Concentration and bioavailability of metals in San Diego Bay, California

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Abstract

The goal of this study is to describe the variation of metal concentrations in the environment of San Diego Bay, California, and assess the biological availability of metals throughout the Bay considering the local environmental characteristics. The concentrations of 15 metals were measured from sediment (top layer) and seawater particulates collected during an 8 week period from June 14 to August 9, 2001 at four sites in San Diego Bay, located near navigation buoys R8 (site A), R16A (site B), G1 (site C) and R34 (site D). Metals were also measured from tissues of the common brittlestar, *Ophiothrix spiculata* (Ophiuroidea, Echinodermata), transplanted from outside to inside the Bay. Experiments with transplants determined the ratio of metal from the environment that is biologically available to fauna. Measuring metal concentrations from disk and arm tissue of transplanted brittlestars determined the variation with time of metal contamination from the diet and/or from the seawater. Indeed, brittlestars can accumulate metals by feeding on metal contaminated food items that will mainly target the disk tissue, while they can also accumulate metals directly from seawater in arm tissue. The report also includes, from each site, environmental parameters such as surface and bottom temperature, salinity, turbidity and tidal height.

Summary

San Diego Bay, California, 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 metal concentrations in seawater particulates were similar throughout the Bay. Metal bioavailability was assessed over 8 weeks by transplant of the local brittlestar, *Ophiothrix spiculata*, from outside to inside the Bay. 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 when placed on the seafloor bottom, to an extent similar between the mouth and the back of the Bay, with greater metal accumulation near the bottom. 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 concentrations 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.

Methodology

Seawater temperature, salinity, and Secchi depth were measured at each site (Table 1; Figure 1). Tidal height was obtained from the NOAA tide data (http://co-ops.nos.noaa.gov) at benchmark station #9410170, and tidal range was calculated as the greatest difference in water level height that occurred during the week preceding each collection time (maximum values of Mean Higher High Water – minimum value of Mean Lower Low Water).

Seawater and the top layer of seafloor sediment were collected by SCUBA diving; seawater particulates were retained on 0.45μm pore size nitrocellulose filters upon filtration of seawater. Brittlestars from outside the Bay were collected by SCUBA at the La Jolla Cove Underwater Reserve (considered a non-contaminated reference), and transplanted in the Bay in PVC cages with open sides covered with 710 μm mesh allowing passage of water and sediment material. Brittlestars were recovered from the cages at weeks 1, 2, 3, 4, 6, and 8, and returned to the laboratory where they were dissected under a compound microscope (the disk and the arms were separated from each other) and processed for metal analysis.

Sediment, seawater particulates, brittlestar disk and arm tissue were then oven dried, weighed using a high precision microscale, and digested in 7 N 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_3) 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),

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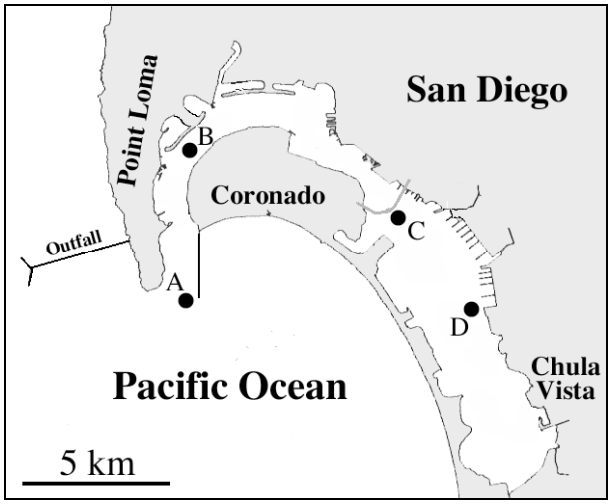

Figure 1: Location of sites of investigation in San Diego Bay, California. The sites were located near navigation buoys R8 (site A), R16A (site B), G1 (site C) and R34 (site D). Depth was similar among sites, being 16 m for site A, 15 m for B, and 11 m for both C and D.

