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RESEARCH NOTES

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ROLE OF CHITIN IN THE ACCUMULATION OF HEAVY METALS IN THE AMERICAN OYSTER

Joseph J. Cooney and Richard A. Smucker

Oysters aren't supposed to turn green. When they do, it usually means they have been growing in copper-saturated waters. This project traced one of the trickiest ways in which copper can cause the greening of oysters. It documented how increasing counts of chitin in the estuary could speed up copper uptake by oysters.

Chitin is a common element in most estuarine systems. When crabs and many zooplankton die, certain microorganisms break down the chitin in their exoskeletons and release large amounts of carbon and small amounts of nitrogen back into the natural energy flows of the ecosystem. Researchers are now reporting that new agricultural pesticides and germicides kill off those chitin-degrading microorganisms, leaving a lot of free floating chitin. This project supports the hypothesis that in addition to keeping a lot of valuable carbon out of the food chain, those chitin fibers may be hooking up with copper and making it easier for this metal to penetrate the food chain and turn oysters green.

Smucker and Cooney suggest that future work could validate their hypothesis further by testing oysters over longer periods of time and at lower copper levels similar to those found in some areas of the estuary.

-- The Editors

INTRODUCTION

There is a paucity of information supporting any working model of the mechanisms of heavy metal transport and of accumulation in the oyster. This study was designed to test the hypothesis that the ubiquitous estuarine polysaccharide, chitin, is a mediator of available copper to the oyster. Chitin was chosen as the study particulate: 1) because it is a common decomposition product of crab, shrimp, zooplankton, and many microorganisms, and 2) because it is one of the most efficient natural polymer chelators of cations.

NOTE: Joseph J. Cooney, professor and laboratory head, and Richard A. Smucker, assistant professor, are faculty members at the Chesapeake Biological Laboratory of the University of Maryland's Center for Environmental and Estuarine Studies.

METHODOLOGY

The hypothesis that the presence of chitin promotes accumulation of copper in the American oyster, *Crassostrea virginica*, was tested on animals collected from Hog Island oyster bar (January 31, 1979), at the mouth of the Patuxent River. Thirty animals were temperature conditioned in our controlled environment laboratory beginning at 0°C and acclimated to 20°C water gradually over a period of five weeks. Animals were depurated in 5 µm filtered water for 24 hr and then selected for inclusion in the experiment on the bases of depuration activity (presence of biodeposits) and uniformity of size. Animals were randomly selected for experimental groupings.

Each oyster was placed in a separate dish with each dish containing the same volume of compressed air-aerated 0.45 µm filtered estuarine water. Animals were exposed to their respective conditions for 17.5 h, in the dark (Table 1). Copper was detected by flame atomic absorption (AA) spectrometry and also by x-ray microprobe analysis. The x-ray microprobe analysis was limited to the surface (1 µm depth) of the respective oyster gill face. AA analysis revealed information of homogenized samples, but in contrast, microanalysis revealed only metal within 1 µm of the respective gill surfaces.

RESULTS AND PLANS FOR FUTURE WORK

Results in Table 1 show that chitin was an acceptable material (pseudofeces dry weight/animal was used as an index of feeding). Also, 1 ppm copper (as chloride) was not inhibitory to feeding. Microanalysis of gill surfaces indicated that presence of chitin-copper complex resulted in higher copper on the gill surfaces than in animals exposed to copper alone.

The conclusion of this experiment is that copper bound to the organic polymer chitin is transported through the gill. Mechanism of the gill copper transport is not known but is consistent with Stauber (1961) and Galtsoff (1964), who showed that particulates are transported to deeper tissues after being phagocytized on the gill surface by wandering amoebocytes.

Presently we are completing our assessment of chitin microparticulate loads available to oysters. Water samples were taken with an especially designed pumping mechanism so as to effectively sample water between 1-1.5 in. from the sediment-water interface. Our assay system for specifically identifying chitin is nearing completion. A major problem is the purification of chitinase. Commercially available enzymes often contain proteinases and are not useful to this work. The fluorochrome DTAF (Calbiochem) has been chosen as the chromophore of choice to obtain suitably reproducible enzyme to fluorochrome ratios.

Future work on the oyster filtration of particulates should use lower, environmentally significant concentrations of copper and longer term experiments would help in validating (or disproving) the chitin-copper transport hypothesis. In addition, other kinds of environmentally significant soluble (<50,000 MW) and particulate substances should be assessed for their roles in cationic and anionic toxicant accumulation in the oyster.

Table 1. Effect of Chitin on Copper Accumulation by *Crassostrea virginica*.

Water: Filtered water plus medium[1]		X-ray microanalysis of gill[2] counts/250s		Copper content (ppm)[3]			Pseudofeces (mg) dry weight
		Internal Face	External Face	Gill	Mantle	Viscera	
No addition							
	A	0	0	388	414	154	6.5
	B	0	0	234	99	81	8.9
	C	0	0	404	172	73	13.0
	D	0	0	434	239	164	2.6
0.375% Chitin							
	A	20	0	162	150	43	22.4
	B	0	0	368	325	180	44.9
	C	0	0	234	149	86	99.7
	D	0	0	207	75	89	39.8
1 ppm Copper							
	A	0	0	584	580	159	10.8
	B	0	0	340	251	134	2.2
	C	0	0	344	158	91	2.6
0.375% Chitin and 1 ppm Copper							
	A	0	0	204	235	66	40.4
	B	1067	0	573	309	230	47.0
	C	1366	2632	638	594	123	38.8

[1] The control seawater basal medium was filtered through 0.45-µm filters. Salinity 8°/$_{oo}$. Animals were exposed for 17.5 h.

[2] Analysis of 1 µm surface layers were standardized conditions.

[3] Bulk analysis of nitric acid digested tissues, by atomic absorption spectrometry.