A RESEARCH PROGRAM IN DETERMINATION OF HEAVY METALS IN SEDIMENTS AND BENTHIC SPECIES IN RELATION TO NUCLEAR POWER PLANT OPERATION

Final Technical Report 1974 - 1982
NASA Grant NGR09-050-019

Dr. Harriette L. Phelps
University of the District of Columbia

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Principal Investigator

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TABLE OF CONTENTS

PAGE

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Heavy metals in the estuarine environment can be toxic to fish and shellfish early life history stages and concentrations build up to levels of concern in marketable shellfish. Oysters near a power plant on the Patuxent estuary for example were found to have high copper levels unfit for human consumption and metal loss from power plant heat exchange systems have been cited as the causitive factor. The present survey was begun just before startup in 1974 of the 1900 megawatt Calvert Cliffs Nuclear Power Plant on the Chesapeak Bay in order to assess and understand factors relating to heavy metal accumulation in estuarine biota. Oysters were collected in large numbers at test and reference sites in June 1974-77 and individually analyzed for copper and zinc. A statistically significant increase in oyster copper concentrations from the power plant site was found after the second unit began operations in 1976, with an increase at the reference site the following year. To examine whether the increase in oyster copper concentrations at the reference site was a local or bay-wide phenomenon, the survey was expanded in 1978 and 1979 to 11 additional mid-Bay sites covering a wide range of salinities. Significant increases in oyster copper concentrations were found at almost all sites in 1979. The survey was repeated in 1981 and significant decreases were found along with increased average salinities. Unexpectedly, highest metal concentrations were in small oysters from an unpolluted site.

Oyster copper and zinc concentrations correlated with salinity

read at time of collection, averaging -16 ug copper/gm oyster wet weight/ppt salinity, and -216 ug zinc/gm oyster wet weight/ppt salinity from 5 - 20 ppt salinity; however, concentrations were shifted according to yearly average salinity making direct year-to-year comparisons impossible.

The relationship of oyster age to metal concentration was examined with two sets of oysters of known age and genetic origin (laboratory spawned), of which one set was stunted. Stunted oysters accumulated copper at 9.1 ug copper/gm wet weight/year while oysters with normal growth had decreasing copper concentrations at -3.8 ug copper/gm wet weight/year.

Investigations of copper sorption by typical mid Bay sediments revealed that clay-silt sediment sorbed 45X as much copper as sand sediment, with sorption directly proportional to clay-silt content and logorithmically proportional to loss of weight on ignition. Test sediment lost copper sorption below pH 3.

Field studies on cadmium revealed that cadmium concentrations in sediments and infaunal benthics were not correlated. From differential sediment extractions, cadmium in sediment appeared to be weakly sorbed to organics.

To study pathways of metal uptake by shellfish young soft-shell clams and radioactive 109Cd were used in static short-term uptake studies. Cadmium uptake was most rapid from water but there was also short-term cadmium uptake from sediment and humic acid. Cadmium uptake by clams was directly proportional to concentration and activity of the divalent ion and inversely proportional to salinity.

Separate studies on chlorination effects revealed that chlorination of suspended sediment and seston at power plant levels released up to 90% of the cadmium sorbed to sediment components.

The present study has accumulated one of the largest data bases on estuarine oyster metal levels in the mid Chesapeake Bay. Results have been used in decisions on the metalurgy of power plant heat exchange systems and in establishing an appropriate methodology in undertaking State of Maryland oyster metal surveys.

PREFACE

The 1670 megawatt Calvert Cliffs Nuclear Power Plant (CCNPP) which began operations May 1975 on the western shore of the Chesapeake Bay (Fig. 1) has copper/nickel condenser tubing in its heat exchange system, and pumps an estimated 3.6 billion gallons of estuary water through its cooling system per day making it the fourth largest water discharge into the Chesapeake Bay (after the Susquehanna, Potomac and James rivers). An agreement between Maryland's Department of Natural Resources and Baltimore Gas and Electric Company resulted in the dredging and removal of the Flag Pond oyster bar, the nearest commercial oyster bed to the nuclear facility. The Flag Pond area was subsequently closed to commercial harvesting.

A previous study of the effects of the nearby Chalk Point Power Plant on the Patuxent estuary found high accumulations of copper ("greening") in nearby oysters (Roosenberg, 1969) with corrosion of the condenser tubing (Leschber, 1972) considered the probable source. A similar accumulation of copper in sediments around the Chalk Point power plant was not found (Somer, e.a., 1977), although elevated copper levels were later reported in the dissolved organic fraction of the power plant effluent (Sigleo, e.a., 1980). Since the CCNPP was also constructed with copper/nickel condenser tubing, a primary concern was the issue of substantial copper enrichment at this site and the possibility of bioaccumulation.

Objective 1, of this study was to assess CCNPP operations with respect to heavy metals, particularly copper, in the nearby Chesapeake

Bay. This was carried out by semiannual sampling and analysis of oysters from the closed Flag Pond site at Calvert Cliffs and a reference station 10 km north ("upstream") at Scientist Cliffs.

Objective 2, to examine copper accumulation in oysters of the wider mid-bay area and determine effects of natural yearly variations in salinity, was accomplished by expanding the field studies to the nearby Patuxent estuary and other areas of the mid-Chesapeake Bay for three years.

Objective 3, to determine the relationship of oyster age and stunting on copper and zinc accumulation, was carried out by examining metal concentrations in laboratory spawned year classes of oysters raised in two different areas of the mid-Chesapeake Bay.

Objective 4, to determine factors affecting copper sorption by typical sediments near the CCNPP, was undertaken by laboratory studies. The biogeochemistry of copper and other trace metals in estuaries is complex and poorly understood. One important stage in movements of dissoved copper is sorption and desorption by sediments since it is known that sediments and soils are capable of sorbing significant quantities of divalent metals (Huckabee and Blaylock, 1973). The overall nature of sediment copper sorption was explored by studying effects of pH, lyophilization and ashing.

Objective 5, to determine the relationship of metals in sediments to metal concentrations of organisms living in the sediments (infauna) was examined through differential extraction of sediments for cadmium (Black, 1956) and correlation with concentrations of cadmium in principal infaunal species. Benthic animals living in sediments are exposed to sediment-sorbed metals, sometimes at high concentrations (Villa and Johnson, 1974) but complexation may prevent uptake.

Objective 6, examination of modes of uptake of heavy metals by shellfish, was undertaken by laboratory studies on uptake of sediment-sorbed cadmium by shellfish.

Objective 7, to examine effects of chlorination on release of metals from suspended sediments, was carried out by laboratory studies. Chlorine releases heavy metals from sewage sludge (Olver, e.a., 1975) and it has been suggested that reduced levels of metals in sediments near the estuarine power plant at Chalk Point, MD, might be due to the oxidizing action of chlorine (Somer, e.a., 1977).

In all of these studies from 1974 to 1981 several students and faculty were involved. Results from this study have been used in decisions on power plant operations at Calvert Cliffs and by the State of Maryland in making oyster metal surveys. A list of publications and presentations resulting from this work is presented in Appendix A. Additional funding, publications and presentations leading from research supported by this study are listed in Appendix B. Literature reprints mentioned in Appendix A and B are included in Appendix C. This report is not a duplication of material already published in the formal literature but rather a summary of the important methodologies, results and conclusions including some that will not be published elsewhere. Some findings still being written up for publication are also reported in greater detail.

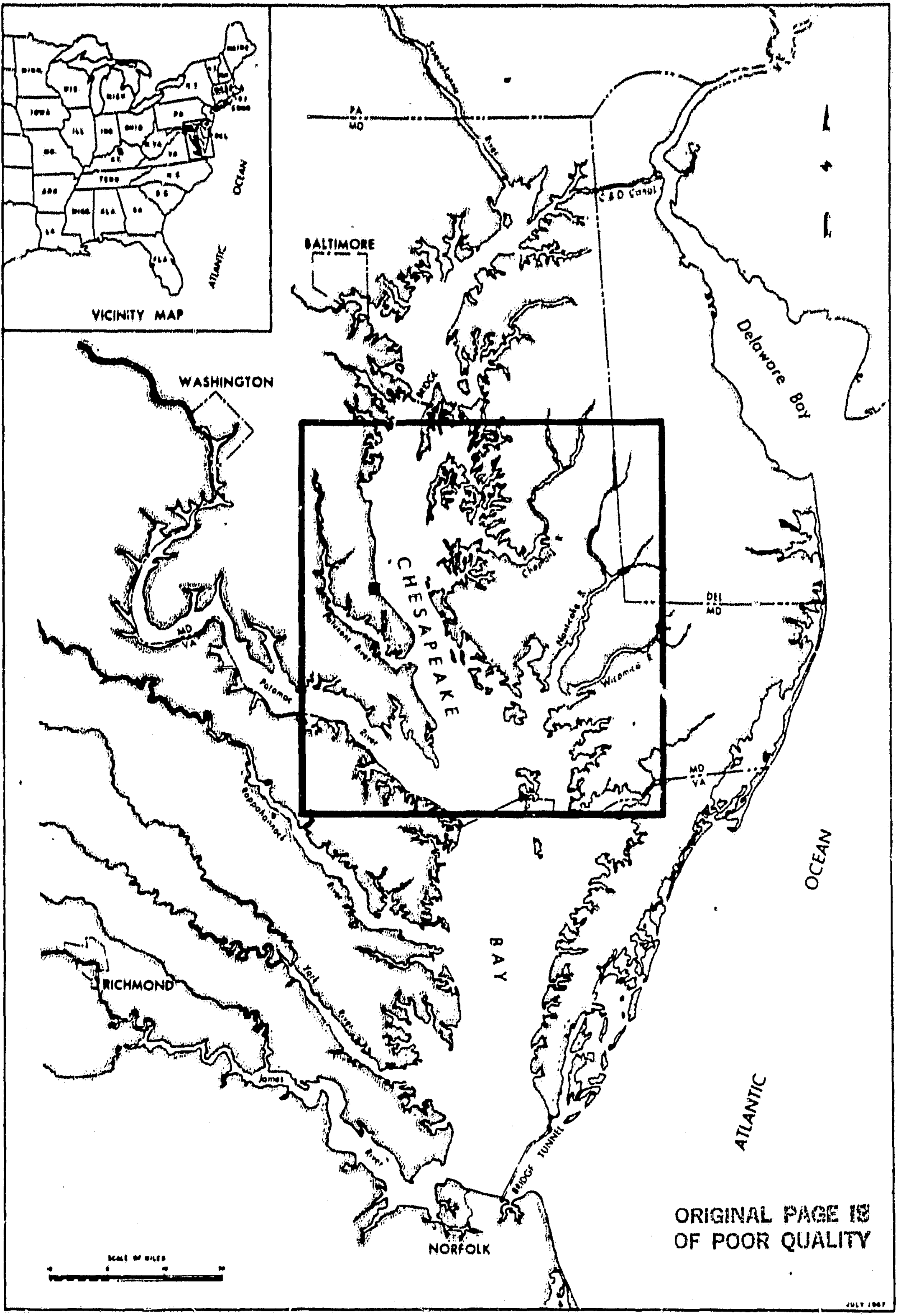

Figure 1: Chesapeake Bay Sampling Program

OBJECTIVE 1

CALVERT CLIFFS NUCLEAR POWER PLANT OPERATIONS AND HEAVY METAL ACCUMULATION IN OYSTERS

Introduction

The Calvert Cliffs Nuclear Power Plant is one of the largest baseload power plants on the Chesapeake Bay with the potential for affecting a large volume of water with its pumped, once-through cooling system. Very low levels of ionic copper can have significant effects on aquatic organisms, for example affecting phytoplankton growth at 10^{-8} to 10^{-10} molar (Sunda and Guillard, 1976; Jackson and Morgan, 1978). The ability of the American oyster (*Crassostrea virginica*) to accumulate high concentrations of metal in its soft body tissue has been well documented by Hiltner and Wichmann (1919), Bodansky (1920), Shuster and Pringle (1968), Wolfe (1970), Windom and Smith (1972) and many others. Therefore, copper concentrations in oysters might be an indication of copper enrichment of the estuary (Huggett, e.a., 1973).

The first unit of the CCNPP began full operation May 8 1975 and the second unit (each 835 megawatts) began generation April 1, 1977. The oyster-metal field study was initiated December 1974 and terminated in June 1981. Objectives of this study were to determine if plant operations affected copper levels in nearby oysters. Zinc concentrations in oysters were considered a baseline indicator because no zinc was expected from power plant sources and as the copper/zinc ratio in oysters has been proposed as an indicator of metal enrichment (Huggett, e.a., 1973).

Methodology

Two natural oyster bars were selected for the oyster sampling program (Fig. 2). One station was a commercial oyster bar that was closed due to possible plant effects near the CCNPP and formerly called Flag Pond. The other station was a commercially harvested oyster bar ten miles north ("upstream") of the power plant at Scientist Cliffs. Oyster and sediment samples were taken quarterly and semi-annually with an oyster dredge and modified van Veen sampler. Oysters were held up to two weeks in a cold room (10 deg. C) before processing. Preparation for analysis involved scrubbing, shucking, rinsing, blotting, weighing and freezing oyster meats individually.

Individual oysters were digested in acid-washed bottles by warming to 70 deg. C with 10-20 ml concentrated HNO3 (Fisher, reagent grade). Oyster digests were filtered through acid washed glass fiber filters and adjusted to 100.0 ml for copper analysis by flame atomic absorption spectroscopy (Perkin-Elmer Model 303 and 440). Fifty microliter aliquots of diluted digests were adjusted to 10.0 ml (deionized water) in polyethylene vials for zinc analysis by Graphite Furnace Atomic Absorption Spectrophotometry (Perkin-Elmer Model HGA 2000). Data were stored and analyzed with BMDP programs and support from the University of Maryland Computer Science Center. Since Frazier (1975) reported significant seasonal variation in oyster metal concentrations and that individual oyster metal concentrations were not normally distributed (Phelps, 1979), statistical comparison was only made between June samplings and using nonparametric tests

(Mann-Whitney).

Results and Conclusions

Copper and zinc concentrations in oysters from sampling trips on 12/74, 6/75/ 10/76, 4/76 and 6/77 (Table 1, Figure 3) indicated Calvert Cliffs oysters had steadily increased average copper concentrations. Significant correlation between copper and zinc concentrations was found for all but one sample (Table 2), similar to results reported by Huggett (e.a., 1973). The copper/zinc ratio in oysters from this study increased steadily from 1974 to 1979 at both reference and plant locations but was not statistically significant until after the beginning of the operation of the second unit at Calvert Cliffs. Huggett also found significant changes in the copper/zinc ratio of James River oysters near industrial sources of metals and proposed the ratio as an indicator of estuarine metal enrichment. Since the Scientist Cliffs station is well outside the calculated thermal effluent plume area (0.5 deg. F isotherm increase, Lantz, 1969), the plume area may not be the standard for assessing the influence of a power plant on copper enrichment in oysters.

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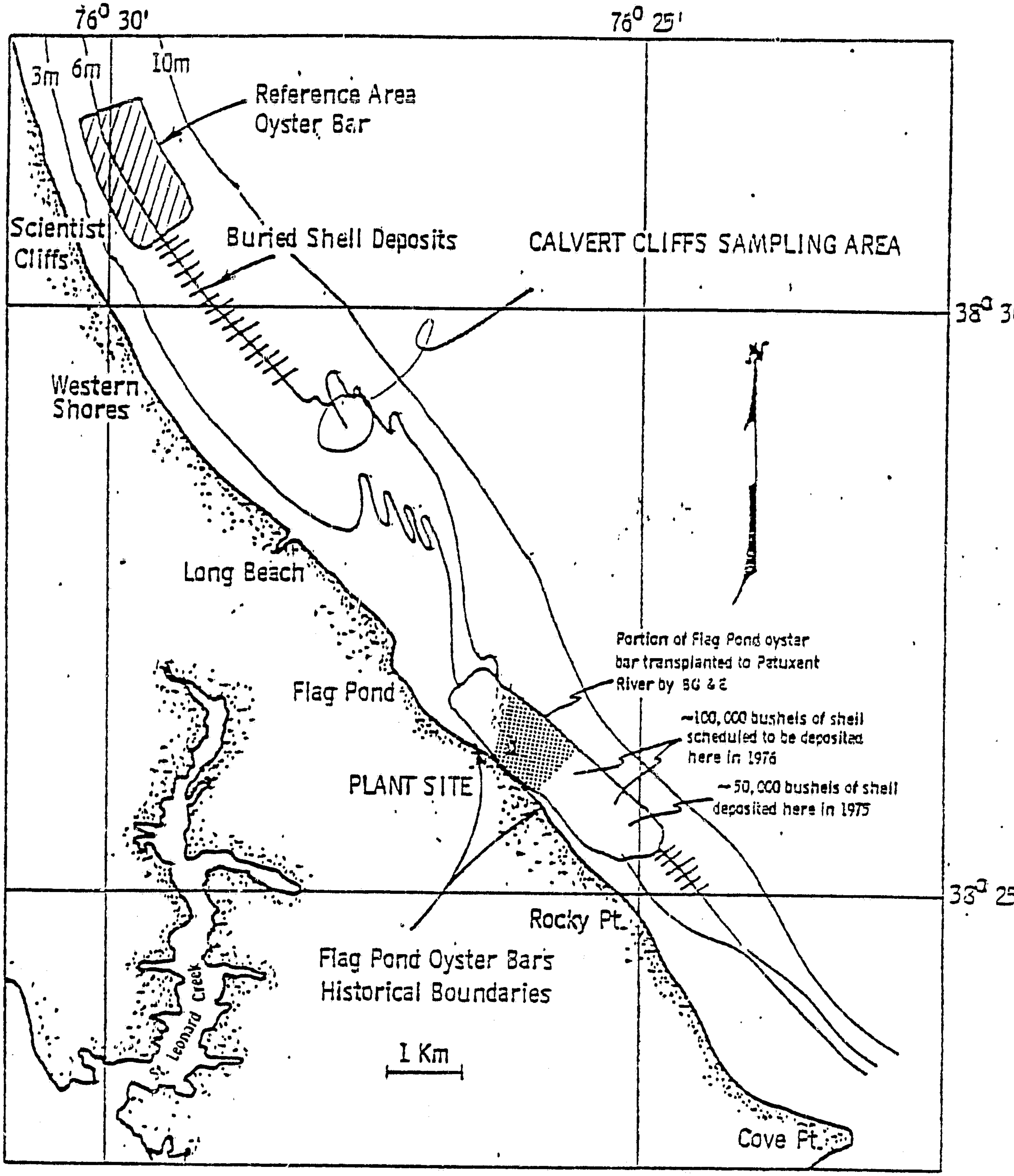