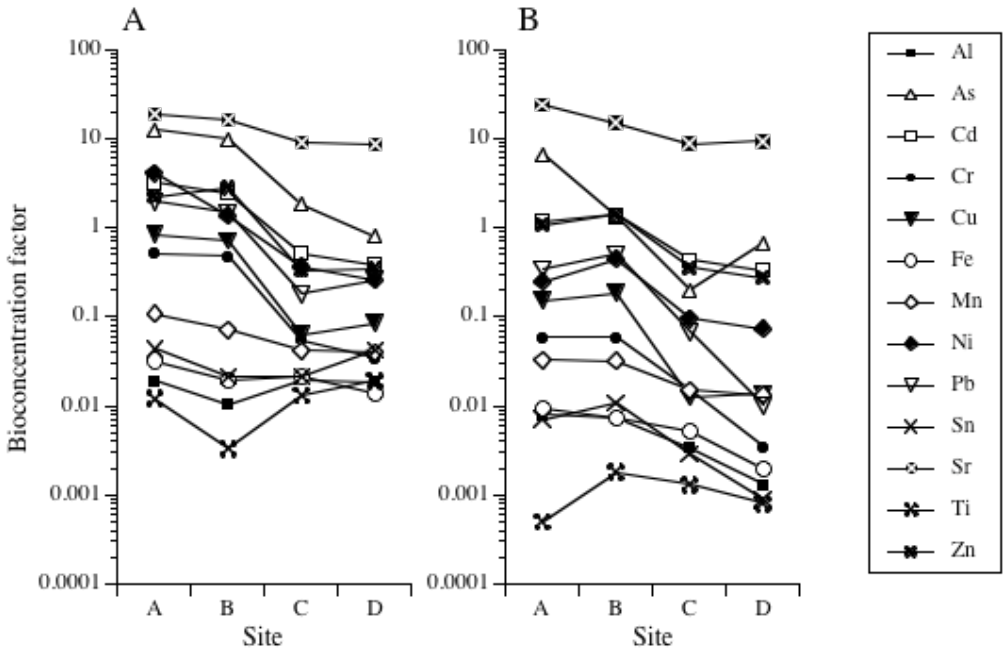

Figure 2. Bioconcentration factor (median transplanted brittlestar metal concentration / median sediment leachable metal concentration) for each metal in (A) disk and (B) arm tissue of transplanted brittlestars, *Ophiothrix spiculata*, as a function of site. Overall, the bioconcentration factor was lower for arm than disk tissue, indicating that the accumulation happens mainly through the diet, and was lower for sites C and D than sites A and B, indicating greater bioavailability at the mouth of the Bay. The bioconcentration factor was < 1, except for Sr, As, Ni, Cd, Zn, and Pb for arm and disk tissue, and Sr, As, Cd, and Zn for arm tissue.

Table 1. Environmental parameters measured at sites A to D in San Diego Bay during the 8-week field transplant experiment (June 14 – August 9, 2001). Both bottom and surface temperatures increased from site A to D. At sites C and D the bottom and surface temperatures were sometimes similar, indicative of an homogenous water column, whereas at sites A and B there was always a difference between bottom and surface temperatures, indicative of a stratified water column. The salinity was similar throughout the Bay. The Secchi depths were greater at site A than the other sites, indicating less seawater particulates at the mouth of the Bay. The tidal range was greater at weeks 1, 2, and 6, indicating enhanced Bay-Ocean water exchange. Height of water level (from http://co–ops.nos.noaa.gov) is given for the closest corresponding time of collection.

Parameter/site		**Week (date)**						
		0 (6/14/01)	1 (6/21/01)	2 (6/28/01)	3 (7/5/01)	4 (7/12/01)	6 (7/26/01)	8 (8/9/01)
T° bottom (°C)	A	15.0	11.0	12.0	14.0	12.0	13.0	12.0
	B	16.0	16.0	17.0	17.0	14.0	15.0	15.0
	C	19.0	20.0	21.5	21.5	20.0	22.5	20.0
	D	18.0	23.0	24.0	21.5	23.0	22.5	24.0
T° surface (°C)	A	18.5	16.0	16.0	16.0	14.0	15.0	14.2
	B	19.5	19.0	19.0	20.0	16.0	18.5	16.9
	C	20.5	22.0	22.0	22.0	21.0	21.0	22.0
	D	21.0	24.0	24.5	22.5	19.0	23.0	24.5
Salinity (PSU)	A	35.0	35.0	35.7	35.7	35.8	34.8	35.0
	B	35.0	35.7	34.7	34.7	35.7	34.8	34.7
	C	35.0	35.0	35.0	35.3	34.0	34.8	36.7
	D	35.3	35.0	35.0	35.0	34.8	34.8	35.8
Secchi depth (m)	A	4.3	5.0	3.2	5.0	5.1	3.5	6.7
	B	2.9	2.8	2.4	2.8	3.7	3.0	4.2
	C	3.2	3.0	3.2	2.8	4.3	3.8	4.6
	D	2.8	2.5	2.0	3.3	3.4	4.0	4.0
Collection time (h) (PST)	A	1300	1200	1040	1030	1030	1000	1030
	B	1400	1310	1140	1130	1140	1140	1115
	C	1720	1515	1340	1340	1350	1320	1345
	D	1845	1610	1440	1430	1500	1410	1430
Water level (m)	A	0.964	-0.309	1.267	-0.200	0.970	1.296	1.013
	B	0.785	-0.019	1.159	-0.170	0.752	0.910	0.766
	C	0.344	0.707	0.702	0.344	0.380	0.605	0.320
	D	0.423	0.999	0.438	0.690	0.299	0.377	0.399
Tidal range (m)		2.1	2.5	2.6	2.2	2.2	2.7	2.1

Table 2. Metal concentrations (x multiplication factor) associated with sediment and in seawater particulates over the 8-week experimental period for each site. Samples were significantly different ($\alpha<0.05$) unless underlined. No Se was detected in samples. – : below detection limit; N: number of measurements; NA: Not Applicable. Overall, concentrations of metals associated with sediment were similar between sites A and B, and sites C and D, yet greater for the latter compared to the former, while concentrations of metals in seawater particulates were usually similar among the different sites. This indicates there was an increasing gradient of metal concentrations associated with sediment from the mouth to the back of the Bay, but not in the seawater particulates. Metal concentrations associated with sediment were always 1 to 2 orders of magnitude greater than in seawater particulates.

