Bull. Environ. Contam. Toxicol. (1990) 44:142–148

Bulletin of Environmental Contamination and Toxicology

Heavy Metals in the Eastern Oyster, *Crassostrea virginica*, of the Mississippi Sound

Thomas F. Lytle[1] and Julia S. Lytle[2]

[1]Analytical Chemistry Section and [2]Environmental Chemistry Section, Gulf Coast Research Laboratory, P.O. Box 7000, Ocean Springs, Mississippi 39564, USA

Bivalves and other sessile estuarine organisms have been increasingly targeted as sentinels of ambient levels of heavy metals in the water column of coastal estuaries. In the most ambitious project to date, the U.S. National Oceanic and Atmospheric Administration (NOAA) inaugurated the National Status and Trends Program in 1984 in which the eastern oyster, Crassostrea virginica, is collected in Gulf of Mexico and Southeastern Atlantic states with other bivalves from other regions to monitor levels of nine heavy metals along these coastlines. Three of the sites of this program are located in the Mississippi Sound extending along the coast of Mississippi from Louisiana to Alabama. In addition to NOAA results (NOAA, 1987) previous metal analyses of C. virginica in the Mississippi Sound have included those of Lytle and Lytle (1982), Harvey et al. (1982) and Harvey and Knight (1978). Levels of metals in oysters in the Sound are of profound interest not only because they document those geographic areas where metal pollution levels may be problematic but because they may disclose possible problems to consumers of oysters. At the present time objective federal standards for heavy metals in oysters and other seafood are restricted to mercury. The closure of Mississippi oyster reefs has been predicated upon bacteriological standards with little if any attention paid to heavy metals. A study of fourteen metals in oysters of the Sound was began in 1988 with objectives differing from that of the Status and Trends Program (STP) in three ways. STP levels are reported on dry weight basis of composites from three sites. In the present study and those by Lytle and Lytle (1982), Harvey, et al. (1982) and Harvey and Knight (1978), oysters were analyzed and reported on a wet weight basis, a reporting method more consistent with consumer needs. Additionally analyses were made of individual specimens to indicate expected specimen to specimen variations and were conducted on oysters from the three STP and two other important oyster reef sites. In the future three or more additional sites will be added to this continuing survey effort. Metals chosen for this study were lead (Pb), cadmium (Cd), iron (Fe), copper (Cu), cobalt (Co), manganese (Mn), zinc (Zn), silver (Ag), nickel (Ni),

Send reprint requests to T.F. Lytle at above address.

mercury (Hg), aluminum (Al), chromium (Cr), molybdenum (Mo) and vanadium (V).

MATERIALS AND METHODS

At each of five sites in January, 1988, 30 oysters were hand collected and placed in polyethylene plastic bags and brought immediately to the laboratory. Two of these sites, Pascagoula River and Twin Island are located at the eastern end of the Sound in the area most heavily industrialized and seriously polluted (Lytle and Lytle, 1985). Further west of the Pascagoula River is the reef in Graveline Bayou, habitually closed because of bacterial levels. In the central area of the Sound oysters were collected at the mouth of Biloxi Bay and also at the mouth of St. Louis Bay at the western end of the Sound.

Six specimens of oysters from each site were selected to be representative of the size of oysters present at each particular site and were placed in an oven at 100°C for 30 seconds or until the oyster could be pried open with a polycarbonate knife. All tissue and liquid was removed, weighed and included for analyses. For all metals but mercury, tissue was digested five minutes at 90°C with 5 mls conc. HNO_3 and 6 mls conc. H_2SO_4. Five mls more HNO_3 was added for another five minute heating period after which 30% H_2O_2 was added dropwise during a one hour digestion at 90°C to maintain a light colored digest. These digests and appropriate reagent blanks were diluted to volume and aspirated directly into the flame of an Instrumentation Laboratory 351 atomic absorption spectrophotometer. The acetylene/air flame was supplemented with nitrous oxide for Al, Cr, Mo and V. Hg was measured by digestion of a separate set of samples. Whole oysters were digested with 8 mls conc. H_2O_4 and 2 mls conc. HNO_3. After reduction with $SnCl_2$, Hg vapor was flushed through a quartz cell in the atomic absorption spectrophotometer.