Figure 2; CCNPP and Scientist Cliffs Sampling Sites

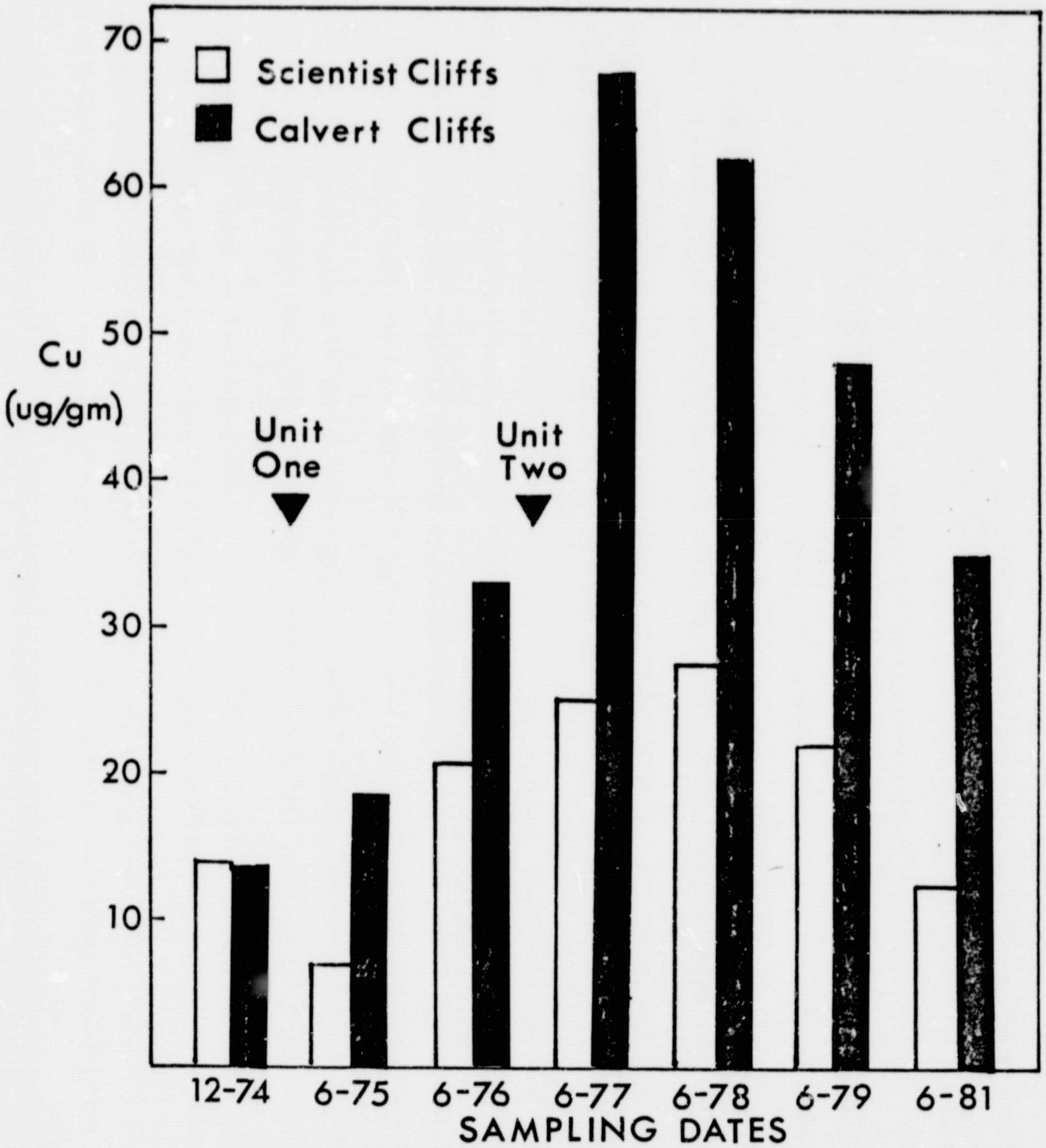


Figure 3; Copper Concentrations in Oysters from CCNPP and Scientist Cliffs

TABLE 1

Oyster Copper and Zinc Concentrations from Calvert Cliffs Nuclear Power Plant and Scientist Cliffs

Sampling Date	Station	n	Wet Weight (gm)		Copper (ug/g)		Zinc (ug/g)		Cu /Zn x10^{-3}
			Aug. ± S.D.	Min/Max	Aug. ± S.D.	Min/Max	Aug. ± S.D.	Min/Max	
12/74	Scientists Cliffs	12	21.8 ± 6.03	13.31/30.65	13.7 ± 6.74	3.18/25.5	2191 ± 1078.	477./4230	6.25
	Calvert Cliffs	11	19.1 ± 3.19	14.52/25.19	13.5 ± 4.19	6.75/19.3	1929 ± 668.	909./3150.	7.00
	(Outfall)								
6/75	Scientists Cliffs	3	31.2 ± 11.7	20.8/43.8	6.9 ± 3.7	3.85/11.0	667 ± 481.	359/1230	10.3
	Calvert Cliffs	9	15.7 ± 7.66	4.5/30.4	18.0 ± 9.3	3.30/38.6	1978 ± 861.	885./3450.	9.11
	(Outfall)								
6/76	Scientists Cliffs	26	22.2 ± 4.58	15.36/30.32	29.5 ± 9.21	16.5/48.0	2180 ± 871.	703./4480.	13.5
	Calvert Cliffs	31	19.3 ± 5.94	9.46/36.14	32.4 ± 16.5	12.3/73.8	2110 ± 884.	556./4210.	15.3
	(Outfall)								
10/76	Scientists Cliffs	35	24.6 ± 4.57	17.20/36.45	18.1 ± 10.1	4.78/39.6	1190 ± 588.	230./2710.	15.2
	Calvert Cliffs	34	19.0 ± 6.95	8.74/36.31	34.2 ± 16.6	12.1/96.7	2970 ± 1243.	1010./6290.	11.5
	(Outfall)								
4/77	Scientists Cliffs	35	28.2 ± 6.64	15.89/39.67	12.8 ± 7.4	2.50/29.7	725 ± 420.	194./1600.	17.7
	Calvert Cliffs	36	17.0 ± 7.84	3.13/34.38	30.2 ± 12.4	5.5 /60.2	1170 ± 879.	293./4220.	25.8
	(Outfall)								
6/77	Scientists Cliffs	35	19.0 ± 5.15	4.7 /30.0	24.2 ± 11.8	5.28/57.4	1140 ± 853.	163./3790	21.2
	Calvert Cliffs	35	18.3 ± 4.87	19.7 /31.0	67.8 ± 27.4	10.5/129.	1390 ± 680.	296./3770	48.8
	(Outfall)								
6/78	Scientists Cliffs	34	20.4	10.8 /38.4	26.9 ±	6.45/42.0			18.6
	Calvert Cliffs	34	17.4	8.98 /26.8	61.2 ±	13.8/103.			48.0
	(Outfall)								
10/78	Scientists Cliffs	34	17.0	6.8 /30.1	36.0 ±	16.5/57.6	696	261./2630	51.7
	Calvert Cliffs	35	18.3	8.8 /33.8	75.8 ±	34.7/173.	1109	351./5057	68.4
	(Outfall)								

OBJECTIVE 2

HEAVY METALS IN MID-BAY OYSTERS AND RELATIONSHIP TO SALINITY

Introduction

The unexpected statistically significant increase in oyster copper concentrations at the reference location in 1976 and 1977 was thought to reflect a power plant effect extending beyond the tidally and thermally affected area. Alternatively, it might be indicative of a wider spread mid-Chesapeake Bay phenomenon.

Methodology

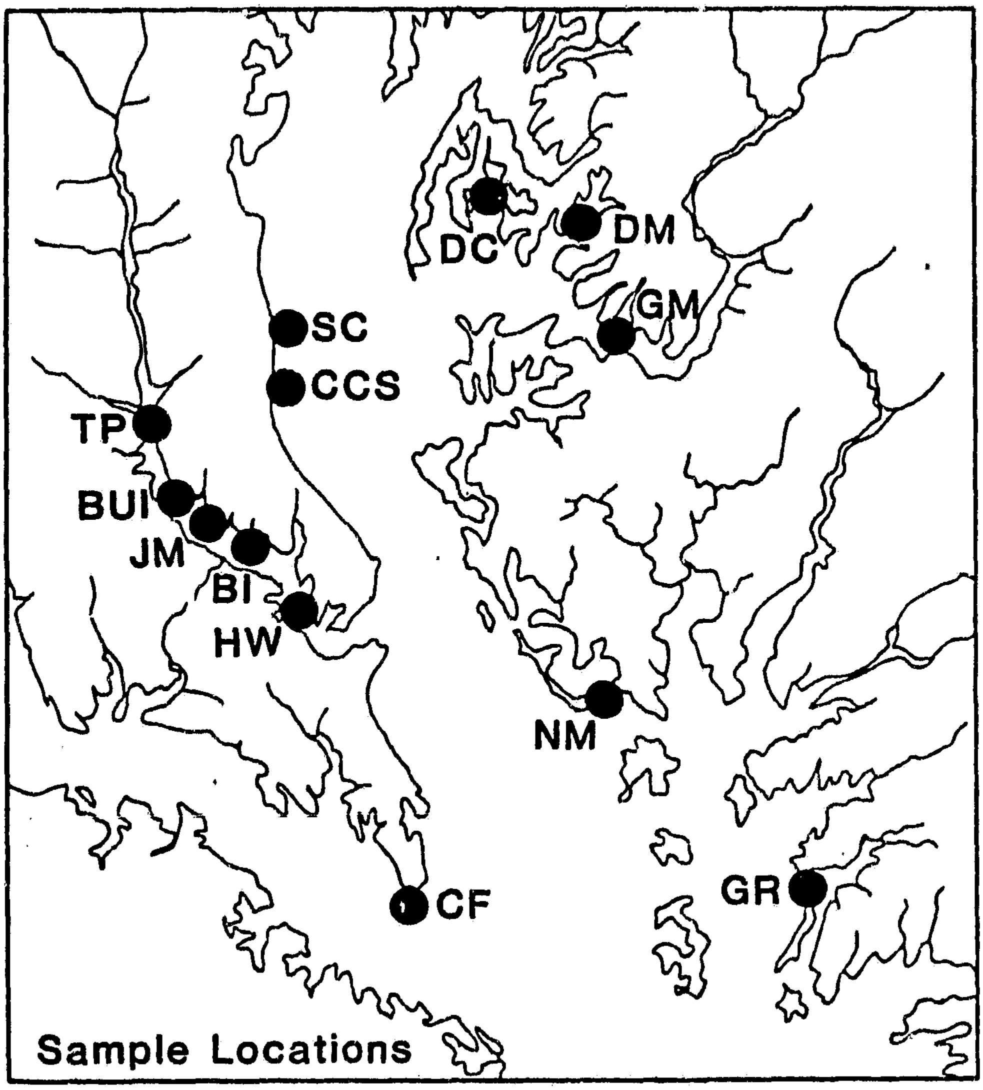
To explore these alternatives, the survey was expanded in 1978, 1979 and 1981 to include 11 additional sites selected in a range of salinities and including "clean" and "contaminated" sites in the mid-Chesapeake bay region (Figure 4). The sampling and analytical methodology was the same as Objective I except all sampling was done in a two day period the second week in June to minimize seasonal variability of oyster metal concentrations.

Results

Comparison of oyster copper and zinc concentrations between the first two years of sampling, 1978 and 1979, found significant increases in copper concentrations at almost all sites (Table 2). When the survey was repeated in 1981, significant decreases in all oyster metal concentrations were found, along with a greatly increased average salinity. Unexpectedly, highest metal concentrations were found in small oysters from an unpolluted seed area at Deep Neck Broad Creek.

It seemed apparent that the relationship of oyster metal concentration to salinity needed to be examined if estuarine oysters were to be useful as indicators of metal enrichment. Copper and zinc concentrations in oysters correlated significantly with salinity read at time of collection (figures 5 and 6). When power plant sites and stunted oyster sites were excluded, results were similar to Maryland Department of Health data correlated with yearly average salinity (Sinex, e.a., 1978). The average correlation of oyster copper concentration with salinity was -16 ug copper/gm oyster wet weight/ppt salinity; the average corrleation of oyster zinc concentration with salinity was -216 ug zinc/gm oyster wet weight/ppt salinity. The significant yearly correlation between salinity and copper and zinc concentrations in oysters shifted each year corresponding to change in average yearly salinity. This may indicate the metal storage compartment in oysters is re-set by yearly average salinity making it difficult to use this assay for year-to-year determination of metal enrichment at any one estuarine location.

Since Sunda and Guillard (1976) reported a relationship between free metal ion activity and toxicity, the free metal ion activity resulting from inorganic complexation due to salinity (Helz, pers. comm.) was compared to oyster metal concentrations at different salinities (Figures 5,6). Zinc concentrations in oysters paralleled divalent zinc ion activity in the range of 5-20 ppt salinity. Copper concentrations in oysters, however, were less than expected from divalent copper ion activity at higher salinities. Since copper is highly sorbed by organic material this may indicate a lower concentration of free copper ion in the organic-rich lower estuary.



Oyster Sampling Sites - Mid Bay

TP: Teagues Point....Patuxent near Chalk Point Power Plant
BUI: Buzzards Island....Patuxent
JM: Jacks Marsh....Patuxent
BI: Broomes Island....Patuxent
HW: Hawks Nest....Patuxent
CF: Cornfield Harbor....Potomac
SC: Scientist Cliffs
CCS: Calvert Cliffs Nuclear Power Plant
DC: Deep Neck Broad Creek
DM: Double Mills...below Easton
GM: Green Marsh....below Cambridge
NM: Normans
GR: Great Rock

Figure 4: Mid Chesapeake Bay Sampling Sites 1978, 1979 and 1981

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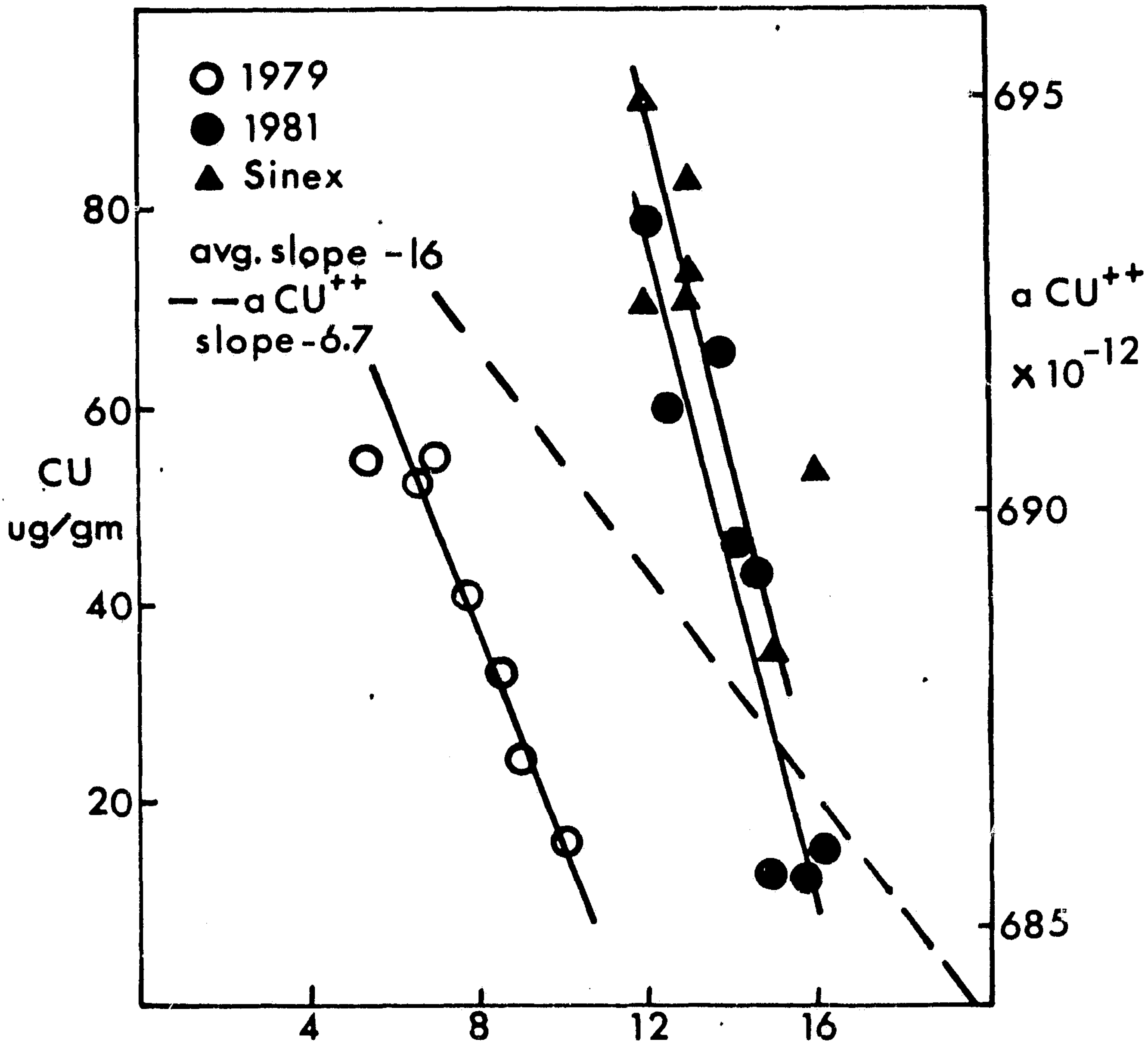

