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RESEARCH NOTES

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THE BIOAVAILABILITY OF COPPER TO THE AMERICAN OYSTER, CRASSOSTREA VIRGINICA: THE IMPORTANCE OF FOOD VS. WATER AND THE ROLE OF DISSOLVED ORGANIC COMPOUNDS

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While the chemical species of a metal is important in determining its bioavailability to estuarine organisms, the relative importance of the different processes by which organisms such as bivalve molluscs accumulate a metal are not well understood. Researchers have characterized several mechanisms to account for metal uptake by molluscs: through ingestion of suspended inorganic particulates and food, as well as through metal ions in the water. In this research note, the authors discuss the importance between food and water on metal uptake--copper, in particular--by the American oyster, Crassostrea virginica. In addition, they also discuss the role of dissolved organic compounds in bioavailability. In their studies, Zamuda, Wright, Saunders and Smucker found that food ingestion can contribute significantly to copper uptake; thus, in the development of water quality criteria, considerations must not only take into account dissolved metal concentrations, but also the presence of food which could lead to an increase in uptake by the organism. By ignoring the presence of food on bioavailability, the toxicity of a particular metal could be seriously underestimated. In their work on the effect of dissolved organic carbon (DOC), the authors found that copper bioavailability increased when DOC levels were reduced, but decreased when DOC levels were added. Furthermore, the presence of chitin--which exists in great quantities throughout the Bay--could significantly reduce bioavailability of copper.

--The Editors

FOOD VERSUS WATER

Introduction

The relative contribution of food vs. water in the accumulation of trace metals will depend upon the concentrations and bioavailabilities of the various chemical

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forms of the trace metal exposed to the organisms. In a previous study (Zamuda and Sunda 1982) we reported that accumulation of dissolved copper by the American oyster, Crassostrea virginica, was a function of the cupric ion activity and that soluble chelates of copper (e.g., with Nitrilotriace acid) are not directly available. In laboratory experiments, we used a chemically defined seawater medium in which the speciation of metals could be calculated, controlled and systematically varied, to investigate the relative importance of food ingestion vs. absorption from solution upon the rate of copper accumulation by Crassostrea virginica.

Methods

Oysters were maintained in flow-through metal-buffered filters (0.45um) seawater at a range of cupric ion activities ($10^{-9.5}$, $10^{-10.5}$ and 10^{-12} M Cu^{2+}). Cupric ion activities in the exposure media containing trace metal-NTA buffers were determined from thermodynamic calculations. Phytoplankton, harvested at stationary phase, were continually pumped into the experimental tanks to yield a constant feeding concentration of 5×10^{4} cells mL^{-1}. For each level of cupric ion activity in the feeding tanks the oysters received cells cultured at 2 different levels, $10^{-9.5}$ and 10^{-12} M Cu^{2+}. Tissue copper content of T. pseudonana has been reported to be a function of cupric ion activity (Sunda and Guillard 1976). Thus, we could expose oysters to different particulate copper concentrations without varying the particle concentration or the food organisms. Exposure treatments also included unfed oysters to enable us to determine the rate of dissolved copper accumulation.

Oysters were sampled periodically during the 14-day exposure period, and analyzed for copper using a wet ashing method (concentrated HNO^{3}) followed by flame atomic absorption spectrophotometry.

Water temperature and salinity were maintained at 20-21°C and 35 ± 0.5 ppt. The tanks were aerated and the pH of each exposure solution measured periodically. The pH was 8.1 and did not vary significantly with either time or treatment. With the use of NTA buffers, small changes in the dissolved copper content of the media associated with copper accumulation by oyster tissue or algal cells should not significantly alter the speciation of copper in the exposure media.