All analyses were conducted in a laboratory especially outfitted to prevent metal contamination throughout analysis. The laboratory was supplied with positive pressure high efficiency filtered air. Water was quartz distilled after batch glass distillation and ion exchange/ultrafiltration. All reagents were of grades commercially purified for metal analysis.

RESULTS AND DISCUSSION

Results of the present study are contained in Table 1. In comparing results among various stations several interesting observations can be made. Mean values of Pb, Fe, Co, Mn, Ni, Al and Cr appear to be elevated in oysters in the Pascagoula River and Twin Island sites compared to other sites. Since Fe, Al and Mn are major elemental constituents of clay minerals, elevations of these metals at these sites might indicate ingestion of sedimentary clay particles by these oysters, a suggestion that is consistent with the intense boating and shipping perturbations that could expose oysters in these two areas to high levels of resuspended clay minerals. The elevated levels of Pb, Co, Ni and Cr might then also be explained as a result of direct sediment exposure except that oyster specimens throughout the study with highest levels of Al, Fe and Mn contained relatively low levels of Pb, Co, Ni and Cr. Apparently these metals are enriched in oysters at these two sites because of enhanced levels in the water column, not ingestion or inclusion of sediment particles. One would expect evaluated levels of metals in these locations as evidenced by the intensity of industrial development in the region.

The smallest oysters were collected at Pascagoula River and Twin Island followed closely by Biloxi Bay oysters and those from St. Louis Bay. Those from Graveline Bayou were significantly larger than any others. As suggested by Wright, 1978 and indicated by the earlier study in St. Louis Bay (Lytle and Lytle, 1982) metal levels in oysters are often inversely proportional to oyster size indicating diminishing uptake with age of the oyster. Not surprisingly the oysters from Graveline Bayou had the lowest overall values of Cd, Fe, Cu, Mn, Zn and Al. The Fe, Mn and Al (from clay minerals) deficiencies however are probably indicative of low sediment perturbations in this fairly isolated region, not size of oyster. It is interesting to speculate whether oyster size might be an indication of general good health attributable to freedom from metal toxicants rather than low levels of metals resulting from oysters with declining ability to accumulate metals. Brouwer et al. (1986) have suggested that oysters, by being able to sequester heavy metals with metal binding proteins, can withstand high accumulation rates of heavy metals with little harm to the oysters discounting any theory that modest increases in metals might inhibit oyster growth. Therefore it is more likely that the cause/effect relationship of heavy metals in Graveline Bayou oysters is size (cause)/metal level (effect).

Biloxi Bay oysters tend to have the highest zinc concentrations, probably reflecting higher levels of zinc in the water column here. St. Louis Bay oysters seem to be registering the most "pristine" of the study areas, having oysters with the highest mean Cd but no other outstanding metal levels. Cu levels in both Biloxi Bay and St. Louis Bay oysters were of equal magnitude and about double that in other areas. Huggett et al. (1973) have suggested that high Cu/Zn ratios in oyster tissue are indicative of Cu enrichment in estuarine waters. If so the ratio of Cu/Zn in St. Louis Bay, being decidedly the highest of any region, would suggest the greatest Cu enrichment occurring in waters of this bay. An alternate explanation for the high Cu/Zn ratios in St. Louis Bay might follow the research findings of Brouwer et al (1986) indicating that Cd (which is highest in St. Louis Bay oysters) induces the production of Cu-binding proteins that would give St. Louis Bay oysters a decided advantage in accumulating Cu to oysters from other collection sites.