Metal	**Value**	**Sediment**				**Seawater particulates**			
	type	Site A	Site B	Site C	Site D	Site A	Site B	Site C	Site D
Ag	median	1.20×10^{-4}	0.56×10^{-4}	–	–	0.42×10^{-4}	0.78×10^{-4}	0.72×10^{-4}	0.65×10^{-4}
	min.	1.20	0.56	–	–	0.049	0.077	0.038	0.120
	max.	1.20	0.56	–	–	1.30	2.50	2.52	2.50
	N	1	1	–	–	11	11	13	11
Al	median	5.77	5.55	16.58	15.03	0.17	0.24	0.34	0.52
	min.	4.19	3.58	7.59	6.26	0.03	0.07	0.07	0.04
	max.	9.69	16.10	22.28	29.67	1.54	1.91	0.87	1.41
	N	21	21	21	21	21	21	20	19
As	median	7.70×10^{-4}	5.84×10^{-4}	24.90×10^{-4}	44.60×10^{-4}	1.59×10^{-4}	2.30×10^{-4}	1.04×10^{-4}	1.04×10^{-4}
	min.	1.09	1.80	13.40	24.70	0.66	0.18	0.005	0.37
	max.	15.00	28.90	129.00	69.20	6.88	4.71	3.08	5.30
	N	11	19	21	21	14	15	12	16
Cd	median	2.96×10^{-4}	2.70×10^{-4}	9.18×10^{-4}	10.80×10^{-4}	0.49×10^{-4}	0.63×10^{-4}	0.23×10^{-4}	0.63×10^{-4}
	min.	1.73	1.08	6.05	9.32	0.083	0.155	0.068	0.153
	max.	4.41	9.34	11.20	12.30	0.895	0.798	0.900	0.900
	N	21	21	21	21	2	3	7	4
Cr	median	8.29×10^{-3}	9.68×10^{-3}	50.10×10^{-3}	55.20×10^{-3}	1.55×10^{-3}	1.47×10^{-3}	–	–
	min.	6.05	3.81	27.60	47.50	0.57	0.99	–	–
	max.	17.80	22.20	56.90	63.00	2.54	1.96	–	–
	N	21	21	21	21	2	2	–	–
Cu	median	4.89×10^{-3}	8.86×10^{-3}	90.80×10^{-3}	140.0×10^{-3}	0.84×10^{-3}	0.96×10^{-3}	0.21×10^{-3}	0.22×10^{-3}
	min.	2.58	2.33	56.00	124.00	0.003	0.198	0.370	0.690
	max.	9.80	20.80	127.00	155.00	2.28	9.80	1.13	1.13
	N	21	21	21	21	16	21	21	21
Fe	median	7.97	8.24	25.51	32.77	0.27	0.38	0.58	0.66
	min.	5.67	3.60	15.99	28.56	0.04	0.13	0.10	0.06
	max.	15.43	20.82	28.86	38.11	2.13	2.85	6.40	8.73
	N	21	21	21	21	21	21	21	21

Table 2 continued

Metal	Value type	Sediment				Seawater particulates			
		Site A	Site B	Site C	Site D	Site A	Site B	Site C	Site D
Mn	median	8.14 x10^{-2}	8.08 x10^{-2}	21.30 x10^{-2}	33.30 x10^{-2}	0.21 x10^{-2}	0.74 x10^{-2}	2.18 x10^{-2}	2.40 x10^{-2}
	min.	5.48	2.56	14.60	25.80	0.063	0.299	0.482	0.320
	max.	16.50	19.70	64.30	62.10	1.47	3.10	6.42	13.9
	N	21	21	21	21	20	21	21	21
Ni	median	3.01 x10^{-3}	3.24 x10^{-3}	12.50 x10^{-3}	15.50 x10^{-3}	0.256 x10^{-3}	0.319 x10^{-3}	0.361 x10^{-3}	0.217 x10^{-3}
	min.	2.07	1.37	7.00	13.60	0.028	0.0005	0.013	0.015
	max.	7.50	7.90	15.00	18.30	1.19	1.81	2.53	3.90
	N	21	21	21	21	16	16	18	19
Pb	median	6.15 x10^{-3}	6.67 x10^{-3}	57.80 x10^{-3}	57.10 x10^{-3}	0.57 x10^{-3}	1.59 x10^{-3}	2.18 x10^{-3}	1.34 x10^{-3}
	min.	3.94	3.06	30.30	41.70	0.02	0.33	0.16	0.05
	max.	75.40	8.91	68.30	71.90	2.47	6.91	11.60	13.30
	N	21	21	21	21	21	21	21	21
Sn	median	1.24	1.35	3.90	5.18	3.68 x10^{-2}	5.58 x10^{-2}	8.93 x10^{-2}	7.44 x10^{-2}
	min.	0.81	0.56	2.23	4.39	0.35	1.68	1.12	0.53
	max.	3.21	2.99	4.49	6.04	31.20	42.40	82.80	127.00
	N	21	21	21	21	21	21	21	21
Sr	median	6.13 x10^{-2}	6.34 x10^{-2}	12.50 x10^{-2}	15.00 x10^{-2}	1.61 x10^{-2}	1.64 x10^{-2}	1.34 x10^{-2}	1.56 x10^{-2}
	min.	3.32	2.43	7.71	13.10	0.94	0.95	0.75	0.97
	max.	12.30	43.20	16.90	18.50	3.01	3.07	4.22	7.63
	N	21	21	21	21	21	21	21	21
Ti	median	5.03 x10^{-1}	4.75 x10^{-1}	4.60 x10^{-1}	4.70 x10^{-1}	0.152 x10^{-1}	0.173 x10^{-1}	0.237 x10^{-1}	0.241x10^{-1}
	min.	3.28	1.57	3.45	3.65	0.028	0.050	0.051	0.027
	max.	14.70	12.10	6.76	7.70	0.983	1.320	1.830	3.540
	N	21	21	21	21	21	21	21	21
Zn	median	2.34 x10^{-2}	3.30 x10^{-2}	19.70 x10^{-2}	25.30 x10^{-2}	0.369 x10^{-2}	0.412 x10^{-2}	0.721 x10^{-2}	0.493 x10^{-2}
	min.	1.49	0.76	12.90	22.40	0.061	0.169	0.204	0.251
	max.	3.87	17.60	22.90	124.00	0.761	1.140	3.370	7.100
	N	21	21	21	21	21	21	21	21

Table 3. Factors influencing metal concentrations associated with sediment and in seawater particulates from San Diego Bay. 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). Additional sources of variation are included in the factor Residual. Each sample shows the Degree of Freedom (DF) associated with the considered factor, the percentage of variation due to a given factor (%) and the associated statistical significance (*P* value, except for the Residual factor). *P* values in bold are significant ($\alpha < 0.05$). Overall, the factor site was the main factor explaining variations among metal concentrations associated with sediment, whereas it was the factor week for seawater particulates.