Results and Discussion

The results of these experiments indicated that oysters fed algae accumulated appreciably more copper than those that were not fed. Copper accumulation also increased with cupric ion activity. Previous attempts to estimate the relative contribution of food to the metal uptake rate of metal accumulation was generally estimated from the difference between the rate of metal uptake in the presence of food and the rate when no food was present. Recent experiments (Zamuda et al. in prep.) have shown that the presence of particles may significantly increase the rate of uptake of dissolved copper. When food particles are present, there is an associated increase in the uptake due possibly to increased pumping rates, filtration rates or increased metabolic activity. To characterize this effect, we designed an experiment utilizing essentially copper-free food, thereby separating the "feeding" effect from any copper uptake attributable to copper-enriched food vs. the dissolved fraction. Oysters were fed algal cells cultured in a media of low copper availability (10^{-12} M Cu^{2+}). An additional set of treatments included oysters fed algal cells at similar particle concentrations as above but from a culture media of increased copper availability ($10^{-9.5}$ M Cu^{2+}). Thus, the difference in copper accumulation rates for oysters in the presence of algal cells with reduced copper content (6.3×10^{-19} moles Cu/cell) vs. unfed oysters would result from stimulation of dissolved copper uptake due to the presence of particles. The difference in copper accumulation rates for oysters in the presence of the relatively metal free cells vs. cells cultured at higher copper availability (2.5×10^{-16} moles Cu/cell) would be due to ingestion and assimilation of the particulate copper. Using this approach we were able to distinguish between the relative contribution of (1) absorption of copper from water, (2) uptake of copper from food, and (3) the stimulation of absorption dissolved copper due to the presence of food particles.

In the absence of food particles copper accumulation increased as a function of the cupric ion activity. The contribution of food ingestion (difference in accumulation rates of copper by oysters in the presence of cells cultured at higher copper availability vs. the relatively copper-free cells) to the overall rate of copper accumulation in absolute terms was constant for the range of treatments. Yet, the relative contribution of food increased with decreases in cupric ion activities. Thus, as the availability of dissolved copper decreases (decreases in cupric ion activity) the relative importance of food ingestion to copper accumulation in oyster increases.

These results indicate for most environmental situations, food ingestion of particulate matter may contribute significantly to the total metal body burden of oysters and possibly other aquatic organism. The generally higher metal levels in oysters from less saline waters of many estuarine systems of the Chesapeake Bay (Huggett et al. 1975; see UM-SG-TS-83-08) may be the response of oysters to fluctuations in metal associated with particulate matter rather than to the soluble component. This explanation seems feasible due to the decreasing particulate concentrations toward the mouth of estuaries (Thayer et al. 1975) and the high particle filtration efficiencies of 75-100% have been reported for Crassostrea virginica (Haven and Morales - Alamo 1970). Thus, the composition and concentration of particulate matter may significantly affect the bioavailability and accumulation of copper.

In summary, the following generalizations can be made:

(1) accumulation of copper increases with cupric ion activity in the activity range of 10^{-12} to $10^{-8.5}$ M Cu^{2+};

(2) the presence of food particles significantly increases the rate of copper uptake by oysters;

(3) the increase in copper uptake may be attributed to not only the ingestion and assimilation of the particulate copper, but also to an associated increase in the uptake of dissolved copper by oysters in the presence of food particles; and

(4) water quality criteria based upon the rates of dissolved metal accumulation by organisms measured in the absence of food organisms may significantly underestimate the bioavailability of not only copper, but probably other toxic trace metals.

THE ROLE OF DISSOLVED ORGANIC COMPOUNDS

Introduction

Considerable uncertainty exists regarding the role of naturally occurring organic compounds in determining the chemical speciation and the bioavailability of trace metals. This uncertainty results in part from a lack of information both on the composition of organic ligands and quantifiable levels of trace metal complexation to these compounds. Despite numerous studies, approximately 90 percent of the dissolved organic compounds in estuarine waters remains uncharacterized; thus, their influence upon trace metal availability is uncertain.

Organic complexation of certain trace metals may control their accumulation and toxicity. For example, previous studies on microorganisms have indicated that increases in copper complexation decreased copper accumulation and toxicity by reducing free cupric ion activity (Sunda and Guillard 1976; Anderson and Morel 1978). As noted previously Zamuda and Sunda (1982) have shown that accumulation of copper by the American oyster, Crassostrea virginica, was related to the cupric ion activity and independent of the concentration of organically complexed copper. However, frequently these studies have utilized synthetic chelators such as Nitrilotriace acid (NTA), or ethylenediamine-tetracetic acid (EDTA) as analogs to natural dissolved organic compounds.

In our study, we have examined the influence of naturally occurring organic compounds upon trace metal availability. Copper accumulation by oysters and the effect of additions of cellular organic compounds (derived from phytoplankton) and chitin, as well as dissolved organic ligands present in estuarine water samples, were measured.