The greatest site to site variations appear to occur with the metals Fe, Cu and Zn. Cu and Zn were also noted by Marcus and

Thompson (1986) as having the greatest station to station variability. These two metals incidentally are most often cited as those for which oysters have a pronounced ability to accumulate from overlying waters (Lytle and Lytle, 1982, Brouwer et al. 1986).

Brouwer et al. (1986) have suggested that genetic differences in differing geographic locations could account for substantial differences in station to station oyster metal levels. Even among individual specimens genetic differences may account for profound differences in metal levels. Note particularly metal levels in Table 1 for specimen No. 4 in Graveline Bayou with its outstanding levels of Pb, Cd, Ag, and Ni; specimen No. 3 from Pascagoula River with high levels of Pb, Cd and Ni; and No. 1 from Twin Island with very high levels of Pb, Cd, Co, Ag and Ni. Apparently very significant specimen to specimen metal level variability can occur with C. virginica and was seen even in the very small sample population used in this study. This facet of heavy metal accumulation is obviously an important consideration in sampling.

Data included in Table 2 permits comparison of the current data to that of earlier surveys. Data from the NOAA Status and Trends Program has been converted to wet weight basis using an average 84.2% moisture content found in oysters in a study at this laboratory (D. Cook, GCRL, personal communication). In comparing this 1988 data to 1982 data in St. Louis Bay virtually no difference is seen in metal levels in oysters attesting to a consistency in estuarine water quality as measured by oyster metal uptake. Oysters taken from Graveline Bayou in 1982 differ primarily from those in this study in having had roughly double the body loads of Zn and Cu compared to the recent survey. There is little relevant data to make a comparison of data for Biloxi Bay oysters though it appears that levels may have increased in the oysters and, by inference, the water column here since 1978. For the three sites sampled by NOAA STP, values of almost all metals on the present study are 1.5 to 2 times those of the STP. Since one of the sites for STP was St. Louis Bay where 1982 and 1988 values are in agreement, it is felt that this discrepancy in 1986 and 1988 values is more a product of an ill-advised mathematical manipulation of STP data than real analytical differences. Another possible method of direct comparison might be to normalize both sets of data to mean concentration values of zinc, a key metal constituent in oysters. Doing so produces a more compatible set of data but is still a rather artificial manipulation. It is suggested that moisture content be measured on samples in the future so that direct conversions and comparison can be made. It is instructive to note that STP data, with variability expressed as % standard deviation for analysis of 3 sets of 20 specimen composites, though showing less variability than specimen to specimen variability in the present study nonetheless is still quite large.

A number of problems have been addressed in this study of heavy metals in oysters and none satisfactorily resolved. Some issues

Table 1. Heavy Metals in Oysters of Mississippi Sound[1]