Figure 5: Oyster Copper Concentrations and Salinity

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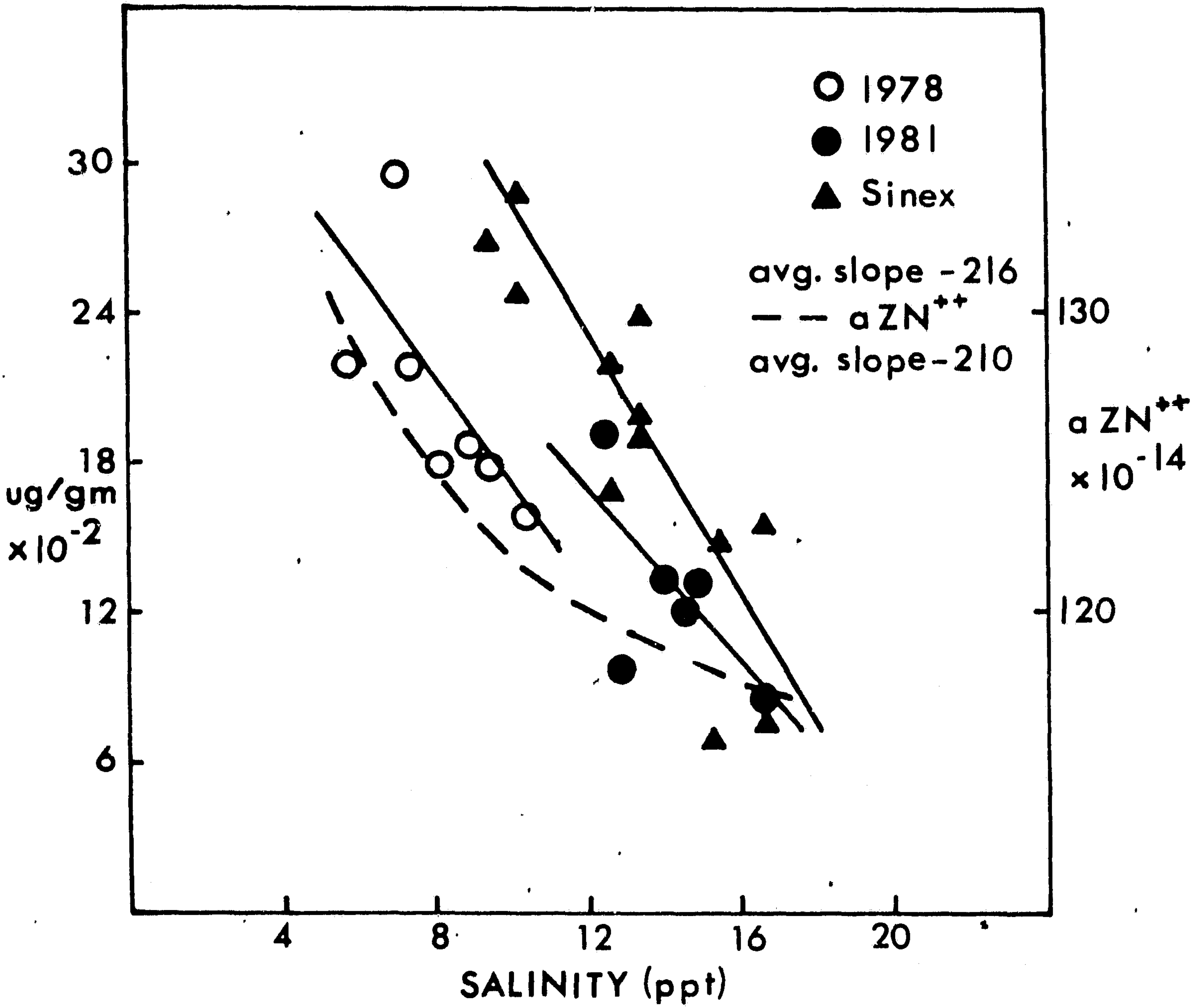

Figure 6; Oyster Zinc Concentrations and Salinity

TABLE 2

1978, 1979 and 1981 Copper and Zinc Concentrations in Mid Chesapeake Bay Oysters

STATION # Location	COPPER ug/gm wet weight '78-'79-'81	ZINC ug/gm wet weight '78-'79-'81	CU/ZN ($x10^2$) '78-'79-'81	SALINITY ppt '78-79-81	WEIGHT gm '78-'79-'81
1. Calvert Cliffs South	61-48-34	1900-610-710	3-8-5	12-9-16	17-24-11
2. Scientist Cliffs	20-21-12	1800-570-370	1-4-3	12-9-16	21-20-17
3. Teagues Point (Px)	70-71-20	1800-570-680	4-12-3	3-5-12	15-14-8
4. Buzzards Island (Px)	55-76-59	2200-730-880	3-10-8	5-7-13	16-15-11
5. Broomes Island (Px)	41-34-44	1700-630-1100	2-5-4	8-8-14	9-18-13
6. Jacks Bay (Px)	27-40	1400-1000	2-[illegible]	7-7	21-18
7. Hawks Nest (Px)	32-33-43	1800-1000-1200	2-3-3	9-10-15	9-12-6
8. Cornfield Harbor	9-16-11	1700-760-630	1-2-2	9-10-15	9-12-6
9. Deep Neck	103-143-82	2100-790-1400	5-18-6	9-9-14	5-6-3
10. Double Mills	73-73-67	2200-1400-1200	2-5-6	16-17-9	7-7-14
11. Green Marsh	53-78-81	3000-1400-1800	2-6-5	7-6-12	12-14-11
12. Normans	14-56-15	1500-610-800	1-9-5	11-15-10	10-11-16
13. Great Rock	11-11-7	660-510-540	2-2-1	12-14	8-9-11
'78-'79					
Calvert Cliffs	decrease	decrease	increase	decrease	increase
Scientist Cliffs	same	decrease	increase	decrease	same
Patuxent	same/increase	decrease	increase	same	same
Mid-Bay	increase	decrease	increase	same	same
'79-'81					
Calvert Cliffs	decrease	same	decrease	increase	decrease
Scientist Cliffs	decrease	same	decrease	increase	same
Patuxent	decrease/same	same	decrease	increase	decrease
Mid Bay	decrease	same	decrease	increase	both

OBJECTIVE 3

OYSTER AGE, STUNTING AND HEAVY METAL ACCUMULATION

Introduction

High metal concentrations were found in small oysters from an unpolluted area similar to a population reported by Burrell (1981) and since it is difficult to determine the exact age of field oysters this was suggested due to stunting (Krantz, pers. comm.). This study was undertaken to determine the relationship of oyster age and stunting to metal accumulation.

Methodology

Two year-class sets of laboratory-spawned oysters which were one through four and one through five years old. One set was spawned by Dr. George Krantz of the University of Maryland Horn Point Laboratory, raised in field trays over the summer and held in flowing estuarine water in the laboratory during the winter. The second set was spawned by Mr. Frank Wilde, Chesapeake Bay Oyster Culture, Shadyside, MD, and raised in field trays at that location. Thirty oysters from each year class were analyzed for copper and zinc as described in Section I; shell length and width and tissue dry weight were measured as growth indicators.

Results and Discussion

Horn Point third year oysters had significantly lower wet and dry tissue weights than second and fourth year oysters, therefore the third year population was not used to calculate trends in metal concentrations (Fig. 7). Multiple regression equations for the remaining year classes indicated Horn Point oysters were significantly stunted with almost no increase in tissue dry weight with age (Table 3) and decreasing shell length/width ratio was also indicative of stunting (V. Burrell, pers. comm.). Shadyside oysters appeared normal with increases in wet and dry tissue weight and shell size with age.

The causes of stunting are not known and could be inherent (genetic) or due to environmental factors. Natural stunted oyster populations such as reported by Burrell (1981) from the Wando estuary and from Deep Neck Broad Creek in the Chesapeake Bay (Phelps, 1980) are associated with high spatfall and have traditionally been used as sources of seed oysters for growth when transplanted, therefore they are genetically competent. Environmental stunting factors might include the high infestation of boring sponge at Deep Neck (though not at the Wando estuary), or the highly variable low salinities present at all three locations that may place osmoregulatory demands on amino acids normally employed for growth. Additional explanation for stunting may involve the high concentrations of copper and other metals in stunted oyster populations.

Normal (Shadyside) oysters had yearly decreases in copper and zinc concentrations while stunted (Horn Point) oysters accumulated copper at 9.8 ug copper/gm oyster wet weight/year and zinc at 167 ug zinc/gm oyster wet weight/year (Figure 7). The natural Deep Neck Broad Creek oyster population near Horn Point had some of the highest copper concentrations in the mid-Bay, averaging 81.5 ug/gm wet weight in 1981. Assuming a copper accumulation history similar to Horn Point oysters, Deep Neck oysters might average 8 + 3.1 years old, which corresponds with estimates of oyster age based on periodicity of recruitment and growth rate at Deep Neck (G. Krantz, pers. comm.).

Concentrations of copper and zinc in normal oysters were recalculated as if they were stunted (Figure 8) which showed normal oysters accumulated about half the metals of stunted oysters. Recalculated metal concentrations in normal oysters also had yearly decline, unlike the contihuous accumulation in stunted oysters, and both differences may be related to the greater surface available for metal depuration (K2 process) in larger oysters.

Both stunted hatchery oysters and Deep Neck (small) natural oysters had similar average copper/zinc ratios (Table 4). Normal field oysters obtained at the same time from a paired-salinity control station across the Bay (Hawks Nest) had copper concentrations similar to three and four year old Horn Point oysters but the absence of stunting gave a much lower copper/zinc ratio. High copper/zinc ratios have been suggested as indicative of metal enrichment (Huggett, 1973; Phelps, 1979) but since this is also found in field-stunted oysters the average oyster weight should be noted in field surveys.

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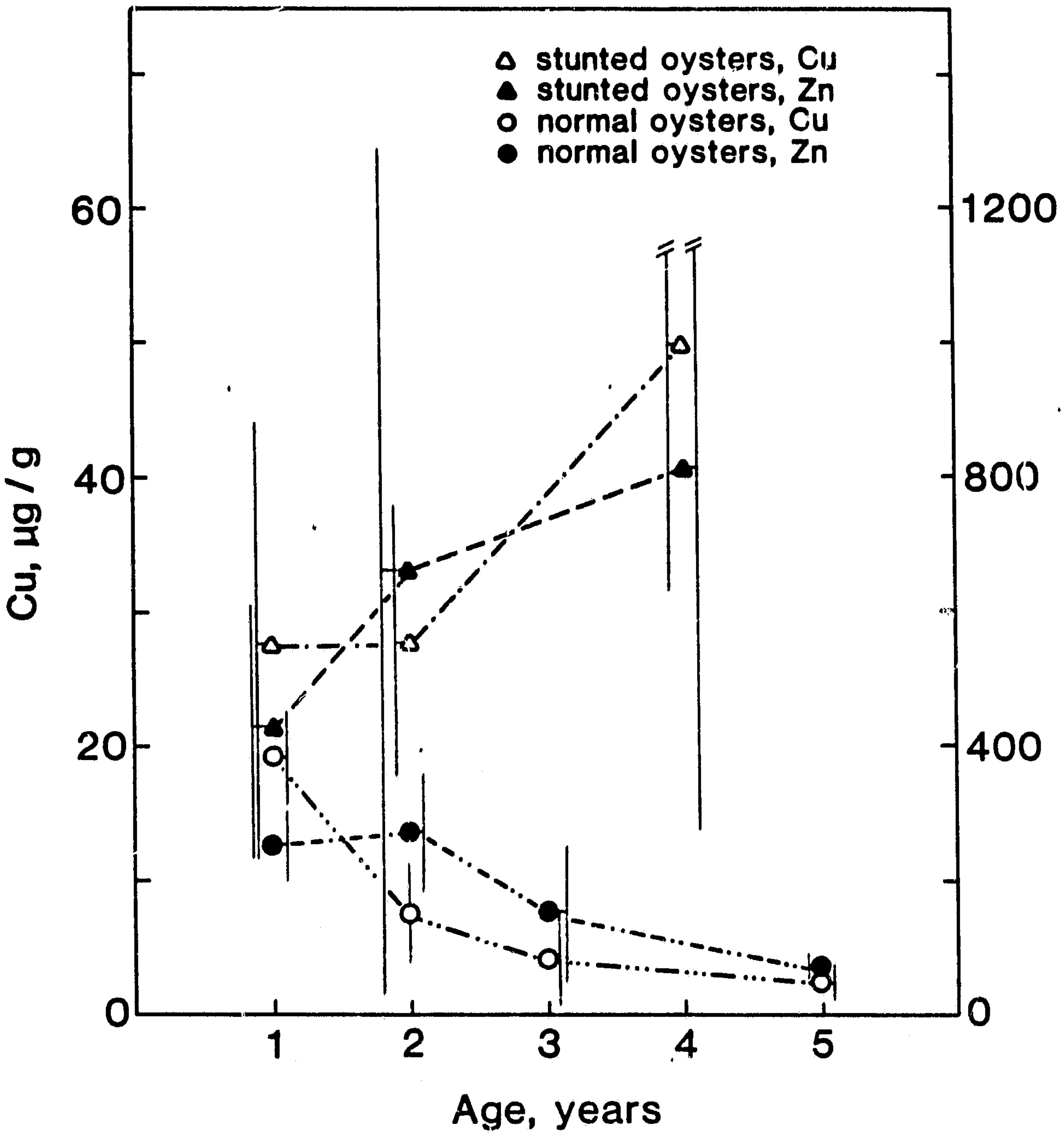

Figure 7; Copper and Zinc Concentrations in Age Classes of Normal and Stunted Oyste

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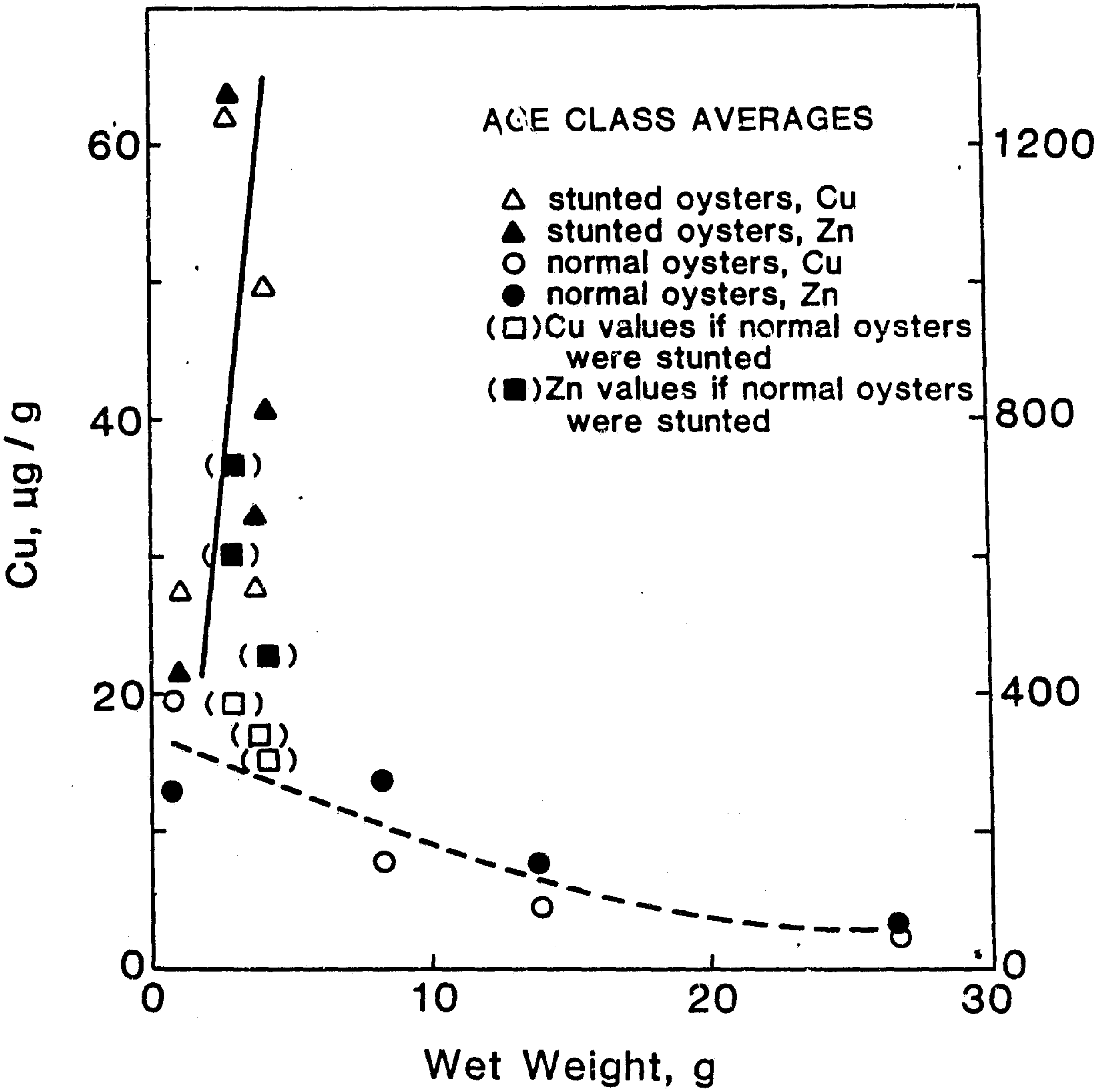

Figure 8: Copper and Zinc Accumulation in Age Classes of Normal and Stunted Oysters

TABLE 3

Multiple Regression Equations For Oyster Year Classes

	Shadyside Oysters	Horn Point Oysters
Wet Weight (gm)	= -4.96 + (6.35 x Age)	= .961 + (.842 x Age)
Dry Weight (gm)	= -.840 + (1.05 x Age)	= .309 + (.0638 x Age)
Shell Length (cm)	= 2.89 + (2.13 x Age)	= 3.64 + (1.00 x Age)
Shell Width (cm)	= 2.33 + (1.37 x Age)	= 1.98 + (.823 x Age)
Shell Length/Width	= 1.28 + (.050 x Age)	= 1.74 - (.072 x Age)
Copper Concentration (ug/gm)	= 18.9 - (3.83 x Age)	= 16.9 + (9.78 x Age)
Zinc Concentration (ug/gm)	= 330. - (51 x Age)	= 362. + (167. x Age)
Copper/Zinc	= .652 - (.084 x Age)	= .558 + (.013 x Age)

TABLE 4

Metal Concentrations in Normal, Field Stunted and Hatchery Stunted Oysters

Station	N	Salinity (ppt)	Wet Weight (gm±S.D.)	Copper Conc. (ug/gm±S.D.)	Zinc. Conc. (ug/gm±S.D.)	Copper/Zinc (x10^2)
Normal (Hawks Nest)	31	14.6	6.0±3.11	43.±24.0	1200±492	3.3
Stunted (Field, Deep Neck)	35	14.3	2.8±1.21	82.±28.9	1434±326	5.7
1 Year (Hatchery Stunted, Horn Point)	35	(5-20)	1.1±.61	28.±9.7	430±192	6.2
2-4 Year (Hatchery Stunted, Horn Point)	105	(5-20)	3.7±1.59	47.±25.5	869±416	5.1

OBJECTIVE 4

COPPER SORPTION CHARACTERISTICS OF CHESAPEAKE BAY SEDIMENTS

Methodology

Sediment samples were collected in 1974 (before the start of plant operations) with an epoxy-coated anchor dredge from four sampling locations in the Chesapeake Bay near Calvert Cliffs (Figure 9). Three stations, 1, 2 and 3 were at 3, 7 and 10 meter depths at Calvert Cliffs, station 4 was at 10 meters depth 3.2 km south of Calvert Cliffs at Rocky Point. Sediment samples were handled in plastic and stored unfrozen at 2 deg. C to preserve colloidal structure. Experiments and analyses were performed within a month of collection.

