rather than the diet. These results may indicate a near-shore upwelling source of metals for the Bay as some of the studied metals such as Al, Cd and Fe are commonly found concentrated in upwelling regimes (Lares et al., 2002).

## 5. Conclusion

Determining contamination levels, bioavailability of local contaminants to organisms, and identifying early signs of toxicity are key components of environmental quality assessment. This study demonstrates the importance of assessing the bioavailability of contaminants, because the total concentration of contaminants in the environment does not reflect the fractions of those contaminants that are bioavailable to the local biota; only the bioavailable fraction can be accumulated by organisms. Bioavailability is highly variable according to the physico-chemical properties of the seawater, biogeochemistry of the sediment and the metals, and biology of the organism. Due to this variability, environment quality assessment should characterize the link between contaminant levels and bioavailability, as well as the environmental factors that may affect these processes. Only bioavailable elements have the potential to be toxic, in that they can trigger deleterious effects at the individual, population, and/or ecosystem level. Environmental quality assessment needs to integrate not only assessments of chemical concentration and bioavailability, as considered in this study, but also incorporate as-

says of toxicity to assess the ultimate ecological impacts of contamination.

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