	Specimen #	Wt.	Pb	Cd	Fe	Cu	Co	Mn	Zn	Ag	Ni	Hg	Al	Cr	Mo	V
Biloxi Bay	1	3.2	1.6	1.2	81	33	nd[2]	9.5	1,100	nd[2]	nd[2]	nd[2]	24	nd[2]	2.6	nd[2]
	2	4.0	nd[2]	1.2	41	48	nd	3.1	1,200	nd	nd	nd	11	nd	1.7	nd
	3	5.6	0.79	0.80	42	49	0.47	4.9	1,000	nd	nd	nd	13	nd	1.8	nd
	4	5.6	nd	0.71	41	55	nd	3.0	1,100	nd	nd	nd	5.8	nd	nd	nd
	5	6.2	nd	0.72	74	44	nd	7.7	980	nd	nd	nd	27	nd	nd	nd
	6	8.3	0.62	0.81	55	30	0.37	3.8	790	nd	nd	nd	10	nd	1.0	nd
Mean, % RSD[3]			1.0,52	0.91,25	56,32	43,22		5.3,50	880,46				15,56		1.8,37	
St. Louis Bay	1	5.2	nd	2.7	77	46	0.50	8.0	590	0.95	2.7	nd	23	nd	nd	nd
	2	5.7	nd	2.4	63	26	0.15	7.4	360	nd	1.6	nd	19	nd	nd	nd
	3	7.4	nd	2.7	50	43	0.35	3.1	670	0.45	nd	nd	9.5	nd	nd	nd
	4	7.5	nd	1.9	37	42	nd	2.7	550	0.89	nd	nd	4.3	nd	nd	nd
	5	7.5	nd	1.9	59	33	nd	7.9	520	nd	0.61	nd	11	nd	nd	nd
	6	12	nd	1.1	36	29	nd	3.3	430	nd	nd	nd	8.9	nd	nd	nd
Mean, % RSD				2.1,29	54,30	36,23	0.33,53	5.4,48	440,50	0.76,36	1.6,64		13,55			
Graveline Bayou	1	12	nd	0.47	29	8.5	0.14	2.6	310	nd	0.74	0.66	6.1	nd	nd	nd
	2	13	nd	0.69	37	7.3	nd	2.0	330	nd	1.0	nd	7.4	nd	nd	nd
	3	16	nd	0.56	48	7.9	0.16	3.6	320	nd	0.94	nd	28	nd	nd	nd
	4	17	2.1	1.6	30	7.4	0.65	3.1	300	0.79	6.1	nd	2.9	nd	nd	nd
	5	17	nd	0.73	36	12.0	0.36	2.7	410	nd	1.1	nd	7.7	nd	nd	nd
	6	20	nd	0.44	29	5.9	0.35	2.5	230	0.085	nd	6.6	nd	nd	nd	nd
Mean, % RSD				0.75,58	35,21	8.2,25	0.33,62	2.8,20	320,18		2.1,120		9.8,93			
Pascagoula River	1	2.6	7.9	1.2	85	28	0.84	5.2	830	nd	2.6	nd	29	1.3	nd	nd
	2	2.8	2.1	0.96	104	34	nd	5.8	910	nd	nd	nd	35	nd	nd	nd
	3	3.6	20	2.9	70	20	3.7	4.4	760	nd	8.9	nd	30	nd	1.8	nd
	4	4.2	nd	0.52	160	42	nd	9.1	1,200	nd	1.1	nd	50	nd	nd	nd
	5	5.8	0.50	0.83	110	24	nd	4.9	690	0.29	1.6	nd	42	nd	nd	nd
	6	6.9	1.7	0.70	97	19	nd	3.5	420	nd	nd	nd	48	nd	nd	nd
Mean, % RSD			6.4,120	1.2,74	100,30	28,32		5.5,35	800,32		3.6,100		39,23			
Twin Island	1	3.6	13	3.5	94	18	2.8	5.7	670	1.9	12	nd	41	nd	nd	nd
	2	3.7	nd	1.0	370	28	nd	18.0	880	nd	nd	nd	200	nd	nd	nd
	3	4.2	nd	0.51	150	6.3	nd	12.0	210	nd	nd	nd	99	1.2	nd	nd
	4	4.2	nd	1.3	93	13	nd	5.4	530	nd	4.4	nd	40	1.2	nd	nd
	5	5.0	nd	1.3	110	15	nd	3.8	650	nd	2.8	nd	52	nd	nd	nd
	6	6.2	nd	0.52	58	12	nd	3.0	510	nd	1.5	nd	18	nd	nd	nd
Mean, % RSD				1.4,82	140,78	15,47		8.0,73	580,39		4.7,91		75,89			

[1]Values of metals in oysters expressed in µg metal/g wet tissue or ppm. Sample collection was made in January, 1988.
[2]Not detected, detection limits for 10g oyster: Pb = 0.7 ppm, Co = 0.5 ppm, Ag = 0.5 ppm, Ni = 0.5 ppm, Hg = 0.02 ppm, Cr = 0.2 ppm, Mo = 0.3 ppm, V = 0.9 ppm.
[3]% relative standard deviation; means and % RSD only calculated when 3 or more detectable values are reported.