Sediment type was determined by lyophilization and dry-sieving for grain size. Percent water content was determined by drying to constant weight at 90 deg. C and confirmed by the results of lyophilization. Percent organic material was estimated by weight loss on ignition to 600 deg. C for one hour (Wakeels and Riley, 1956). Humic acid content was estimated by shaking one gm sediment with 20 cc 0.5 N NaOH overnight at room temperature, filtering (0.2 u filter) and reading at 400 nm (Beckman Acta III Spectrophotometer) with humic acid (Aldrich) standards (Schnitzer and Kahn, 1972).

Influence of salinity on sediment copper sorption was estimated by comparing station 3 sediment copper sorption in distilled water and filtered (0.45 u) bay water from the collecting site (14 ppt salinity). Centrifugation (1000 G, 10 minutes) removed sediment and supernatent was analyzed for remaining copper by flame atomic absorption spectroscopy.

Influence of time on copper sorption was determined by sequential sampling of two concentrations of station 3 sediment/copper slurry (50 gm/l and 100 gm/l; 10 mg/l Cu). Remaining copper sorption studies were performed with Station 3 (unless otherwise noted) sediment slurries 1:20 (50 gm/l) in distilled water sampled at 10.0 minutes.

Influence of pH on copper sorption was examined by adjusting pH of sediment slurries to values from 1 to 9 with 0.2 N NaOH or 0.1 N HCl.

Maximum sorptive capacity of sediments for inorganic copper was determined by slurrying sediment with up to 740 ug/cc copper ($Cu(N0_3)_2$, Fisher, reagent grade). Sediment concentration from stations 1, 2 and 3 (sand) was calculated as weight of damp sediment corrected for previously measured water content; station 4 sediment concentration (clay-silt) was measured by drying and weighing a 10 ml slurry aliquot. Station 3 sediment was also tested for maximum copper sorption following lyophilization and following ashing.

Results and Conclusions

Grain size analysis and water content of sediment from stations 1, 2 and 3 indicated they were of the sand type characteristic of the shore and neighboring shelf zone of the Chesapeake Bay (Table 5) while station 4 sediment was similar to clay-silt sediment described by Biggs (1967) as comprising the majority of central Bay sediments.

Organic carbon contents of sediments were higher than reported by Biggs (1967) for similar sediment types, which may have been due to Biggs' pretreatment of sediment samples with HCl digest.

Copper sorption was the same in distilled water and 14 ppt salinity implying Ca++ and Mg++ did not compete for copper binding sites in sediment since both Ca++ and Mg++ in 14 ppt salinity water exceed experimental copper concentration by 10 to 100 times. These results also suggested complexing between copper and inorganic ligands such as chlorine was not critical to sorption behavior.

Copper sorption in wet sediment slurries increased asymtotically with time approaching a maximum in 40 minutes (Figure 10).

The slurry pH had a pronounced effect on sediment copper sorption (Figure 11) with loss of sorption below pH 3. Teleosts have a gastric pH of 1 while invertebrates are reported to have a gastric pH of 3 (Prosser, 1973) thus it might be possible for fish but not invertebrates to release copper sorbed to ingested sediment. This pH-Cu desorption curve is similar to pH titration curves for dissociation of humic acid-metal complexes reported by Schnitzer and Kahn (1972) and dissimilar to dissociation curves for ligand-free Kaolinite clay-copper complexes having loss of sorption below pH 5 (Payne and Pickering, 1975). Therefore, the primary sorber of copper in sediment may be humic acids or other organic molecules as suggested by Steger (1973), but supported by clays since maximum sediment copper sorption was linearly related to percent clay-silt content (Figure 12) like most sorbed metals as reported by Sinex (1981).

The two types of sediment had widely varying copper sorption capacity, with clay-silt sediment (station 4) sorbing 18-44 times more copper per gram than sand sediment (Figure 13). Both lyophilization and ashing reduced sediment maximum copper sorption by about 80% (Figure 14), indicating the majority of copper sorptive behavior depended on colloidal structure, which implicates both organics and iron and manganese hydroxides. Supporting this conclusion is the positive correlation between sediment copper sorption and weight loss on ignition (Figure 15).

Extractable humic acids ranged from 0.12 mg/gm in sand sediment to 5.0 mg/gm in clay-silt sediment, nearly 5 percent of the total weight loss on ignition (Table 5). Assuming one mole-equivalent of humic acid copper-binding capacity has a molecular weight of 1000 (Schnitzer and Kahn, 1972; also personal observation) the theoretical copper binding capacity of sediment humic acid could account for only 1 percent of the maximum sorptive capacity. Other types of organics known to bind large amounts of metals such as polyanionic polysaccharides (Rendleman, 1978) may be important. Identification of copper-accumulating fractions in sediment will be important in tracing copper movement and biogeochemistry in estuaries.

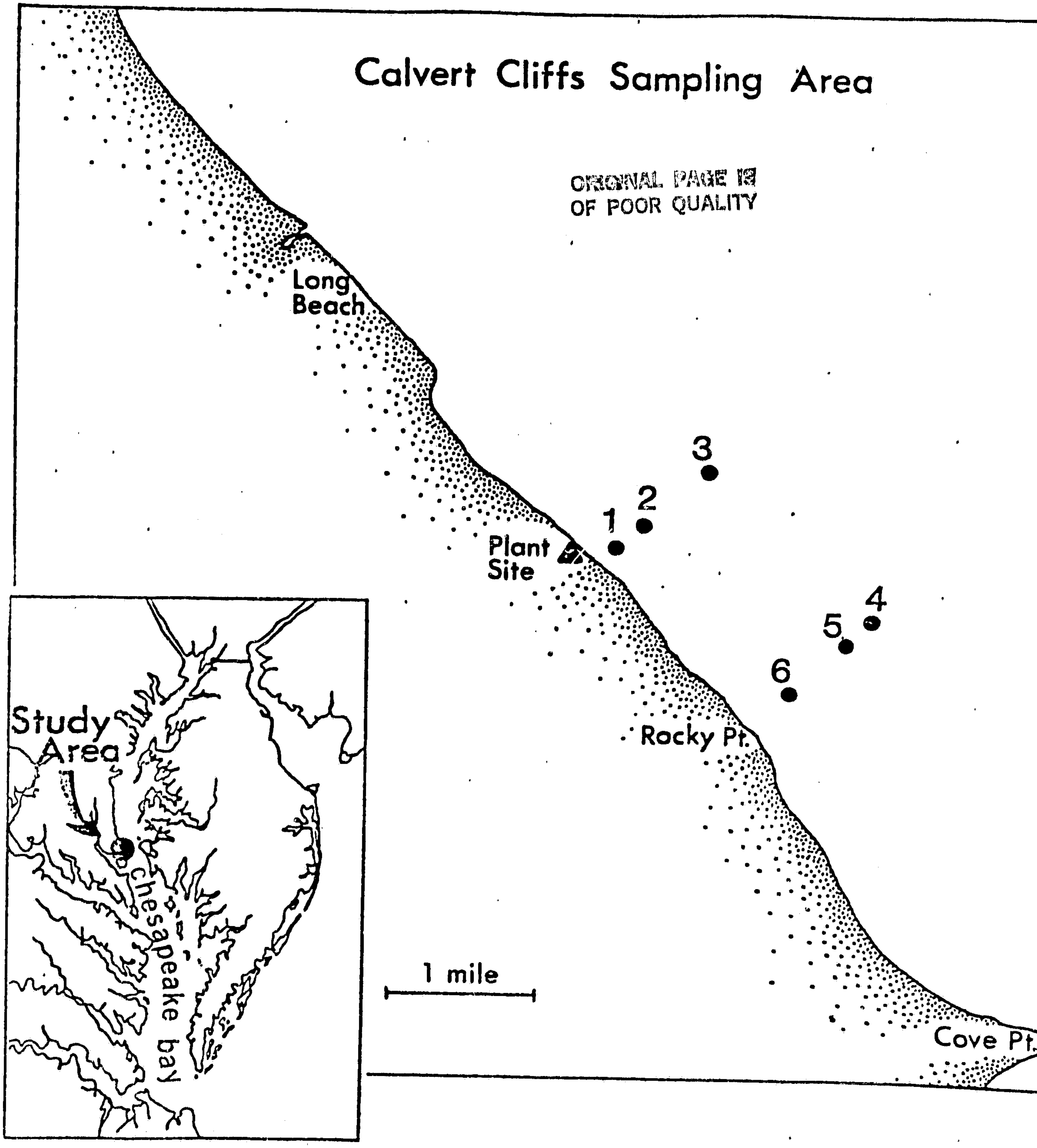

Figure 9; Chesapeake Bay Sediment Sampling Sites Near CCNPP

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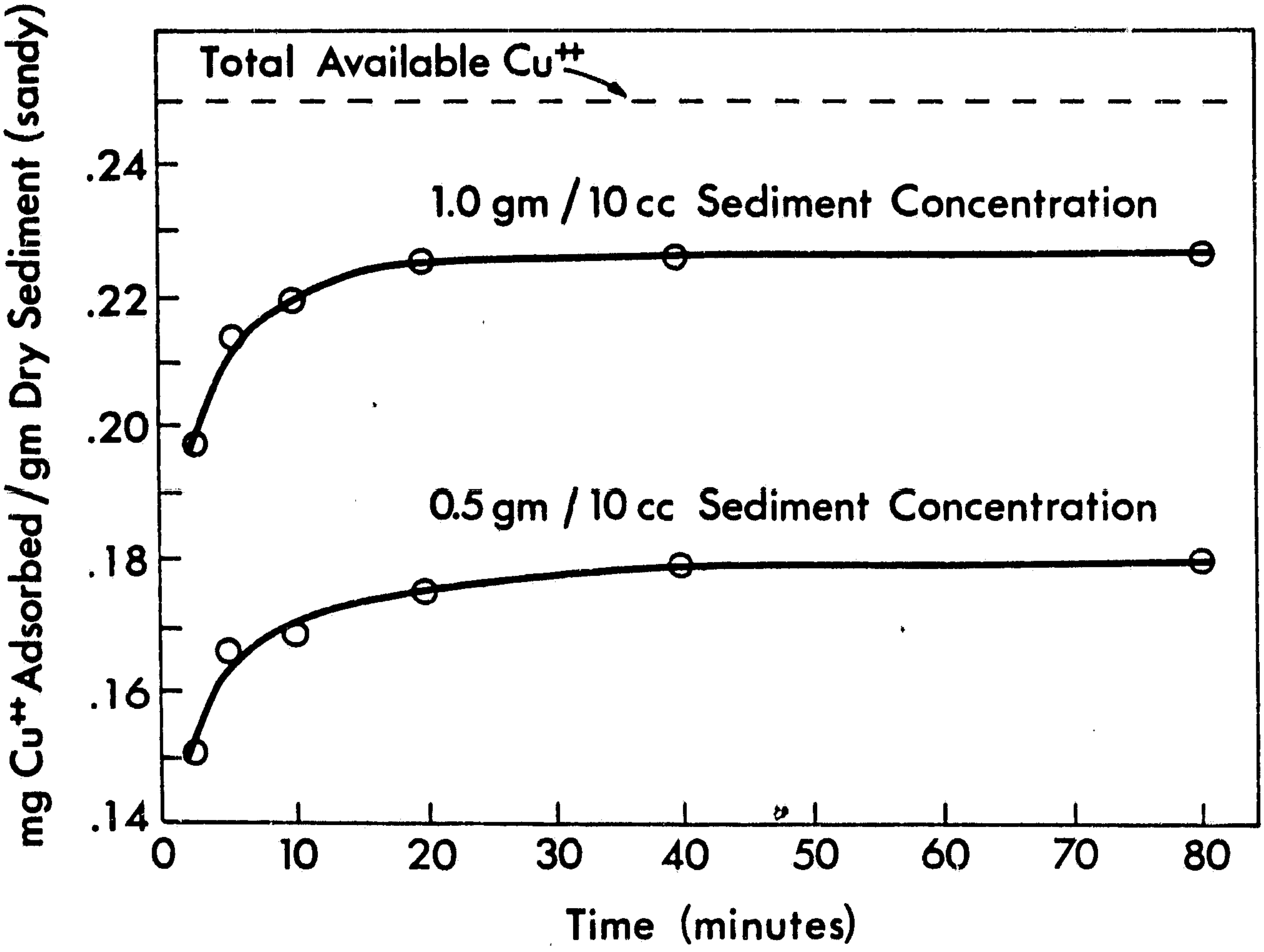