The results provide an improved understanding of the potential biological regulation of copper speciation and bioavailability, which can occur by the release of extracellular organic compounds or the presence of chitin. Although the influence of chitin in altering trace metal bioavailability is uncertain, significant concentrations do occur naturally in estuarine environments. This study provides an increased understanding of the factors that control metal accumulation under natural conditions and in evaluating the biological impact of anthropogenic metal inputs in estuarine environments.

Methods

The adult oysters we used were hatchery reared and of common genetic stock. The accumulation of copper by oysters was measured for a range of concentrations in the presence of various dissolved organic compounds. Treatments included: (1) cellular organic compounds derived from Thalassiosira pseudonana, (2) chitin, (3) uv-treated filtered estuarine water, and (4) filtered estuarine water.

To determine the effect of adding natural cellular organic compounds and chitin or the effect of prior photo-oxidation of naturally occurring organic matter in estuarine water upon the availability of copper to oysters, we used ^{64}Cu as a radiotracer. The ^{64}Cu was added to all treatments and equilibrated (24 h) with the exposure media prior to introduction of the test organisms. Three levels of ^{64}Cu addition were chosen to yield final total copper concentrations in the exposure medium of 10, 30, and 100 μg Cu L^{-1}. Twenty ml samples of each exposure tank were taken for initial ^{64}Cu analysis and again after the 24 h exposure period. Samples were taken from each treatment tank for DOC analysis. Dissolved organic carbon analysis employed the persulfate oxidation technique of Menzel and Vacarro (1969).

The 4 liters of exposure medium in each polyethylene tank were aerated and the water temperature and salinity maintained at 18-20°C and 20 ppt. The pH was 8.0 and did not vary appreciably with either time or treatment.

The results from the total copper concentration and ^{64}Cu analysis for the treatment media were used to convert radioisotope accumulation rates in oyster tissue to total dissolved copper accumulation rates.

Results

Oyster accumulation of copper (^{64}Cu) at a given concentration of dissolved copper varied significantly among the different estuarine water-dissolved organic carbon treatments (Fig. 1). Copper accumulation rates were greater in the uv-treated

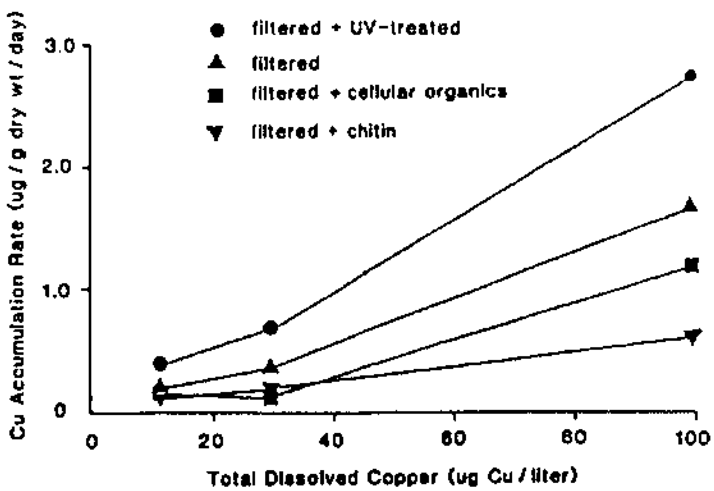

Figure 1. Crassostrea virginica. Net copper accumulation rates as a function of total dissolved copper in uv-treated estuarine water, estuarine water and estuarine water amended with cellular organic compounds (Thalassiosira pseudonana) or chitin.

filtered estuarine water than the non uv-treated filtered estuarine water. With the addition of dissolved organic compounds, the accumulation rates decreased: copper was less available in the presence of chitin than algal cellular organic compounds.

Increasing the total dissolved copper (64Cu) concentration increased the copper accumulation rates for oysters in a non-linear fashion. The apparently exponential increase in copper accumulation rates associated with increases in total dissolved copper at constant dissolved carbon levels was greatest for the uv-treated filtered (<10,000 NMW) estuarine water and decreased in the order: filtered (<10,000 NMW) estuarine water > algal cellular organics + filtered (<10,000 NMW) estuarine water > chitin + filtered (10,000 NMW) estuarine water.