Metal	**Factor**	**DF**	**Sample**			
			Sediment		Seawater particulates	
			%	*P*	%	*P*
Ag	Site	3	2.7	0.5324	3.5	0.4868
	Week	6	6.4	0.5226	5.6	0.6784
	Site*Week	18	22.8	0.4302	11.8	0.9632
	Residual	56	68.1		79.1	
Al	Site	3	72.8	**<.0001**	0.5	0.8899
	Week	6	4.0	**0.0342**	10.6	**0.0424**
	Site*Week	18	8.0	0.0814	46.6	**0.0002**
	Residual	56	15.2		42.3	
As	Site	3	62.4	**<.0001**	0.3	0.9599
	Week	6	3.8	0.2070	6.3	0.4299
	Site*Week	18	9.6	0.2643	35.2	**0.0376**
	Residual	56	24.2		58.2	
Cd	Site	3	89.1	**<.0001**	1.7	0.4916
	Week	6	1.3	**0.0176**	46.7	**<.0001**
	Site*Week	18	5.4	**<.0001**	11.9	0.5452
	Residual	56	4.2		39.7	
Cr	Site	3	94.5	**<.0001**	3.9	0.2271
	Week	6	0.5	**<.0001**	23.5	**0.0009**
	Site*Week	18	4.1	**<.0001**	23.5	0.1288
	Residual	56	0.8		49.1	
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	**0.0002**
	Residual	56	0.4		24.5	
Fe	Site	3	89.4	**<.0001**	5.4	**0.001**
	Week	6	1.2	**0.0108**	45.4	**<.0001**
	Site*Week	18	5.7	**<.0001**	32.8	**<.0001**
	Residual	56	3.7		16.3	

Table 3 continued

Metal	Factor	DF	Sample			
			Sediment		Seawater particulates	
			%	*P*	%	*P*
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	**0.0481**
	Residual	56	2.9		33.3	
Ni	Site	3	93.4	**<.0001**	2.2	0.4614
	Week	6	0.4	0.2055	21.6	**0.0015**
	Site*Week	18	3.9	**<.0001**	28.0	**0.0471**
	Residual	56	2.3		48.2	
Pb	Site	3	91.0	**<.0001**	11.3	**0.0002**
	Week	6	2.3	**<.0001**	40.3	**<.0001**
	Site*Week	18	4.4	**<.0001**	20.6	**0.0093**
	Residual	56	2.4		27.8	
Se	Site	3	NA	NA	2.9	0.4161
	Week	6	NA	NA	22.1	**0.0036**
	Site*Week	18	NA	NA	19.2	0.4009
	Residual	56	NA		55.8	
Sn	Site	3	85.2	**<.0001**	4.6	**0.0124**
	Week	6	0.8	0.2815	43.1	**<.0001**
	Site*Week	18	7.8	**<.0001**	30.7	**<.0001**
	Residual	56	6.2		21.6	
Sr	Site	3	25.5	**0.0001**	0.6	0.7812
	Week	6	2.0	0.8428	26.4	**<.0001**
	Site*Week	18	15.7	0.5757	11.8	0.2972
	Residual	56	56.8		61.2	
Ti	Site	3	6.6	**0.0227**	3.2	0.1858
	Week	6	25.1	**<.0001**	38.7	**<.0001**
	Site*Week	18	33.3	**0.0010**	29.0	**0.0004**
	Residual	56	35.0		29.0	
Zn	Site	3	90.4	**<.0001**	9.7	**0.0029**
	Week	6	0.8	0.4863	30.3	**<.0001**
	Site*Week	18	2.9	0.1572	25.5	**0.0094**
	Residual	56	5.9		34.6	

Table 4. Metal concentrations (median values) in disk and arms of the brittlestar *Ophiothrix spiculata* transplanted at sites A, B, C, and D in San Diego Bay during the 8-week experimental period. Values in bold indicate that the levels are significantly different ($\alpha \leq 0.05$) from those of week 0. - : below detection limit, NA: Not Analyzed. Number of replicates is 1 to 3. Overall, metal accumulation started at week 1 for both arms and disk, and was highly variable from one week to another. Metal concentrations were usually greater in disk than arm tissue, suggesting that accumulation through the diet was greater than from seawater. There was no clear trend of metal concentrations among sites, with higher concentrations being found either at sites A or B, or sites C or D, depending on the metal, the week and the tissue considered.