Figure 10; Sediment Copper Sorption

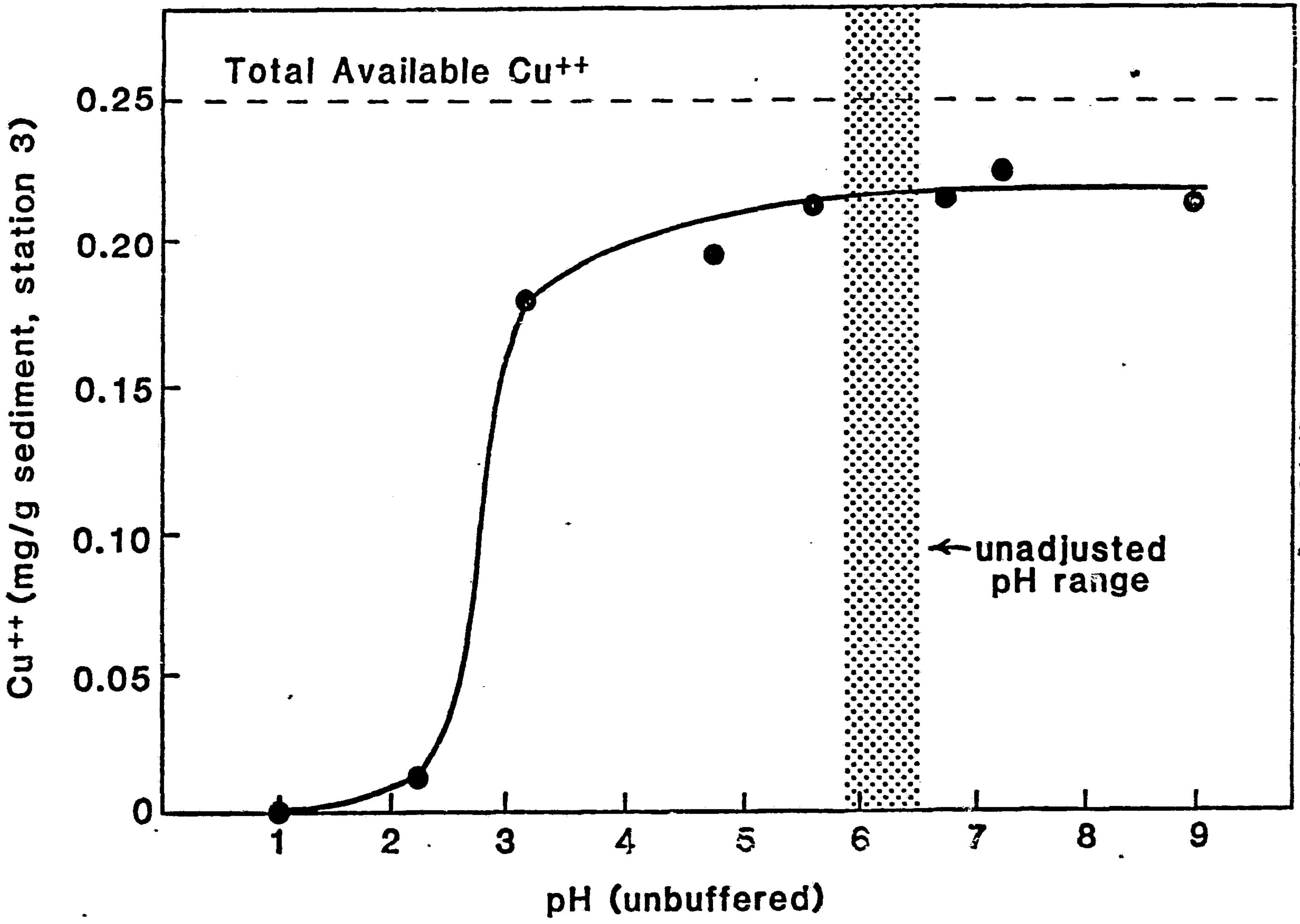

Figure 11: Sediment Copper Sorption and pH

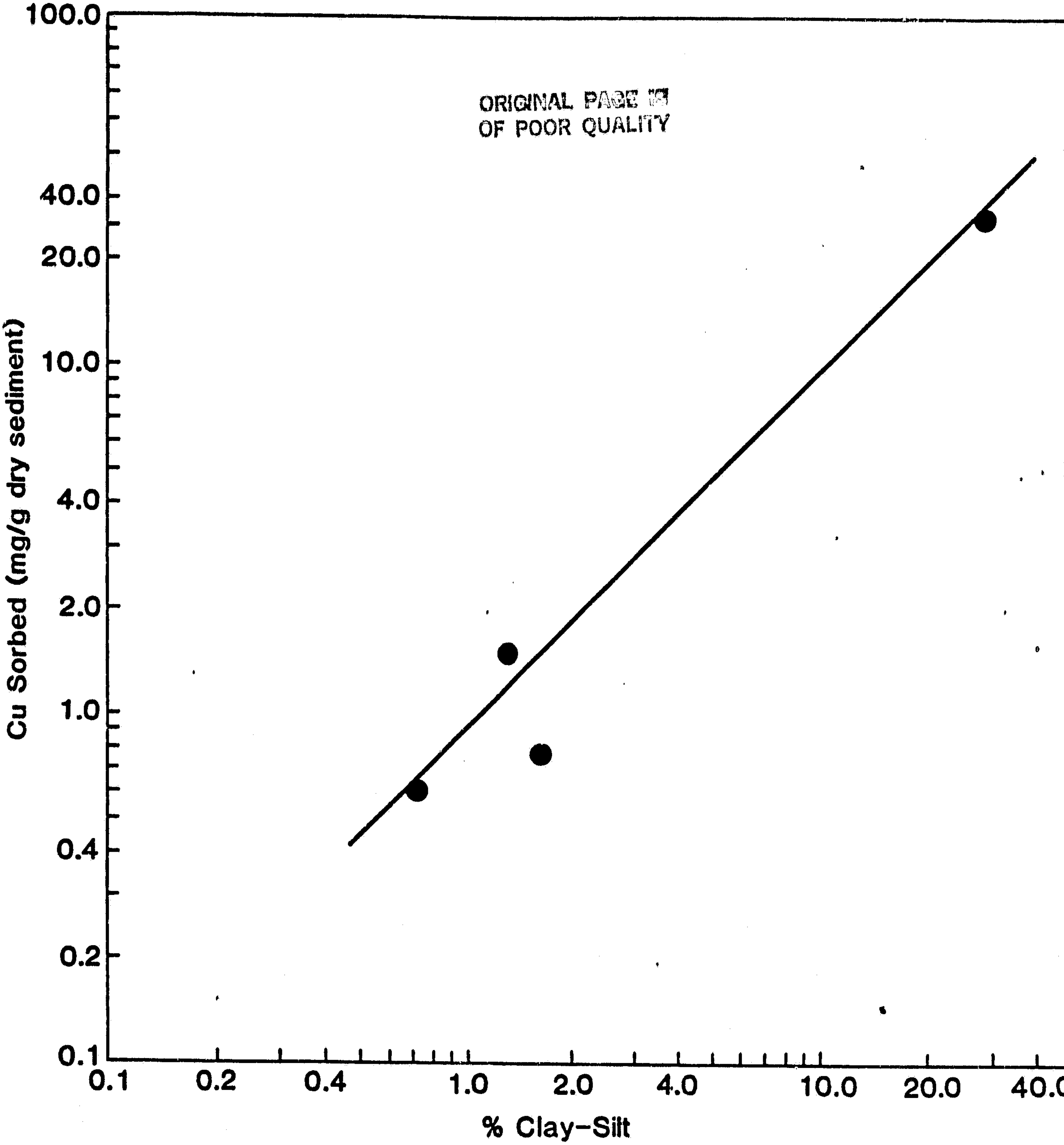

Figure 12: Sediment Copper Sorption and Percent Clay-Silt

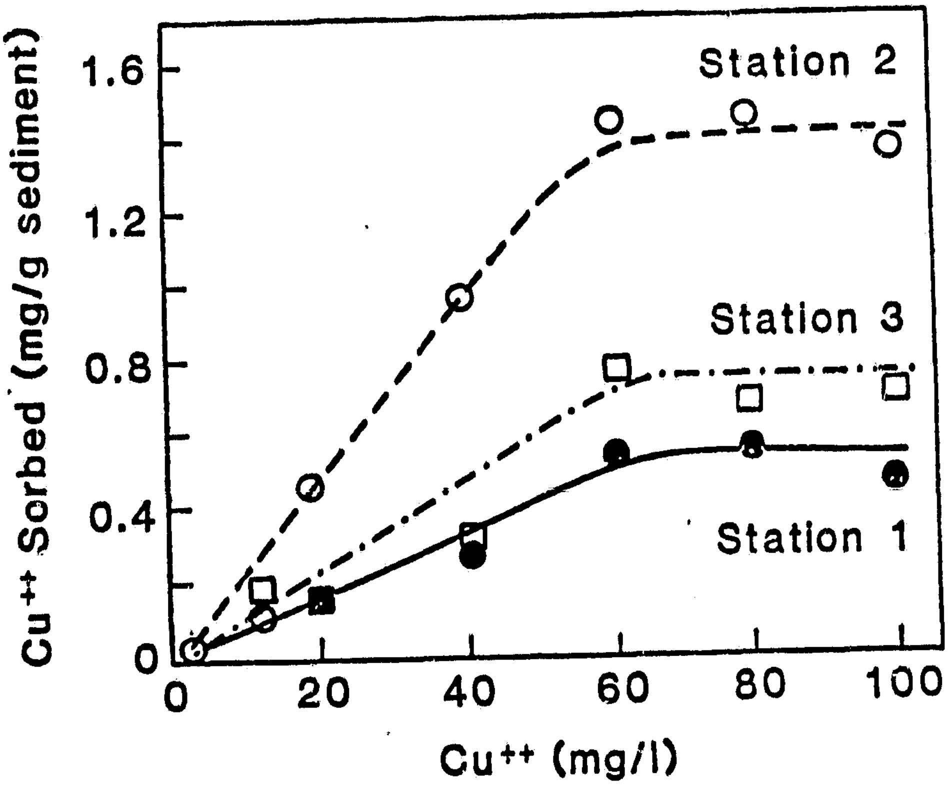

Figure 13: Sand Sediment Copper Sorption

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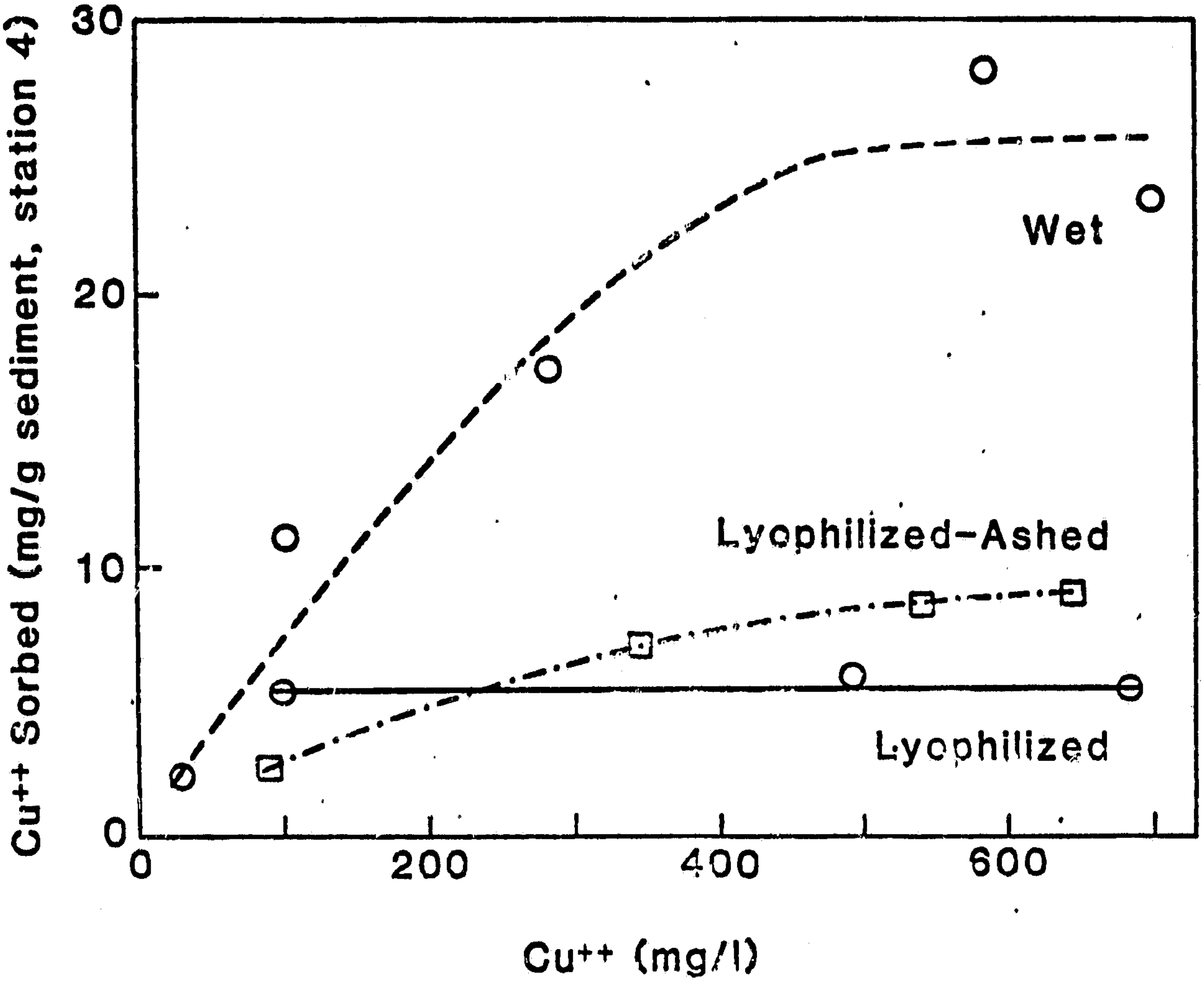


Figure 14: Clay-Silt Sediment Copper Sorption

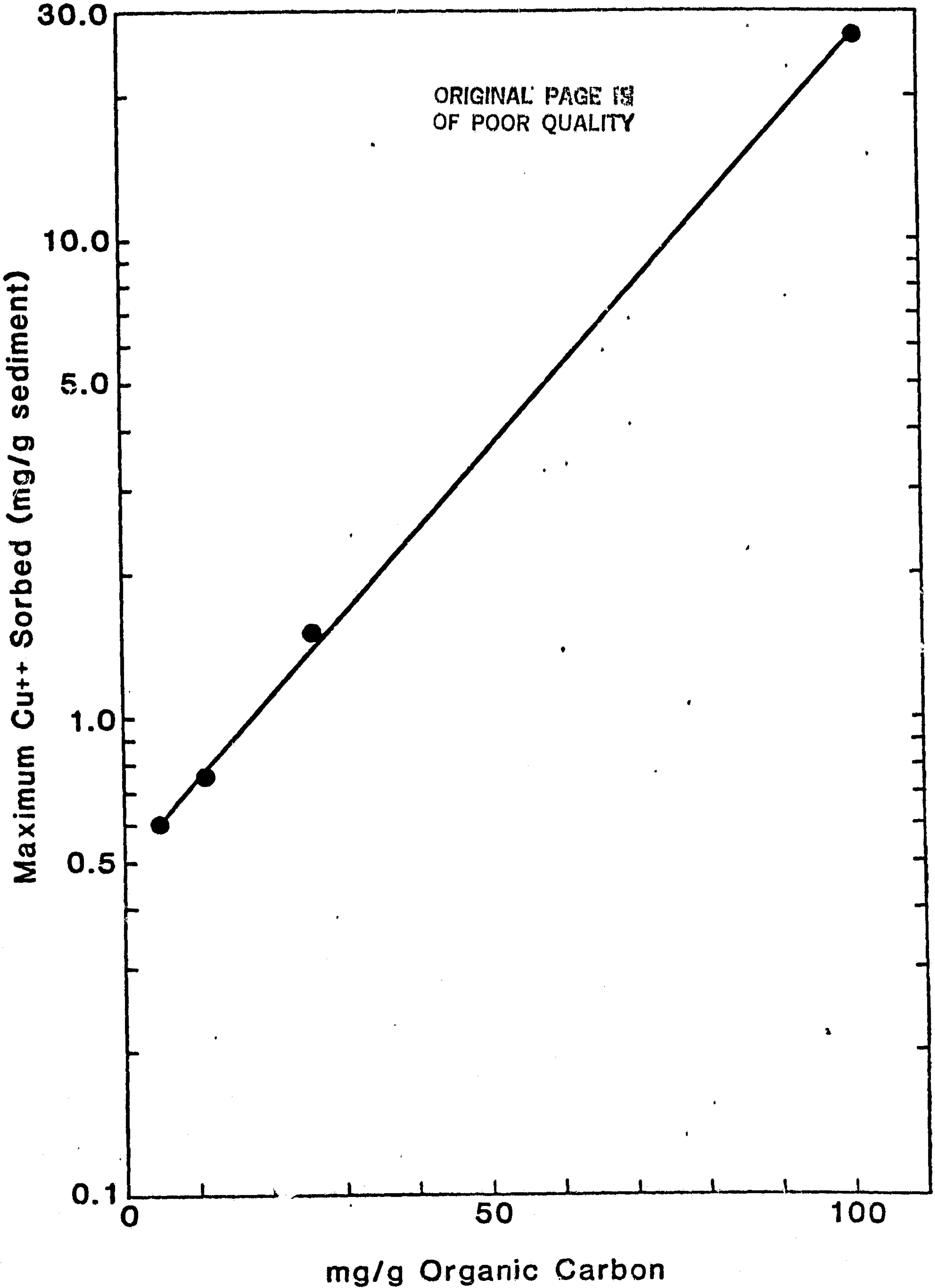


Figure 15: Sediment Copper Sorption and Organic Content

TABLE 5

Sediment Parameters

Station	% Water	%< 74 u (clay-silt)	Humic Acid (mg/gm sed)	% Weight Loss on Ignition (organic C)	Maximum Cu Sorption (mg/gm sed)
1	21. +.8	0.71	0.12	1.6 +.33	0.60
2	22. +.6	1.30	0.15	1.3 +.01	1.50
3	20. +3.8	1.60	0.13	.91 +.23	0.75
4	79. +.1	29.0	5.0	10.3 +3.1	26.5

OBJECTIVE 5

CADMIUM IN SEDIMENTS AND BENTHIC ORGANISMS

Introduction

Objectives of this study were to determine the relative mobility of cadmium in types of sediment near CCNPP through differential extraction procedures used for soil analysis (Black, 1965), and the relative bioavailability and bioaccumulation of sediment-bound cadmium to principal infaunal benthic species through individual analysis and size correlation.

Methodology

Sediments and infaunal benthics were sampled on June 1976 on a transect at Rocky Point (Figure 9), south of Calvert Cliffs. Transect stations were selected with the three characteristic sediment types and benthic communities of that region (Mountford, e.a., 1977): station 4, ten meters depth, clay-silt sediment; station 5, six meters depth, mud-sand transition sediment; station 6, two meters depth, sand sediment. Sediment was obtained with an epoxy-coated anchor dredge, an aliquot reserved for cadmium extractions, and the remainder sieved through one-millimeter plastic sieving to obtain benthic macrofauna.

Sediment samples were lyophilized and analyzed as described in Objective 4 for grain size, water content, percent organic material and humic acid content. Differential sediment extractions were performed according to Black (1965). Ionic (water-soluble) cadmium was extracted from 2.0 gm lyophilized sediment by shaking overnight with 10 ml deionized water in acid-cleaned polycarbonate flasks. The supernatent from centrifugation (1000 G, 10 minutes) was analyzed for cadmium by graphite furnace atomic absorption spectroscopy (GFAAS). Electrostatically (weakly) bound cadmium was extracted using the same procedure with 0.1 N NH4Ac; organically bound cadmium with 0.5 N HCl; humic acid bound cadmium with 0.5 N NaOH, and the extract additionally filtered (0.2 u) before analysis; total bound cadmium with concentrated HNO3 at 85 deg. C for two hours.

Immediately upon return to the laboratory one set of benthic organisms was rinsed, blotted live, placed in pre-weighed plastic vials and weighed. Another set of organisms was held for six days at 10 deg. C with daily changes of estuarine water to achieve gut clearing before analysis (Bryan, e.a., 1973). Individual organisms were digested with Ultrex nitric acid at room temperature for 24 hours and diluted 1:4 with deionized water for cadmium analysis by GFAAS. All determinations were made in triplicate with one to three percent precision.

Results and Conclusions

Chemical and physical analysis of sediments confirmed the types sampled (Table 6).

Cadmium concentrations were below detection limits in sediment extracts with deionized water and with 0.1 N NH4Ac, but cadmium was extracted from all sediments in almost equal amounts by 0.1 N HCl, concentrated HNO3 and 0.5 N NaOH (Table 6). This uniformity suggests cadmium was bound loosely to the organic fraction of sediments, possibly to the humic acid fraction as proposed by Gardiner (1974) for riverine sediments.

Station 4 clay-silt sediment had 1300 times more cadmium than sandy sediment but there was no comparable increase in cadmium concentration in clam worms (Nereis succinea) or the Baltic macoma clam (Macoma balthica) (Figure 16). This lack of correlation may indicate sediment sorption limits cadmium bioavailability. Bryan (1973) reported correlation between log of cadmium concentration in estuarine sediments and in a similar worm species (Nereis) over a narrower range of sediment cadmium concentrations. One possible explaination might be that Bryan obtained worms from several different estuarine locations and did not record salinity, which has a profound effect on cadmium uptake rates (Objective 6) and cadmium concentrations in shellfish (Objective 2).

Mean cadmium concentrations in all benthic species ranged from 0.1 to 0.3 mg/gm wet weight (Figure 16), corresponding to values reported in the literature for similar species (Mullin and Riley, 1956). Holding N. succinea to eliminate gut contents had little effect on mean cadmium concentration, and though holding young soft-shell clams (Mya arenaria) decreased average cadmium concentration, range and standard deviation, the differences were not statistically significant. Large standard deviations indicated lack of normality for cadmium concentrations in all species so nonparametric statistical tests (Mann-Whitney) were used.

Cadmium concentations in N. succinea and M. arenaria had no significant correlation with weight which indicated lack of bioaccumulation in those common species. The bimodal weight distribution in M. balthica suggested two year classes, and significantly increasing cadmium concentrations showed long-term cadmium accumulation (Figure 17). Such differences may be related to different feeding strategies: N. succinea ingests buried sediment, M. arenaria filters food from the water column while M. balthica feeds on surface sediments more likely to be enriched in sorbed metals (Swope, 1978). This difference may pose a metal accumulation hazard for animals such as diving ducks that feed extensively on M. balthica in the Chesapeake Bay.