Dissolved organic carbon values for the various exposure treatments were significantly different. The results indicate that greater than 50 percent of the natural dissolved organic carbon is filtered (<10,000 NMW) estuarine water was photo-oxidized by exposure to high intensity ultraviolet radiation. Dissolved organic carbon concentrations were significantly greater for the chitin and cellular organic compound amended treatments as compared to the filtered (<10,000 NMW) estuarine water.

Discussion

The bioavailability of dissolved copper to Crassostrea virginica is effected by dissolved organic carbon. Our results indicate that reductions of DOC associated with uv-photooxidation of filtered estuarine water increased copper bioavailability while additions of natural dissolved organic compounds (chitin and algal cellular organic compounds) decreased copper uptake by oysters. The variations in copper availability to oysters may be the result of changes in the chemical speciation of copper due to organic complexation of copper. Previous studies using synthetic chelators such as NTA have indicated that accumulation of copper was related to the cupric ion activity and not the concentration of organic-copper complexes (Zamuda and Sunda 1982). However, the influence of natural dissolved organic compounds upon copper bioavailability was not examined. Our work suggests that the relationship between copper accumulation and organic complexation is applicable for a wide range of organic compounds, including natural and synthetic chelators.

The increase in copper accumulation in uv-treated water can be explained by reductions in copper chelation and subsequent increases in cupric ion activity due to photooxidation of natural dissolved organic compounds. Gillespie and Vaccaro (1978) have shown that prior photo-oxidation of organic matter in coastal seawater increased cupric ion inhibition of bacteria incorporation of glucose at a given concentration of copper. Sunda and Guillard (1976) demonstrated that copper addition was more toxic to phytoplankton in photooxidized coastal seawater than in natural coastal seawater. Although only approximately 50 percent of the natural DOC was photooxidized in our uv-treated estuarine water this reduction in DOC, as compared to the non uv-treated estuarine water, was sufficient to result in an increase in copper uptake for each level of copper addition.

The increase in copper accumulation rates associated with increases in total copper concentrations was significantly greater for the uv-treated estuarine water as compared with the other treatments. This may be the result of insufficient chelation capacity of the photooxidized water in contrast to the other treatments. Analogous to a simple titration, the fraction of dissolved copper present as cupric ion will increase with increasing copper concentrations, due to saturation of binding sites of the natural organic compounds. As the complexation capacity of the medium is exceeded, copper accumulation rates would be expected to increase dramatically. This was evidenced in the addition of copper to the uv-photooxidized estuarine water in which the increase in the accumulation rate was significantly greater than similar copper additions to other treatments with higher concentrations of dissolved organic carbon.

The addition of algal cellular organic compounds significantly reduced the accumulation of copper by Crassostrea virginica. The explanation that the added organic compounds complex copper and make it less available for uptake is supported by previous studies. Exudates of living cells of the phytoplankton species, Cricosphaera elongata have been reported to decrease the amounts of Cu and Cd accumulated (Gnassia-Barelli et al. 1978). This phenomenon was attributed to a change in the chemical form of the metal. Several studies have reported that certain algal species may release extracellular organic compounds that complex copper (Swallow et al. 1978; Van Den Berg et al. 1979; McKnight and Morel 1980). Considerable variation in the composition of excreted organic compounds have been reported (Hellebust 1965).

Variations in the concentration and composition of excreted organic compounds may result from changes in light intensity, growth stage, nutrient conditions and species composition. Changes in the quantity and quality of organic compounds will result in variations in metal binding capacity of estuarine water and subsequently determine the detoxifying action of products liberated by phytoplankton. For example, Myklestad (1979) observed that polysaccharides released from two diatoms changed with the physiological state of the cells. Fisher and Fabris (1982) have reported that the copper complexing capacity of exudate form Skeletonema costatum, Asterionella japonica and Nitzchia closterium differed between log and stationary phase cells, suggesting differences in exudate composition between the growth phases.

The addition of chitin to estuarine water also resulted in the reduction of copper accumulation by oysters, and to a greater extent than the addition of algal organic compounds. At dissolved copper concentrations of 100 µg Cu L^{-1}, copper accumulation rates by oysters in the chitin amended medium were approximately 60 percent of the copper accumulation rate for oysters in the algal organic compound amended treatment. In addition, dissolved organic carbon concentrations were greater for the algal organic compound treatment (7.4 mg L^{-1}) compared with the chitin amended estuarine water (3.9 mg C L^{-1}). If the copper accumulation rates are standardized on a per milligram DOC basis, then the difference in the copper accumulation rate for oysters in the presence of chitin vs. algal organic compounds is even greater. This suggests that DOC compounds can differ markedly with respect to strength of copper complexation. The difference in copper accumulation rates recorded in our studies indicate more ligands per unit weight of chitin than for the algal organic compounds.