Metal	Site	Week													
		0		**1**		**2**		**3**		**4**		**6**		**8**	
		Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm
Ag ($x10^{-3}$)	A	3.38	-	**5.20**	**2.21**	-	0.002	**25.60**	0.27	-	0.49	**2.92**	**1.30**	NA	NA
	B	3.38	-	2.95	**1.98**	-	-	6.88	1.60	0.11	0.54	7.07	1.97	35.50	0.24
	C	3.38	-	**1.64**	**2.39**	-	-	3.49	1.00	0.93	0.54	10.80	2.86	0.70	0.30
	D	3.38	-	**3.99**	**2.25**	-	-	-	-	1.78	1.10	-	1.19	NA	NA
Al ($x10^{-2}$)	A	-	-	1.34	**1.05**	11.70	9.29	29.90	4.47	-	0.04	11.20	4.76	NA	NA
	B	-	-	**29.50**	2.03	6.10	7.57	6.88	**3.10**	0.24	0.15	42.70	56.40	0.85	5.54
	C	-	-	**30.30**	**4.98**	63.00	5.60	138.60	7.27	0.68	0.07	25.00	32.50	0.89	0.14
	D	-	-	**76.60**	1.38	83.10	2.77	38.50	**2.73**	0.07	0.20	**28.10**	**7.98**	NA	NA
As ($x10^{-3}$)	A	9.50	-	92.90	-	-	-	7.02	-	1.97	**2.54**	6.18	3.24	NA	NA
	B	9.50	-	5.09	3.00	-	-	-	**0.64**	**17.70**	1.13	5.59	1.78	7.73	0.80
	C	9.50	-	-	-	-	-	-	0.30	3.54	0.09	3.01	3.21	8.90	0.57
	D	9.50	-	3.45	1.91	-	-	-	-	11.30	3.69	2.70	3.27	NA	NA
Cd ($x10^{-4}$)	A	2.00	1.00	6.45	**4.19**	3.39	**4.60**	**30.70**	2.17	1.01	0.92	14.40	3.46	NA	NA
	B	2.00	1.00	**9.27**	**5.25**	4.03	2.68	24.00	3.20	5.15	0.15	5.13	6.59	1.26	6.99
	C	2.00	1.00	**7.72**	**5.36**	3.59	3.77	3.86	2.85	2.95	0.38	24.30	14.90	2.34	0.45
	D	2.00	1.00	3.62	4.00	4.90	3.48	2.89	2.97	4.36	0.14	3.93	**6.74**	NA	NA

Table 4 continued

Metal	Site	Week													
		0		**1**		**2**		**3**		**4**		**6**		**8**	
		Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm
Cr ($x10^{-3}$)	A	2.00	0.38	3.67	0.43	0.13	-	5.86	0.69	1.46	0.33	5.65	-	NA	NA
	B	2.00	0.38	**3.79**	0.81	-	0.34	-	0.74	1.59	0.38	4.02	**0.73**	5.86	0.03
	C	2.00	0.38	3.99	2.11	2.87	**0.42**	1.03	0.23	2.81	**0.60**	-	3.67	1.16	0.90
	D	2.00	0.38	0.59	0.38	3.03	0.18	4.38	0.01	0.48	0.90	1.85	0.11	NA	NA
Cu ($x10^{-3}$)	A	-	-	4.42	**0.47**	**4.10**	**2.66**	10.80	1.34	0.25	0.28	0.16	1.45	NA	NA
	B	-	-	-	0.43	**6.31**	2.09	3.34	**0.92**	2.20	-	1.86	1.90	6.55	1.69
	C	-	-	-	0.06	**11.80**	**3.15**	6.35	1.11	2.35	0.66	2.85	2.09	-	0.22
	D	-	-	3.38	**1.74**	**19.60**	3.46	**157.0**	**29.10**	-	0.10	1.18	0.84	NA	NA
Fe ($x10^{-2}$)	A	-	0.23	34.20	**6.23**	**23.20**	**15.20**	**167.10**	**4.58**	0.28	0.10	**26.60**	**16.90**	NA	NA
	B	-	0.23	**48.10**	4.70	**7.66**	10.30	25.90	**6.90**	0.30	0.03	164.10	96.50	0.78	0.50
	C	-	0.23	**57.90**	**9.93**	83.90	**13.70**	156.60	8.16	**0.95**	0.05	58.10	54.50	0.27	0.19
	D	-	0.23	**105.90**	**3.44**	27.00	7.12	48.70	**6.53**	**0.07**	0.01	**45.70**	12.90	NA	NA
Mn ($x10^{-3}$)	A	-	0.42	34.40	-	**5.74**	**2.66**	8.28	-	0.51	0.36	**11.90**	**3.25**	NA	NA
	B	-	0.42	6.06	9.05	**4.04**	3.60	13.00	1.23	1.62	0.38	12.20	19.80	0.86	0.30
	C	-	0.42	2.05	3.66	13.50	**4.35**	21.00	3.62	0.0001	0.38	8.78	8.88	3.18	-
	D	-	0.42	**62.20**	**3.45**	20.80	4.15	10.10	**6.36**	0.45	1.13	14.40	**6.45**	NA	NA
Ni ($x10^{-3}$)	A	10.20	0.22	-	0.18	-	**1.67**	22.20	0.47	4.46	0.87	14.00	**0.92**	NA	NA
	B	10.20	0.22	-	1.14	0.54	1.57	6.76	1.87	7.00	1.35	1.21	**2.82**	5.22	0.50
	C	10.20	0.22	-	0.67	0.57	1.08	8.29	1.21	7.16	**3.01**	1.25	**1.52**	4.61	0.89
	D	10.20	0.22	3.53	0.24	3.92	**1.10**	0.48	**0.83**	10.60	0.50	-	3.79	NA	NA