Table 2. Previous metal surveys of *C. virginica* in Mississippi Sound[1]

Location	Pb	Cd	Fe	Cu	Co	Zn	Ag	Nrl	Hg	Cr	Mo	V	Reference
Biloxi Bay	0.12[2] (14.1)	0.45 (21.7)		23 (26.4)		600 (14.7)	0.46 (26.6)	0.39 (10.3)	0.021 (33.7)	0.11 (101.6)			NOAA, 1987
	0.12	0.13							0.13				Harvey and Knight, 1978
	0.05	0.04							0.30				Harvey and Knight, 1978
St. Louis Bay	0.053 (44.2)	1.4 (9.5)		23 (4)		260 (6.2)	0.63 (24.4)	0.39 915)	0.016 (7.5)	0.098 (55.6)			NOAA[3]
	<0.5	1.6	57	32	<0.4	820		<2	<0.2	<0.1	<2	<2	Lytle and Lytle, 1982
Graveline Bayou	1.1	0.58		22		620				3.0			Harvey *et al.*, 1982
Pascagoula River	0.098[2] (12.3)	0.48 (20)		17 (31.1)		520 (34)	0.51 (30)	0.37 (11.5)	0.025 (18.7)	0.17 (68.7)			NOAA, 1987[3]
	0.35	0.16							0.25				Harvey and Knight, 1978

[1]Values expressed in µg metal/g wet tissue.
[2]Values in ( ) are reported % relative standard deviations.
[3]Dry wt. concentrations converted to wet wt. basis with assumed moisture content of 84%.

that must be considered in surveys of this type are: size variations in organisms, geographical and genetic differences, individual variability in metal uptake ability, ingestion of sediment particles, and induction of metal binding proteins. In spite of problems with these surveys it still appears that Waldichuk (1985) is correct in his assessment that there is "...no real substitute for chemical analysis of tissues of organisms exposed to metals in various forms in a particular situation ..." to assess the status of metals in the estuarine environment.

Acknowledgments. The authors wish to thank D. Cook, J. Cirino and S. Breland for collection of specimens and F. Mallette for technical assistance.

REFERENCES

Brouwer M, Engel DW, Bonaventura C, Bonaventura J (1986) Toxic trace metals and trace metal binding proteins in marine organisms. In: Thompson MF, Sarajini R, Nagabhushanum R (eds) Biology of benthic marine organisms, Proceedings International Conference. Oxford and IBH Publ. Co., New Delhi, p 97

Harvey EJ, Herrington R, Kabir A (1982) Concentrations of five metals in the oysters (Crassotrea virginica) and clams (Rangia cuneata) of the Graveline Lake and Bayou system of Ocean Springs, Mississippi. J Miss Acad Sci 27:68

Harvey EJ, Knight LA (1978) Concentration of three toxic metals in oysters (Crassotrea virginia) of Biloxi and Pascagoula, Mississippi estuaries. Water Air Soil Pollut 9:255-261

Huggett RJ, Bender ME, Slone HD (1973) Utilizing metal concentration relationships in the eastern oyster (Crassostrea virginica) to detect heavy metal pollution. Water Res 7:451-460

Lytle TF, Lytle JS (1982) Heavy metals in oysters and clams of St. Louis Bay, Mississippi. Bull Environ Contam Toxicol 36:587-594

Lytle TF, Lytle JS (1985) Pollutant transport in Mississippi Sound. Mississippi-Alabama Sea Grant Consortium, Publ. No. MASGP-82-038

NOAA (National Ocean and Atmospheric Administration) (1987) National status and trends program, Progress Report. NOAA Technical Memorandum NOS OMA 38, Rockville, MD

Waldichuk M (1985) Biological availability of metals to marine organisms. Mar Pollut Bull 16:7-11

Wright, DA (1978) Heavy metal accumulation by aquatic invertebrates. In: Coaker TH (ed) Applied Biology, Vol III. Academic Press, New York, p 331

Received May 16, 1988; accepted August 4, 1989.