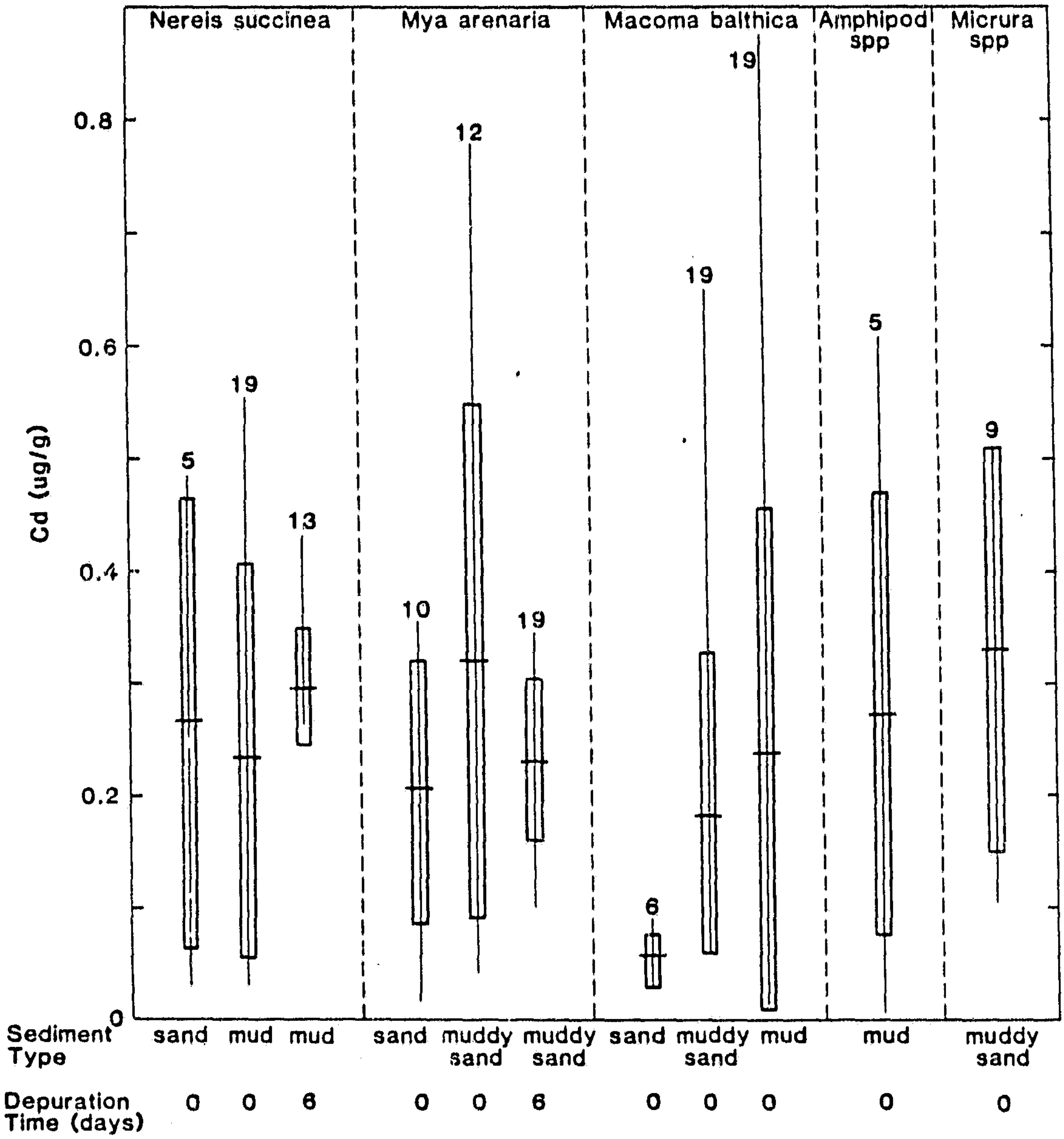


Figure 16: Cadmium in Benthic Organisms and Sediment Type

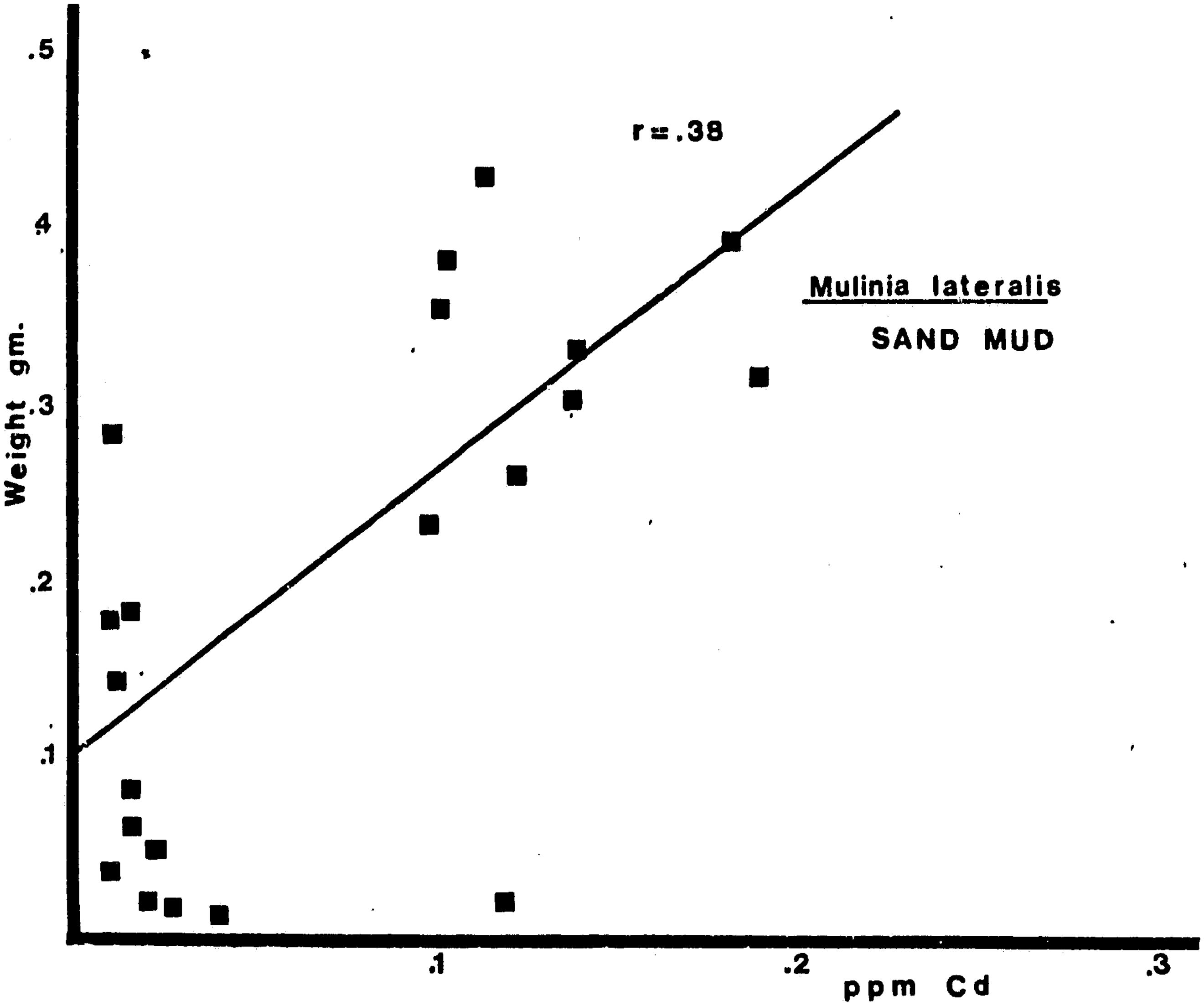


Figure 17: Cadmium Accumulation in *Mulinia lateralis*

TABLE 6

Differential Sediment Extraction for Cadmium

	Station 6 (sand)	Station 7 (sand-silt)	Station 8 (clay-silt)
Water Content (%)	22.	21.	80.
Ignition Wt. Loss (%)	0.1	0.8	11.0
Humic Acid (mg/gm sed)	0.12	0.15	4.1
Mg Cd/gm sed (0.1N HCl)	0.05	0.06	0.40
Mg Cd/gm sed(conc. HNO3)	0.04	0.03	0.37
Mg Cd/gm sed(0.5N NaOH)	0.01	0.01	0.41

OBJECTIVE 6

CADMIUM UPTAKE BY SHELLFISH

Introduction

The pathways of metal uptake by marine organisms are not clear and may include both water and ingested components. Metal uptake rates in benthic molluscs were studied by short-term static uptake experiments using cadmium because of its convenient radioisotope (109Cd). Small soft shell clams (*Mya arenaria*) were used in most experiments because they are easily observed when pumping water which is critical to short-term uptake studies. Small oysters were used in some experiments. Factors studied affecting cadmium uptake included salinity, concentration, sorption to seston/sediment components and sorption to a chelating resin (Chelex 100).

Methodology

In the spring, small (1-2 cm) first-year *M. arenaria* were field collected by anchor dredge from mid-Chesapeake Bay and hand collected from the flowing seawater system at the Chesapeake Biological Laboratory, Solomons, MD. For experiments with oysters, small (1-2 cm) culchless oysters (*Crassostrea virginica*) were purchased from Mr. Frank Wilde, a commercial grower at Shadyside, MD. Clams and oysters were held in shallow trays at 10 deg. C up to two months before experiments. Water was replaced periodically and this treatment did not affect subsequent viability. Clams were acclimated for one week in filtered (Chelex-100) artificial seawater (Kester, e.a., 1977) for variable salinity experiments.

Cadmium uptake experiments used thirty M. arenaria in 500 ml aerated filtered (Chelex-100) estuarine water in plastic containers. Clams were warmed gradually to room temperature over three hours and when siphoning vigorously, sorbing suspensions with radioactive cadmium were added for a final concentration of 1 mg sorbing agent/ml, similar to moderate Bay seston concentrations (Clarke and Palmer, 1972).

Cadmium sorbing suspensions were prepared of 100 mg/ml in distilled water of fresh clay-silt sediment collected from Rocky Point, bentonite clay (Fisher), humic acid (Aldich), albumin (Fisher) and a control (deionized water). Radioactive 109Cd (New England Nuclear) was diluted with stable $Cd(NO_3)_2$ to give a final concentration of 10 ug Cd/l (within EPA guidelines) and added to sorbing suspensions. Cadmium complexation was measured with an Orion Model 94-48 specific-ion electrode and Orion Model 90-01 single-junction reference electrode, and was 97% or greater.

For analysis of 109Cd uptake, clams were removed, rinsed, blotted, tissues dissected from shells and re-rinsed. Shells and tissues were weighed separately and digested by warming in mini-vials with 1 ml concentrated HNO_3. Radioactivity was measured by double-vial counting which eliminated problems with quenching, chemiluminescence or geometry and gave 10% counting efficiency (Packard Model 3000 Scintillation Counter). Three clams were analyzed for each point unless otherwise noted. Radioactivity and pH were monitored in the medium.

The effect of salinity on cadmium uptake rate was studied with clams acclimated to 5, 10 and 15 ppt salinity artificial estuarine water before exposure to 109Cd.

The effect of concentration on cadmium uptake rate was studied by exposing clams to cadmium concentrations ranging from 10^{-10} to 10^{-6} *M*.

The effect of chelation by a readily sorbed food molecule, glycine, on cadmium uptake was studied by determining uptake rates of 109Cd in various concentrations of glycine, and uptake rates of 14C-glycine in various concentrations of cadmium.

The effect of digestive processes on bioavailability of Chelex-100 sorbed cadmium was examined with an artificial oyster food prepared by grinding Chelex-100 resin with liquid nitrogen and wet-sieving with Nytex screening to isolate grain sizes 0 - 15 u (Electrozone Celloscope, Particle Data, Inc.). 109Cd was sorbed to the artificial food which was presented to small oysters for three hours, after which feces and pseudofeces were collected and analyzed for 109Cd by extraction with 1 ml 0.1 *N* HNO3 and centrifugation (Misco micro-centrifuge). Particle size distribution in feces and pseudofeces was determined and oysters were analyzed for incorporated 109Cd.

Results and Discussion

M. arenaria showed uptake of 109Cd at 3.5 hours in both shells and tissues (Figure 18). Shell cadmium uptake was not correlated with weight but tissue cadmium uptake was significantly negatively correlated with weight, indicating surface sorption (Cunningham and Tripp, 1975).

Short-term cadmium accumulation by *M. arenaria* was greatest in the absence of sorbing agents and when cadmium was sorbed to bentonite which desorbs cadmium in salt water (Figure 19) as reported in Objective 7. Humic acid and sediment-sorbed cadmium had a small initial uptake followed by much greater uptake at five hours (Figure 20), about the time required for labelled amino acids to be assimilated from mucus sheets by mussels (Pequinat, 1973), which indicates the feeding process may be involved. Albumin-sorbed cadmium was not accumulated but albumin was observed to form sticky strings and may not have been ingested by clams.

The rate of cadmium uptake by clam tissue varied inversely with salinity (Figure 21) and was directly related to the calculated activity of the divalent cadmium ion as a result of inorganic complexation (Table 7) by the equation:

$$R = Ka \qquad (r^2 = .88)$$

where R = uptake rate of cadmium (mols Cd/hr, x 10^{-11}; K = 5.1 x 10^{-5}; and a = activity of Cd^{++} x 10^{-7}. Jackim, e.a. (1977) also observed a negative relationship between cadmium uptake by shellfish and salinity. Cadmium uptake rate was directly proportional to concentration over a wide range (Fig. 22) but not a simple function of divalent cadmium ion activity calculated from dilution (Table 7).

Both cadmium and glycine were absorbed from water by *M. arenaria* (Figure 23) but cadmium uptake was 10^5 mols/gm tissue/hr more rapid than glycine (Table 8). Levelling of glycine accumulaton at 12 hours and similar concentrations in tissues and liver suggests onset of metabolic processing and excretion processes since dissolved amino acids can be absorbed and used as food by marine invertebrates (Stephens and Schinske, 1961; Sorokin, 1973). Cadmium was linearly accumulated by clams to the end of the experiment, 50 hours, and was concentrated in surface tissues suggesting cadmium loss (K2 process) had not yet started. Jackim, e.a. (1977 reported linear cadmium accumulation by molluscs over weeks of exposure.

Since the presence of chelating amounts of glycine in water (50% of cadmium is chelated in 0.1 M glycine), did not change cadmium uptake rate by M. arenaria (Table 8), perhaps chelation by another sorbed molecule changes the previously reported relationship between metal speciation and toxicity (Sunda, e.a., 1978). It was unexpected to find increasing glycine uptake with increased cadmium concentrations as cadmium might have been expected to inhibit rather than enhance the active transport enzyme systems required for glycine uptake.

In seven oyster feeding experiments with cadmium sorbed to artifical seston (Chelex-100) only trace amounts (0.4% - 5%) were found in body tissues but up to 45% was in feces,indicating resistance of the Chelex-100 cadmium-inimodiacetic bond to digestive breakdown. Particle size analysis found no significant difference betweeen feces and pseudofeces (Fig. 24).