Table 4 continued

Metal	Site	Week 0		1		2		3		4		6		8	
		Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm
Pb ($x10^{-3}$)	A	1.86	2.48	-	**0.97**	13.80	0.83	-	6.85	15.10	**4.66**	11.90	2.58	NA	NA
	B	1.86	2.48	7.16	2.50	0.29	0.13	14.50	1.42	**12.80**	**15.30**	2.98	**5.10**	**14.90**	4.18
	C	1.86	2.48	2.23	0.92	-	0.51	28.70	-	30.30	**13.60**	1.92	**4.52**	11.40	12.10
	D	1.86	2.48	5.39	0.47	-	0.04	27.60	2.24	13.50	5.06	**19.80**	0.62	NA	NA
Se ($x10^{-3}$)	A	-	0.38	11.20	**1.52**	2.59	-	-	1.06	-	-	7.80	-	NA	NA
	B	-	0.38	16.60	1.22	3.75	-	-	-	6.85	-	0.54	0.46	14.90	-
	C	-	0.38	**6.53**	0.58	-	-	-	-	-	-	-	-	11.40	-
	D	-	0.38	-	0.75	3.70	0.60	-	2.50	-	-	4.64	0.52	NA	NA
Sn ($x10^{-2}$)	A	-	5.17	4.27	0.68	2.12	2.10	**37.50**	1.37	0.23	0.33	**5.65**	4.18	NA	NA
	B	-	5.17	3.89	12.70	-	1.85	3.31	1.26	0.65	0.04	19.20	27.20	0.54	0.06
	C	-	5.17	**8.12**	0.64	**8.56**	3.71	41.30	0.96	0.83	0.05	16.50	17.00	1.60	-
	D	-	5.17	**28.00**	**0.53**	19.80	0.44	82.70	0.52	0.81	0.08	**12.10**	3.37	NA	NA
Sr ($x10^{0}$)	A	-	1.19	1.25	**1.57**	**1.15**	**1.47**	**12.80**	0.16	**0.03**	0.01	**0.82**	1.36	NA	NA
	B	-	1.19	**1.36**	**1.53**	**1.18**	0.88	6.33	1.22	**0.02**	0.01	**1.11**	1.53	0.02	0.02
	C	-	1.19	**1.18**	1.49	**1.26**	**1.42**	**1.24**	0.77	**0.01**	0.01	1.33	1.60	0.02	0.01
	D	-	1.19	**1.41**	**1.64**	1.21	1.13	**1.33**	**1.55**	0.02	0.01	**1.49**	**1.64**	NA	NA
Ti ($x10^{-3}$)	A	0.11	1.43	18.70	0.14	6.12	1.55	**6.67**	1.95	**0.38**	0.14	5.22	0.27	NA	NA
	B	0.11	1.43	**8.12**	0.48	1.09	3.28	1.97	1.08	**0.97**	0.15	14.80	2.31	0.68	0.11
	C	0.11	1.43	**9.07**	0.73	28.30	1.04	57.50	**1.78**	0.79	0.26	4.28	0.63	0.77	0.05
	D	0.11	1.43	**29.10**	0.96	5.40	0.05	16.30	0.71	**1.38**	0.33	10.90	0.45	NA	NA

Table 4 continued

Metal	Site	Week													
		0		**1**		**2**		**3**		**4**		**6**		**8**	
		Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm	Disk	Arm
Zn ($x10^{-2}$)	A	-	-	**4.31**	**6.43**	5.55	**6.00**	**64.70**	2.43	0.07	**0.05**	125.50	3.40	NA	NA
	B	-	-	**5.79**	**5.51**	9.81	3.09	28.90	**5.88**	0.47	0.03	1.51	7.40	72.20	0.06
	C	-	-	**5.47**	**6.81**	**8.18**	**7.71**	9.45	5.62	0.06	**0.06**	26.80	28.30	0.05	0.09
	D	-	-	**5.66**	**6.28**	8.26	6.53	**19.90**	**9.79**	0.07	0.05	15.60	6.64	NA	NA

Table 5. Factors influencing metal concentrations in disk and arm tissue of the brittlestar *Ophiothrix spiculata* transplanted in San Diego Bay. Week 8 was not included because of missing samples at sites A and D. 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 ($\alpha < 0.05$). Overall, the variation in metal concentrations in arm and disk tissue was mainly due to the factor week, and not to the factor site.

			Sample			
Metal	**Factor**	**DF**	Disk tissue		Arm tissue	
			%	*P*	%	*P*
Ag	Site	3	6.4	**0.0083**	3.0	0.1252
	Week	5	22.7	**<.0001**	64.2	**<.0001**
	Site*Week	15	48.2	**<.0001**	9.4	0.2625
	Residual	47	22.7		23.4	
Al	Site	3	8.3	0.0683	5.3	0.1999
	Week	6	22.3	**0.0037**	32.2	**0.0002**
	Site*Week	18	18.1	0.3746	13.2	0.6294
	Residual	56	51.3		49.3	
As	Site	3	5.0	0.3918	1.7	0.6886
	Week	6	8.1	0.4292	34.4	**0.0002**
	Site*Week	18	20.8	0.6206	11.3	0.7948
	Residual	56	66.1		52.6	
Cd	Site	3	9.4	0.0526	1.8	0.5609
	Week	6	11.4	0.0922	47.3	**<.0001**
	Site*Week	18	26.1	0.1301	9.4	0.7604
	Residual	56	53.1		41.4	
Cr	Site	3	1.2	0.7968	12.0	**0.0136**
	Week	6	10.1	0.1428	20.4	**0.0040**
	Site*Week	18	34.5	**0.0367**	19.9	0.2369
	Residual	56	54.2		47.7	
Cu	Site	3	12.0	**<.0001**	10.3	**<.0001**
	Week	6	20.0	**<.0001**	19.3	**<.0001**
	Site*Week	18	52.0	**<.0001**	53.7	**<.0001**
	Residual	56	16.0		16.7	