The role of chitin in trace metal transport and bioaccumulation has heretofore been generally overlooked. Although ambient chitin concentrations in estuarine systems are poorly documented, chitin is found in wall surface structures of spores of filamentous bacteria and many fungi and is also a component of the exoskeleton of several marine invertebrates such as crustacea. Viable fungal spores, filamentous bacterial spores and eubacteria are all at high concentrations in near-sediment water. Actinomycetes are often at concentrations of 10^5 CFU mL^{-1}. Other microbial groups are at similar concentrations. It is likely, then, that large quantities of chitin-based materials are present in the vicinity of an oyster bar and may significantly influence the bioavailability of copper to oyster populations.

Our results indicate that the presence of chitin reduces the bioaccumulation of copper to oysters. This reduction in copper availability is attributed to copper complexation by chitin and subsequent reductions in cupric ion activity. Evidence supporting this hypothesis is given by Muzzarelli (1977) who described the copper chelating ability of chitin.

The presence of natural organic compounds such as chitin and exudates from algal cells, which strongly bind copper, has important implications upon the bioavailability of copper, and perhaps other trace metals, to estuarine organisms. Our results indicate that the reduction of natural dissolved organic matter in estuarine waters can increase the accumulation of copper by oysters. In addition, the presence of chitin and algal organic compounds can significantly reduce the bioavailability of copper. This research examining the influence of organic compounds that occur naturally in estuarine waters supports the results of previous studies examining the effect of complexation upon copper accumulation employing synthetic chelators (Zamuda and Sunda 1982).

REFERENCES

Anderson, D.M. and F.M. Morel. 1978. Copper sensitivity of Gonyaulax tamarensis. Limnol. Oceanogr. 23:283-295.

Gnassia-Varelli, M., M. Romeo, F. Laumond and D. Pesando. 1978. Experimental studies on the relationship between natural copper complexes and their toxicity to phytoplankton. Mar. Biol. 47:15-19.

Haven, D.S. and R. Morales-Alamo. 1975. Filtration of particles suspension by the American oyster Crassostrea virginica. Biol. Bull. 139:248-264.

Hellebust, J.A.. 1965. Excretion of some organic compounds by marine phytoplankton. Limnol. Oceanogr. 10:192-206.

Huggett, R.J. F.A. Cross and M.E. Bender. 1975. Distribution of copper and zinc in oysters and sediments from three coastal plain estuaries, P. 224-238. In F.G. Howell, J.B. Gentry and M.H. Smith (eds.) Mineral cycling in southeastern ecosystems ERDA (Energy Res. Dev. Adm. Symp. Ser., CONF 740513).

McKnight, D.M. and F.M.M.M. Morel. 1980. Copper complexation by siderophores from filamentous blue-green algae. Limnol. Oceanogr. 25:62-71.

Menzel, D.W. and R.F. Vaccaro. 1980. The measurement of dissolved organic and particulate carbon in seawater. Limnol. Oceanogr. 9:138-142 (1964).

Myklestad, S. 1977. Production of carbohydrates by marine planktonic diatoms. J.Exp. Mar. Biol. Ecol. 29:161-179.

Sunda, W. G. and R.R.L. Guillard. 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. J. Mar. Res. 34, 511-529.

Swallow, K.C., J.C. Westall, D.M. McKnight, N.M.L. Marel and F.M.M. Marel. 1978. Potentiometric determination of copper complexation by phytoplankton exodotic. Limnol. Oceanogr. 23, 538-542.

Van Den Berg, C.M.G., P.T.S. Wong and Y.K. Chau. 1979. Measurement of complexing materials excreted from algae and their ability to ameliorate copper toxicity. J. Fish. Res. Board Can. 36:901-905.

Zamuda, C.D. and W.G. Sunda. 1982. Bioavailability of dissolved copper to the American oyster Crassostrea virginica. I. Importance of Chemical Speciation. Mar. Biol. 66:77-82.

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