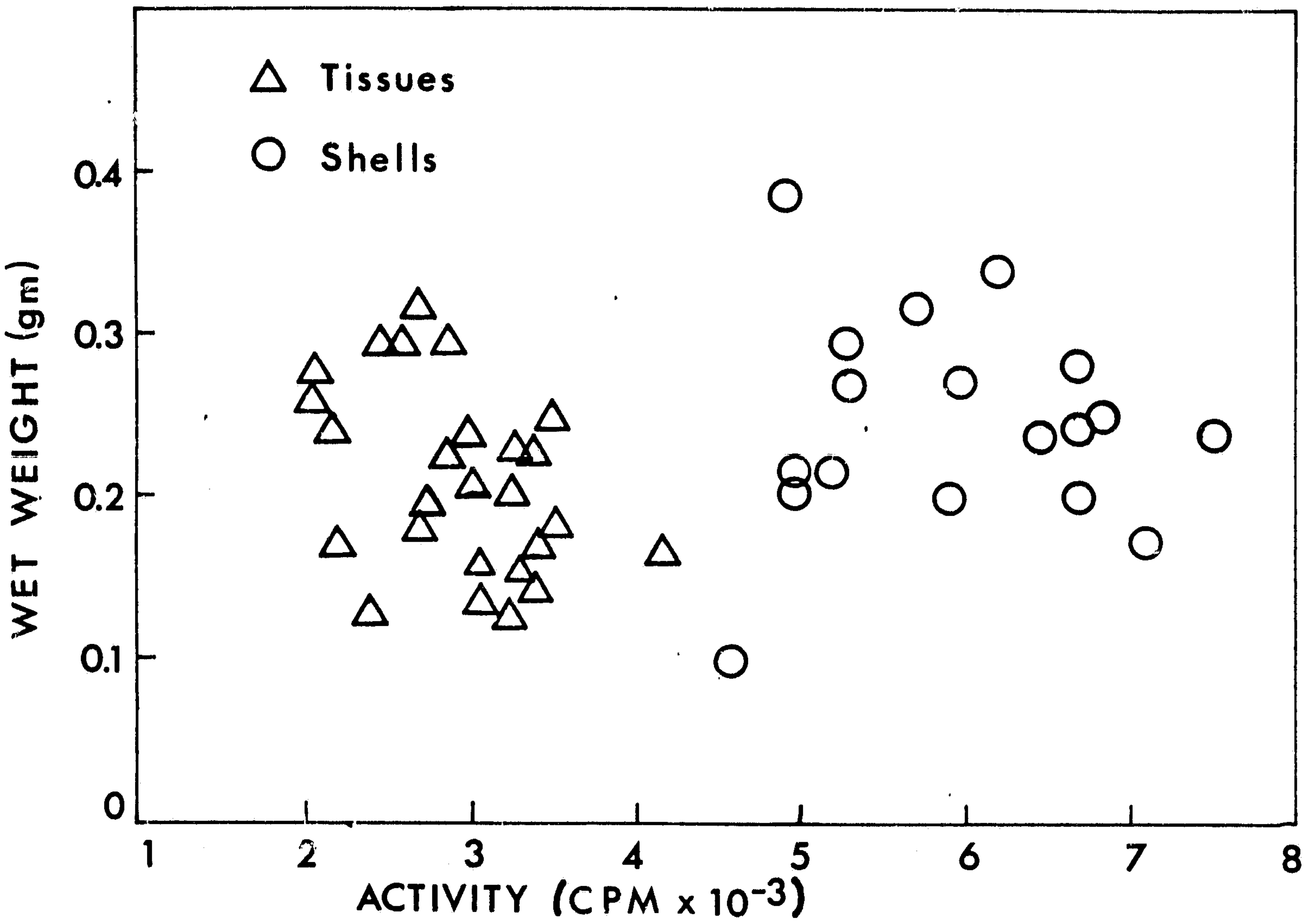


Figure 18: Cadmium Sorption by Shells and Tissues of Mya arenaria

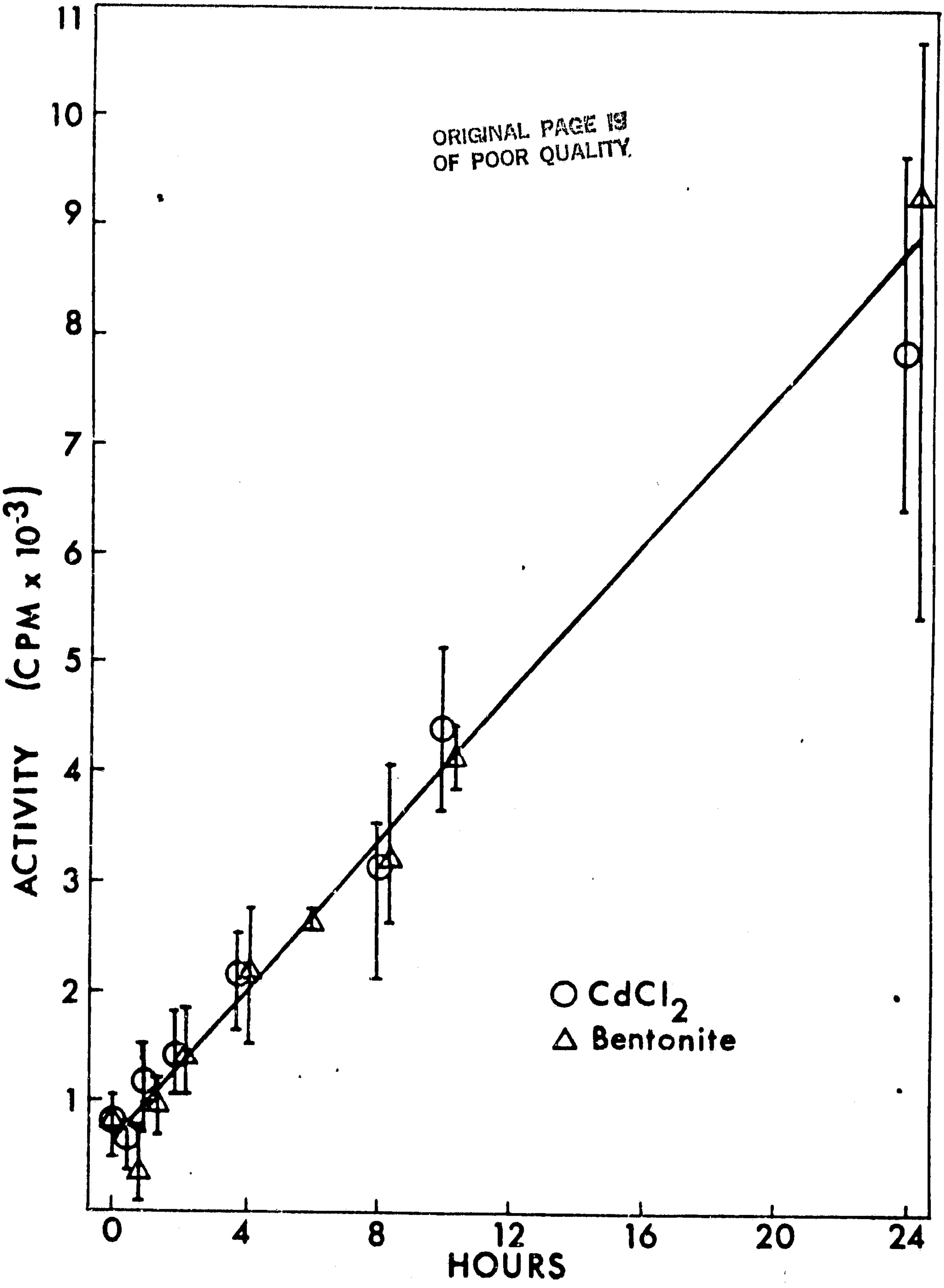
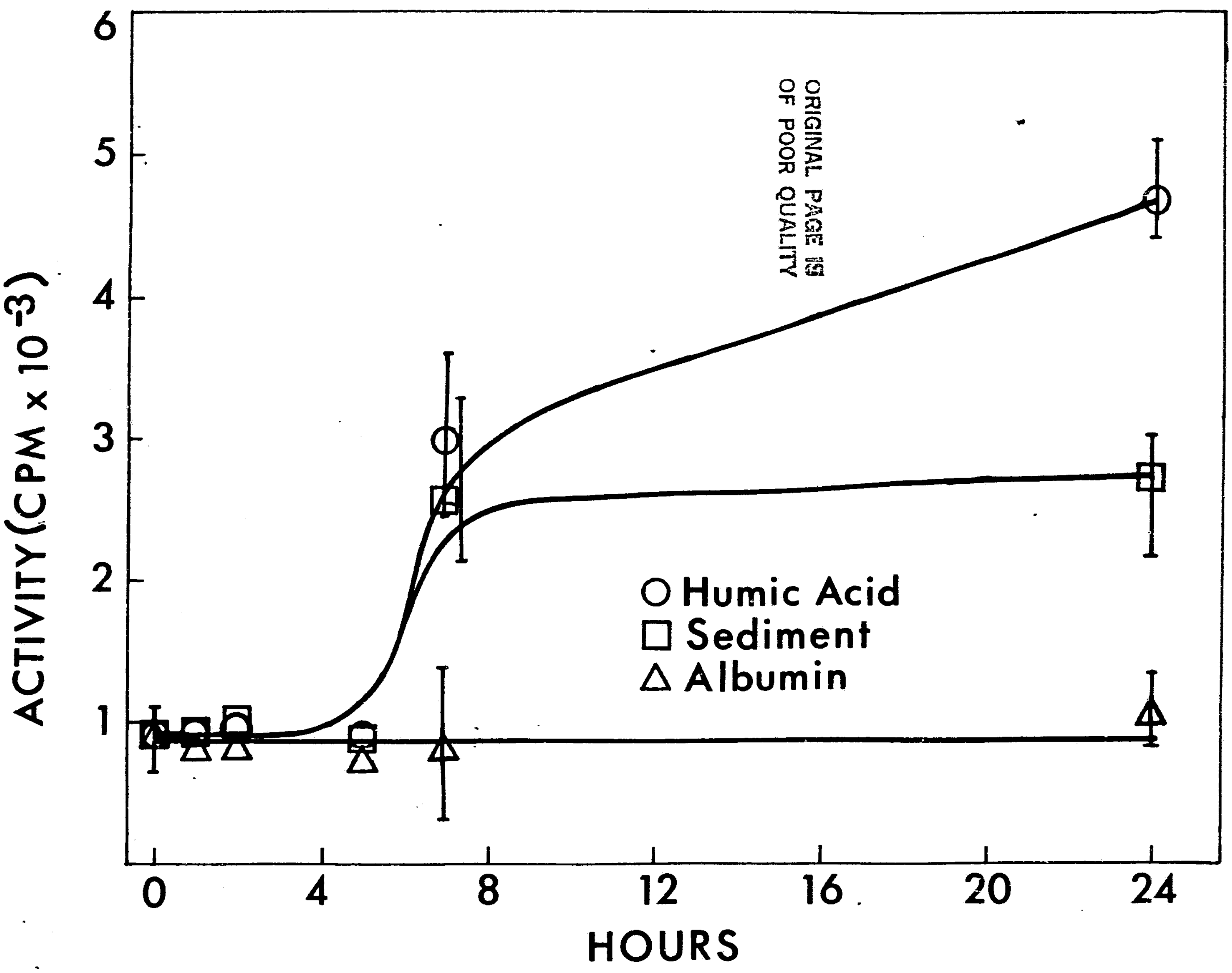


Figure 19: Cadmium Uptake from Bentonite and Water by _Mya arenaria_


6
5
4
3
2
1
ACTIVITY (CPM x 10⁻³)
0
4
8
12
16
20
24
HOURS
○ Humic Acid
□ Sediment
△ Albumin

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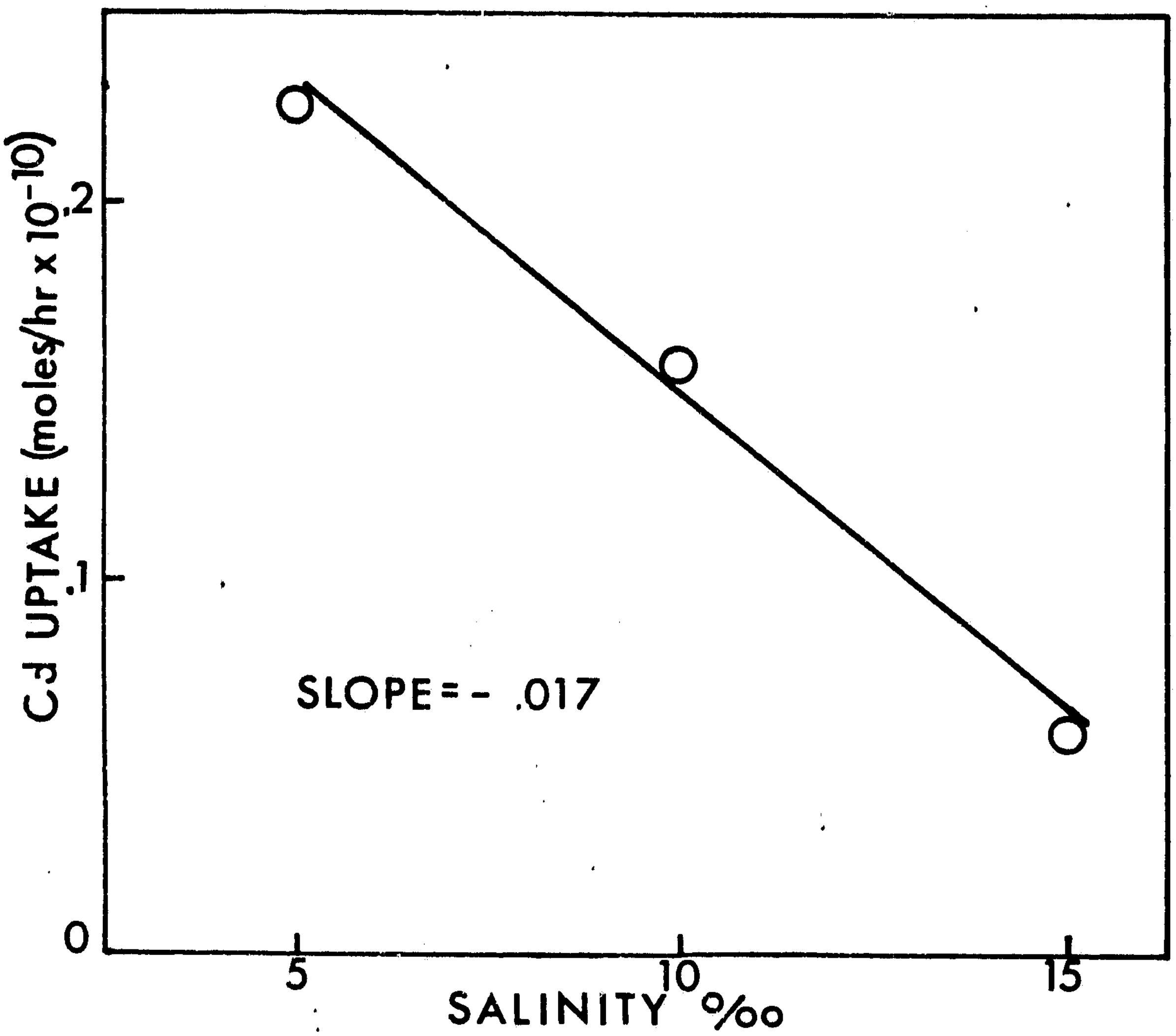

Figure 21: Clam Cadmium Uptake Rate and Salinity

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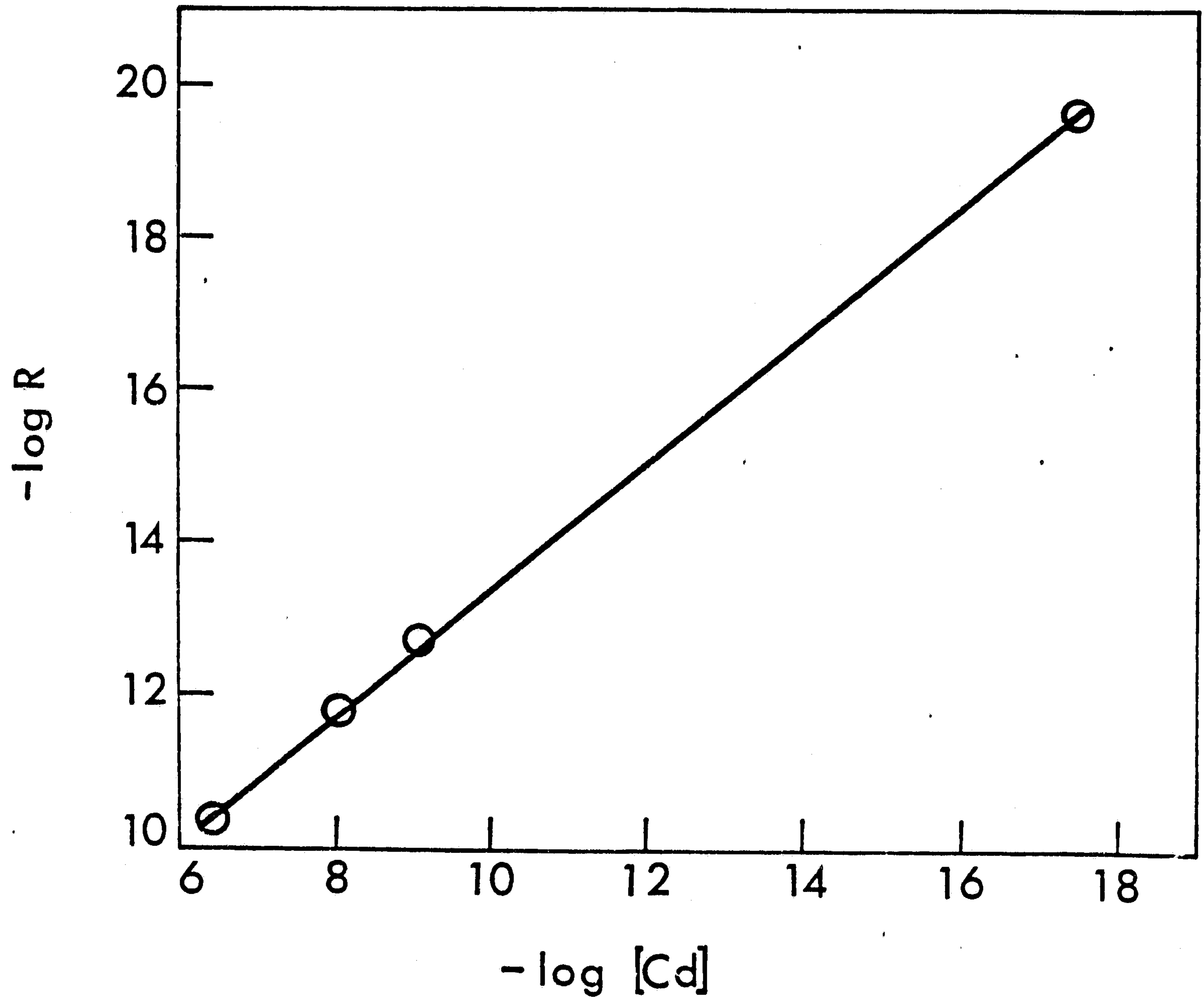