Table 5 continued

Metal	Factor	DF	Sample			
			Disk tissue		Arm tissue	
			%	*P*	%	*P*
Fe	Site	3	3.6	0.2578	2.7	0.4210
	Week	6	33.2	**<.0001**	44.6	**<.0001**
	Site*Week	18	23.0	0.0651	9.5	0.7771
	Residual	56	40.2		43.2	
Mn	Site	3	16.7	**0.0008**	4.9	0.1447
	Week	6	16.7	**0.0011**	38.0	**<.0001**
	Site*Week	18	33.3	**0.0071**	16.6	0.2511
	Residual	56	33.3		40.5	
Ni	Site	3	6.2	0.1303	4.2	0.2642
	Week	6	10.8	0.0877	22.9	**0.0021**
	Site*Week	18	33.4	**0.0258**	24.5	0.1141
	Residual	56	49.5		48.4	
Pb	Site	3	0.7	0.8506	1.2	0.6757
	Week	6	23.9	**0.0005**	52.0	**<.0001**
	Site*Week	18	17.1	0.2325	11.3	0.4719
	Residual	56	58.3		35.5	
Se	Site	3	2.7	0.5866	3.0	0.4943
	Week	6	18.3	**0.0322**	25.1	**0.0040**
	Site*Week	18	15.0	0.7378	13.2	0.7674
	Residual	56	64.0		58.7	
Sn	Site	3	3.3	0.4090	2.1	0.4782
	Week	6	23.8	**0.0020**	38.3	**<.0001**
	Site*Week	18	22.5	0.1907	8.5	0.8737
	Residual	56	50.4		51.1	
Sr	Site	3	3.6	0.0541	1.3	0.4799
	Week	6	50.4	**<.0001**	60.3	**<.0001**
	Site*Week	18	25.7	**0.0002**	14.4	**0.0495**
	Residual	56	20.4		24.0	
Ti	Site	3	6.9	0.1700	8.0	0.1144
	Week	6	18.6	0.0699	8.8	0.2449
	Site*Week	18	18.6	0.5502	23.4	0.2871
	Residual	56	55.9		59.8	

Table 5 continued

Metal	Factor	DF	Sample			
			Disk tissue		Arm tissue	
			%	*P*	%	*P*
Zn	Site	3	8.8	0.0591	2.8	0.5077
	Week	6	20.6	**0.0065**	34.9	**0.0003**
	Site*Week	18	18.4	0.3778	6.4	0.9795
	Residual	56	52.2		56.0	

Table 6. For each metal and site, parameters of the exponential model "y=a*exp(b*x)" that describe the correlation between metal accumulation in disk or arm tissue of the brittlestar *Ophiothrix spiculata* and the tidal mixing in the Bay. "a" was the initial metal concentration in brittlestar before transplant, and "b" the coefficient of the variation, thus representing the rate at which metal concentration changed with tidal range. Results shown only for $N > 3$; $p \leq 0.05$ for models with R^2 in bold. The hypothesis was that the greater the tidal range, the greater the movement of metal contaminated Bay seawater particulates, and the greater the metal exposure to brittlestars, resulting in greater metal concentrations in their tissues. The results supported the hypothesis of a positive correlation between tidal mixing and metal concentration in brittlestar arm and disk tissue for all metals, except Ni and Pb. Coefficient of determination R^2 was usually similar or greater for arm than for disk tissue at sites A and B, indicating greater accumulation at the mouth of the Bay through seawater; it was usually similar or greater for disk than arm tissue at sites C and D, indicating greater accumulation through the diet at the Back of the Bay.

		Model parameters							
		Disk tissue				Arm tissue			
Metal	**Site**	N	a	b	R^2	N	a	b	R^2
Ag	A	3	NA	NA	NA	4	1.11×10^{-6}	2.72	0.56
	B	5	1.15×10^{-3}	0.48	0.004	5	1.38×10^{-6}	2.79	0.64
	C	5	7.15×10^{-7}	3.40	**0.69**	5	3.97×10^{-7}	3.35	**0.89**
	D	2	NA	NA	NA	3	NA	NA	NA
Al	A	3	NA	NA	NA	5	7.45×10^{-9}	5.94	0.42
	B	6	1.63×10^{-8}	6.23	**0.66**	6	4.26×10^{-7}	4.75	0.41
	C	6	5.69×10^{-7}	5.16	0.36	6	2.04×10^{-10}	7.71	**0.69**
	D	5	6.59×10^{-10}	7.82	0.39	5	3.39×10^{-7}	4.4	**0.59**
As	A	4	2.94×10^{-5}	2.39	0.14	2	NA	NA	NA
	B	4	1.39×10^{-1}	-1.21	0.42	5	2.62×10^{-5}	1.65	0.51
	C	3	NA	NA	NA	4	5.03×10^{-8}	3.96	0.61
	D	3	NA	NA	NA	3	NA	NA	NA
Cd	A	5	1.97×10^{-5}	1.41	0.06	5	1.06×10^{-6}	2.26	**0.66**
	B	6	1.17×10^{-4}	0.65	0.04	6	2.23×10^{-6}	1.99	0.13
	C	6	8.41×10^{-7}	2.69	**0.67**	6	1.51×10^{-9}	5.00	**0.82**
	D	5	1.94×10^{-4}	0.28	0.14	5	1.28×10^{-9}	4.90	0.58