Figure 22: Clam Cadmium Uptake Rate and Cadmium Concentration (R) ug/ml

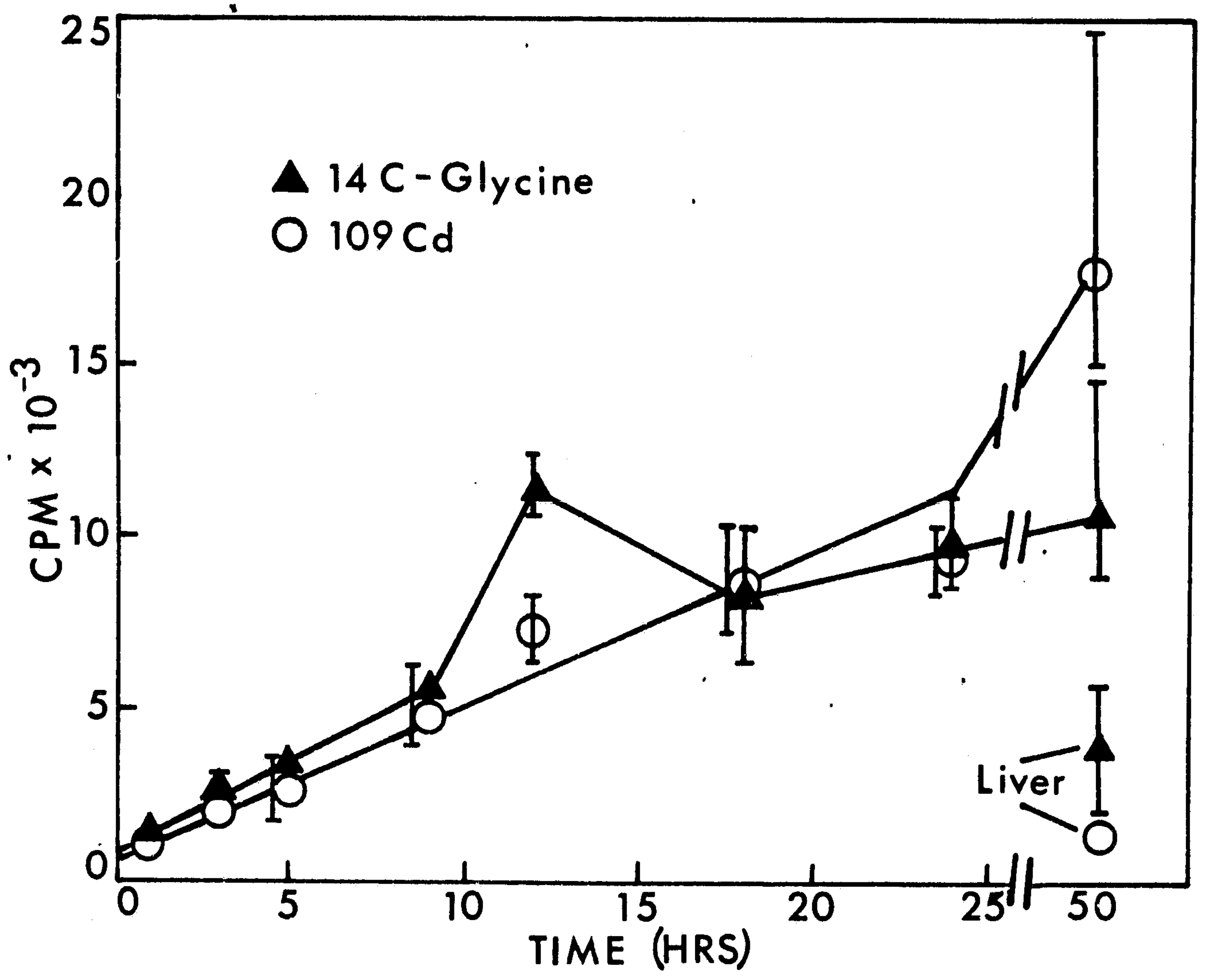

Figure 23: Cadmium and Glycine Uptake Rates in *Mya arenaria*

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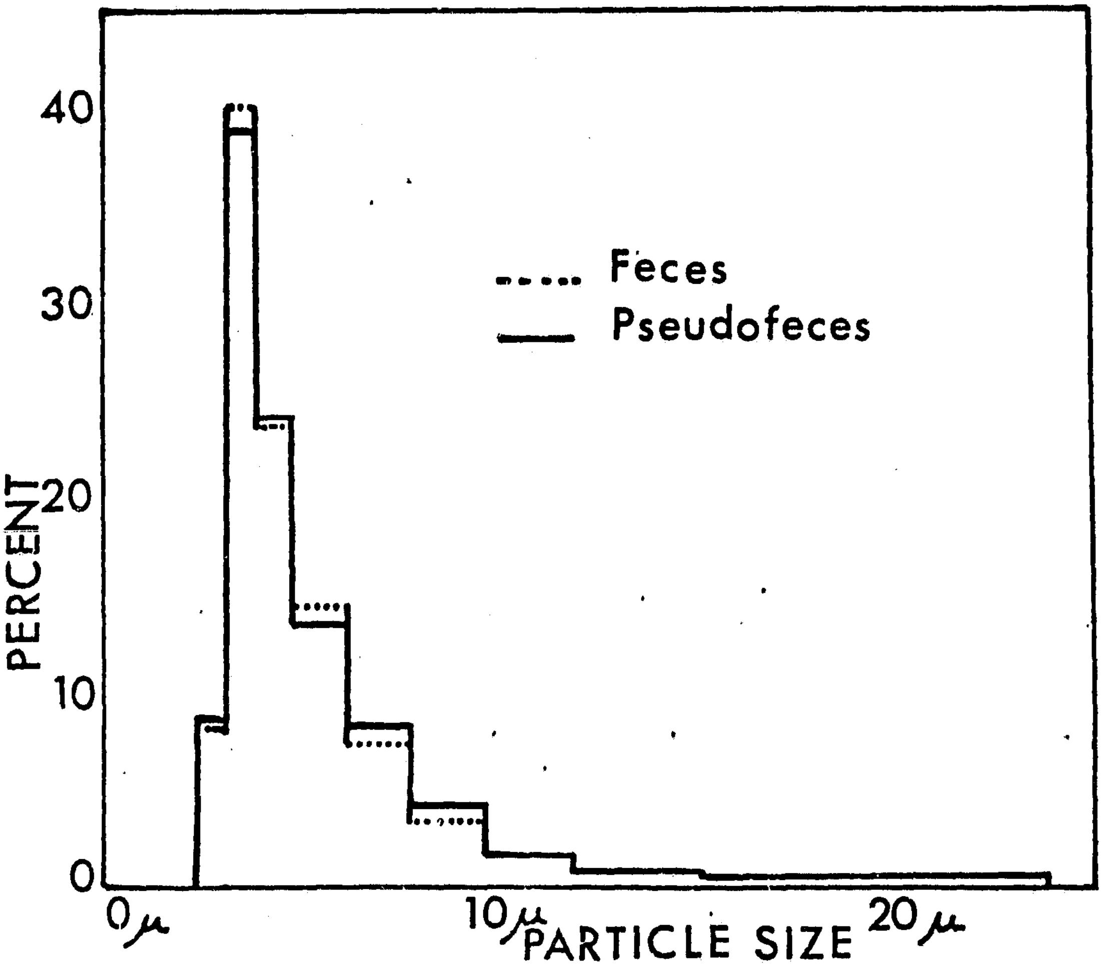

Figure 24: Nytex Particle Size Distribution in Oyster Feces and Pseudofeces

TABLE 7

Effect of Salinity and Concentration on Cadmium Uptake by *Mya arenaria*

Salinity (ppt)	Cadmium (ug/ml)	Activity aCd++(x 10^{-7})	Uptake Rate (R) (mols Cd/hr,x 10^{-11})	R/aCd++(x 10^{-5})
5	10	5.82	2.63	4.5
10	10	2.50	1.57	6.3
15	10	1.39	0.679	4.9
10	3.5x 10^{-9}	8.75x 10^{-9}	1.84x 10^{-8}	21.0
10	1	0.250	0.181	7.2
10	10	2.50	1.57	6.3
10	436	53.2	40.6	0.64

TABLE 8

Interaction of Cadmium and Glycine on Uptake Rates

Cadmium (ug/ml)	Glycine (mols)	Cadmium Uptake (mols/hrx10^{-11})	Glycine Uptake (mols/hrx10^{-6})
10	0	1.6	
10	0.01	1.3	
10	0.02	1.4	
10	0.1	1.5	
1	0.01		0.41
10	0.01		1.2
100	0.01		1.8

OBJECTIVE 7

CHLORINATION AND SEDIMENT CADMIUM DESORPTION

Introduction

The accumulation of copper by oysters (Roosenberg, 1969) and other molluscs (Swope, 1978) near the estuarine Chalk Point power plant has not been explained in terms of corrosion of metal from the power plant alone (Hill and Helz, 1973). Chlorination of Chalk Point cooling water has been shown to cause degradation of organic molecules while increasing concentration of some metals in the remaining colloidal fraction (Sigleo, e.a., 1980) and was suggested as a factor in desorbing metals from nearby sediments (Somer, e.a., 1974). Although chlorination was not originally permitted at CCNPP, the State has been asked to allow chlorinaton to control biofouling. This study examined the ability of chlorine to desorb cadmium sorbed to suspended sediment and metal sorbing components of sediments: clay, humic acid and protein.

Methodology

In experiments the natural concentrations of suspended seston and levels of chlorination at power plants were simulated as much as possible. A trace amount of radioactive 109Cd (2×10^{-16} M) was added to 60 ml filtered (0.45 u) Chesapeake Bay water (14 ppt salinity). Three 20 ml aliquots were put in polyethylene bottles: two bottles receiving 0.4 ml diluted (1:10) fresh clay-silt sediment, for a final concentration of 0.4 - 0.7 mg/l corresponding to ambient Bay seston levels, and one bottle as control receiving no sediment. Bottles were shaken for 30 minutes for cadmium sorption by suspended sediment, then one bottle with sediment and the control received 20 microliters 5% NaOCl (Fisher) and shaken for ten minutes. Chlorine levels measured by DPD test in the bottle without sediment corresponded to power plant chlorination concentrations, 1.5 - 2 ppm. All suspensions and the control were filtered through weighed 0.2 u Nucleopore filters and the filters were then dried and weighed to determine actual sediment concentrations. Filtrates were analyzed for pH, residual oxident (DPD test) and 109Cd.

Dialysis studies used suspensions of 1 mg/ml bentonite clay (Fisher), humic acid (Aldrich), albumin (Fisher), fresh clay-silt Bay sediment and deionized water control in 20 ml filtered (0.45 u) Chesapeake Bay water (14 ppt salinity) in polycarbamate flasks. 109Cd was added, flasks shaken for 30 minutes, and a 1 ml aliquot taken for determination of total 109Cd. Three, three-ml aliquots of each suspension were placed in dialysis bags of Spectropore #1 which has a molecular weight cutoff of 8000, and the remainder of each suspension received 100 microliters of 5% NaOCl (Fisher) with three three-ml aliquots placed in dialysis bags. Chlorinated and unchlorinated dialysis bags were dialyzed separately in polycarbamate flasks against 200 ml filtered estuarine water. Chlorinated and unchlorinated controls indicated completion of dialysis at three hours, at which time dialysis bags, their contents, and dialysates were analyzed for 109Cd.

Results and Discussion

The low concentrations of suspended clay-silt sediment in estuarine water sorbed a majority of the cadmium, 66-82% (Table 9). The ability of sediments and seston to readily sorb cadmium in solution is considered one of the major transport mechanisms of this toxic metal (Friberg, e.a. 1971). Since oxident in the sediment-free system ranged from 1.5 - 2 ppm and residual oxidant in the presence of sediment was 0.1 - 0.5 ppm, sediment-related oxidation was taking place. Sediment chlorination released 11 - 45% of sorbed cadmium in 30 minutes (Table 9). Assuming the lowest release (11%), chlorination of 1 mg/gm suspended clay-silt sediment carrying 91 mg/gm Cd could release 5 ug/l Cd in marine waters, the EPA suggested maximum. Clarke and Palmer (1972) reported most cadmium concentrations in upper Chesapeake Bay sediment were considerably lower (0 - 36 mg/gm) except in the Baltimore Harbor area (192.3 mg/gm). Since cadmium released in estuarine waters is tolerated by and readily concentrated in shellfish (Friberg, e.a., 1971), estuarine reaches receiving chlorinated power plant effluents should periodically be examined for cadmium accumulaton in nearby shellfish.

Sediment component dialysis experiments indicated that equal amounts of humic acid, sediment, bentonite and albumin had widely differing 109Cd sorption capacity (Table 10). The untreated dialysis bags sorbed 18 - 44% of available 109Cd and of the remainder, bentonite clay sorbed less than 1%, fresh sediment 13%, albumin 16% and humic acid 76%. Chlorination for 3 hours at an initial level of 21 ppm released 90% of albumin bound cadmium, 90% of sediment bound cadmium, and 16% of humic acid bound cadmium.

TABLE 9

Sediment Chlorination and Cadmium Desorption

	Test 1	Test 2	Test 3
Cd sorbed/ug sediment (cpm)	6108	20220	3560
Percent Cd sorbed	82	78	66
Cd sorbed/ug sediment (cpm) (post-chlorination)	5412	18075	1950
Percent Cd desorbed	11	11	45
Chlorine added (mg/l)	2.0	1.5	1.7
Residual oxident (mg/l)	0.5	0.1	0.2
pH	7.1	7.2	7.2

TABLE 10

Chlorination and Cadmium Desorption by Sediment and Sediment Components

Suspension (1 mg/ml)	109Cd in Solution (dpm/ml)	109Cd Sorbed Pre Cl (dpm/mg)	% 109Cd Sorbed	109 Cd Sorbed Post Cl (dpm/mg)	% 109Cd Desorbed
Bentonite	60300	537	0.89	671	-26
Humic Acid	29381	22330	76	18780	16
Sediment	40377	5600	13	557	90
Albumin	57563	9210	16	938	90

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SUMMARY

In conclusion, statistically significant increases in oyster copper concentrations were found at the Calvert Cliffs Nuclear Power Plant site after the second unit became operational. Copper levels in oysters incrased a year later at the reference site. Oyster copper concentrations at the power plant site declined in years following metallurgical loss of copper from new condenser system tubing, and again were paralleled by declines at the reference site one year later. This observation, along with laboratory demonstration of high copper sorptive capacity of clay-silt Bay sediment and lack of evidence that sediment-sorbed metal is accumulated by infaunal benthics, suggests a fixed sediment sink for copper at the plant site with a sorption/release mechanism causing slow spreading of copper accumulation in oysters beyond the tidally and thermally affected area moving about 10 km/year "upstream" in the Bay. Though widespread delayed increased oyster copper concentrations are lower than ones near the source because of dilution they must be recognized as a biotic impact from estuarine metal release by power plant systems.

Copper and zinc concentrations in estuarine oysters were found highly variable and nonparametric even in laboratory bred oysters. Reasons for the high variance within cohorts and within a community are not clear but probably are due to unknown micro-habitat phenomena rather than genetic differences. In order to make valid comparisons between sites large samples with individual oyster analysis (around 30 individuals) and nonparametric statistics should be employed.

Oyster copper and zinc concentrations were surprisingly consistently negatively correlated with 5 - 20 ppt salinity: -16 ug copper/gm oyster wet weight/ppt salinity, and -216 ug zinc/gm oyster wet weight/ppt salinity. This correlation parallels relative concentration of the divalent zinc metal ion resulting from inorganic (saline) complexation, and in the case of copper an additional, probably organic, complexation is indicated in higher salinity waters. This suggests control of oyster metal concentrations through the metal uptake (K1) process. However, oyster metal concentrations also varied according to yearly average salinity, suggesting a second, slower salinity response, probably taking place in the depuration (K2) process in oysters. The above renders year-to-year or even season-to-season comparison of oyster metal concentrations at a site difficult without appropriate reference sites.

Shellfish accumulated metal under laboratory conditions from water much more rapidly than from seston. Metal accumulation rates in shellfish were inversely proportional to salinity and directly proportional to divalent ion activity and concentration, also indicating the importance of salinity which must be taken into account before site-to-site and year-to-year field concentration differences can be interpreted.

Field and laboratory stunted oysters exhibited high metal levels with abnormal copper accumulation. Such innate unexplained characteristics of these rare populations should be recognized in field surveys.

Results from this study have been used in the decision to change the condenser tubing metallurgy at the Calvert Cliffs Nuclear Power Plant, and in sampling methodology of the Maryland State Dept. of Health for surveys of heavy metal levels in Chesapeake Bay shellfish.

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APPENDIX A

PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

REFEREED PUBLICATIONS:

Phelps, H. 1979. Cadmium sorption in estuarine mud-type sediment and cadmium accumulation in the soft-shell clam, Mya arenaria. Estuaries 2(1):40-44.

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Jones, C., D. Williams and H. Phelps. April 1977. Mid-Atlantic Regional MBS Symposium, Petersburg, VA. "Accumulation of zinc and copper in shellfish near a nuclear power plant." Abstract.

Phelps, H. L. Oct. 1977. Mini-Symposium on Biology in the District of Columbia, American University, Wash., DC. "Copper-zinc ratios in oysters from selected Chesapeake Bay locations." Abstract.

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Phelps, H.L. Nov. 1978. Savannah River Ecology Laboratory Symposium on Microcosms in Ecological Research, Augusta, GA. "Some factors affecting cadmium accumulation by estuarine molluscs." Abstract.

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Phelps, H.L. Jan. 1981. Mid-Atlantic Regional Meetings, American Chemical Society, Wash., D.C. "Effects of Mg and Ca on 109Cd uptake in the estuarine clam, Mya arenaria." Abstract.

Phelps, H.L. Sept. 1982. 1982 Interstate Seafood Seminar, Annapolis, MD. "Oysters and heavy metals in the mid Chesapeake region." Abstract.

Phelps, H.L. Nov. 1982. Society of Environmental Toxicology and Chemistry, Arlington, VA. "Environmental and ecological factors related to heavy metals in estuarine shellfish." Abstract.

Phelps, H.L. Nov. 1983. Seventh Biennial International Estuarine Reseach Federation Meetings, Virginia Beach, VA "Factors affecting heavy metal accumulation in estuarine shellfish." Abstract.

ADDITIONAL PRESENTATIONS:

Phelps, H. Nov. 1974. Atlantic Estuarine Research Society, St. Michaels, MD. "Binding of selected heavy metals by sediments from the Chesapeake Bay near the Calvert Cliffs nuclear power plant."

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APPENDIX B

ADDITIONAL ACTIVITIES RELATED TO AND GENERATED FROM THIS NASA SUPPORTED PROGRAM

GRANTS:

1975-76. "A Study of the Isozyme and Allozyme Adaptations of an Estuarine Clam Population to Nuclear Power Plant Thermal Discharge". Principal Investigator. NIH MBS Supplemental Grant. $17,000.

1975-76. "A Study of the Isozyme and Allozyme Adaptations of an Estuarine Clam Population to Nuclear Power Plant Thermal Discharge". Principal Investigator. NIH MBS Supplemental Grant. $17,000.

1977-80. "Interactions of Chlorine with Estuarine Sediments and Subsequent Effects on Clams and Oysters". Principal Investigator. NIH MBS Grant. $86,000.

1977-78. MARC Faculty Fellowship. University of Maryland. NIH. $28,000.

1980-81. "The Effect of Copper on Spat Settlement of the Oyster, *Crassostrea virginica*." Principal Investigator. Water Resources Center, Wash., DC. $15,000.

1982, 1983. Summer Faculty Fellowships, NORCUS. DOE. Battelle Northwest Marine Laboratory, Sequim, WA..

1984. Faculty Senate Research Grant, UDC. $3,000.

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Settlement of the Oyster, Crassostrea virginica. Report to Water Resources Research Center, Wash., DC. 37pp.

ABSTRACTS AND ADDITIONAL PRESENTATIONS:

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