Table 6 continued

		Disk tissue				Arm tissue			
Metal	Site	N	a	b	R^2	N	a	b	R^2
Cr	A	5	$1.89x10^{-2}$	-0.95	0.019	3	NA	NA	NA
	B	4	$2.00x10^{-3}$	0.23	0.016	6	$3.40x10^{-7}$	2.90	0.36
	C	5	$2.87x10^{-5}$	1.85	0.46	6	$5.63x10^{-6}$	2.11	0.29
	D	5	$1.59x10^{-4}$	0.91	0.049	5	$1.97x10^{-4}$	-0.09	0.002
Cu	A	5	$7.53x10^{-2}$	-1.59	0.04	5	$4.23x10^{-6}$	2.20	0.33
	B	5	$1.37x10^{-2}$	-0.57	0.08	5	$5.50x10^{-4}$	0.33	0.02
	C	4	$1.32x10^{-3}$	0.53	0.04	6	$3.81x10^{-6}$	2.14	0.14
	D	3	NA	NA	NA	4	$4.09x10^{-9}$	4.89	0.57
Fe	A	5	$1.53x10^{-5}$	3.77	0.14	5	$9.14x10^{-10}$	7.15	**0.65**
	B	6	$2.31x10^{-9}$	7.30	**0.61**	6	$2.77x10^{-11}$	8.67	**0.64**
	C	6	$4.84x10^{-9}$	7.22	0.50	6	$1.80x10^{-11}$	8.88	**0.72**
	D	5	$9.37x10^{-10}$	7.67	0.37	5	$1.13x10^{-11}$	8.66	0.49
Mn	A	5	$4.58x10^{-7}$	3.89	0.36	3	NA	NA	NA
	B	6	$6.23x10^{-6}$	2.73	0.43	6	$8.77x10^{-10}$	6.16	**0.90**
	C	6	$9.79x10^{-10}$	5.99	0.18	5	$1.63x10^{-7}$	3.99	0.64
	D	5	$3.92x10^{-8}$	5.07	0.43	5	$5.64x10^{-5}$	1.70	0.33
Ni	A	3	NA	NA	NA	5	$1.07x10^{-4}$	0.73	0.04
	B	5	$1.17x10^{1}$	-3.53	**0.75**	6	$3.25x10^{-5}$	1.56	0.49
	C	5	$9.58x10^{0}$	-3.43	**0.71**	6	$2.60x10^{-3}$	-0.31	0.02
	D	4	$6.86x10^{-4}$	0.61	0.01	5	$9.33x10^{-7}$	2.96	**0.75**
Pb	A	3	NA	NA	NA	5	$1.22x10^{0}$	-2.56	0.43
	B	6	$1.12x10^{2}$	-4.19	0.51	6	$7.40x10^{-1}$	-2.42	0.15
	C	5	$1.50x10^{2}$	-4.17	**0.70**	5	$1.65x10^{1}$	-3.53	0.45
	D	4	$3.53x10^{-2}$	-0.38	0.02	5	$6.63x10^{2}$	-5.65	0.50
Se	A	3	NA	NA	NA	2	NA	NA	NA
	B	4	$6.01x10^{1}$	-3.85	0.41	2	NA	NA	NA
	C	1	NA	NA	NA	1	NA	NA	NA
	D	2	NA	NA	NA	4	$3.17x10^{0}$	-3.26	**0.91**
Sn	A	5	$1.01x10^{-3}$	1.43	0.03	5	$3.92x10^{-6}$	3.28	0.63
	B	5	$1.57x10^{-7}$	5.10	**0.88**	6	$3.86x10^{-12}$	9.12	**0.77**
	C	6	$5.56x10^{-5}$	2.95	0.28	5	$4.07x10^{-11}$	7.95	**0.78**
	D	5	$1.04x10^{-3}$	1.99	0.08	5	$6.60x10^{-8}$	4.59	**0.67**

Table 6 continued

		Disk tissue				Arm tissue			
Metal	Site	N	a	b	R^2	N	a	b	R^2
Sr	A	5	$6.90x10^{-3}$	1.95	0.04	5	$1.17x10^{-9}$	7.97	**0.75**
	B	6	$1.21x10^{-6}$	5.34	0.34	6	$3.60x10^{-8}$	6.66	**0.58**
	C	6	$1.59x10^{-8}$	6.98	**0.60**	6	$1.94x10^{-9}$	7.84	**0.69**
	D	5	$1.40x10^{-6}$	5.27	0.43	5	$9.59x10^{-8}$	6.32	0.41
Ti	A	5	$1.27x10^{-6}$	3.32	0.30	5	$4.88x10^{-4}$	-0.04	0.001
	B	6	$4.69x10^{-7}$	3.57	0.56	6	$1.39x10^{-8}$	4.49	**0.70**
	C	6	$6.12x10^{-6}$	2.87	0.18	6	$7.43x10^{-7}$	2.71	0.32
	D	5	$8.24x10^{-5}$	1.88	0.15	5	$9.15x10^{-3}$	-1.34	0.08
Zn	A	5	$2.30x10^{-9}$	7.01	0.32	5	$1.02x10^{-8}$	5.86	0.49
	B	6	$3.48x10^{1}$	-2.60	0.13	6	$2.80x10^{-10}$	7.30	**0.58**
	C	6	$1.43x10^{-11}$	8.77	**0.68**	6	$2.56x10^{-11}$	8.55	**0.74**
	D	5	$2.98x10^{-8}$	5.76	0.34	5	$4.24x10^{-8}$	5.46	0.35